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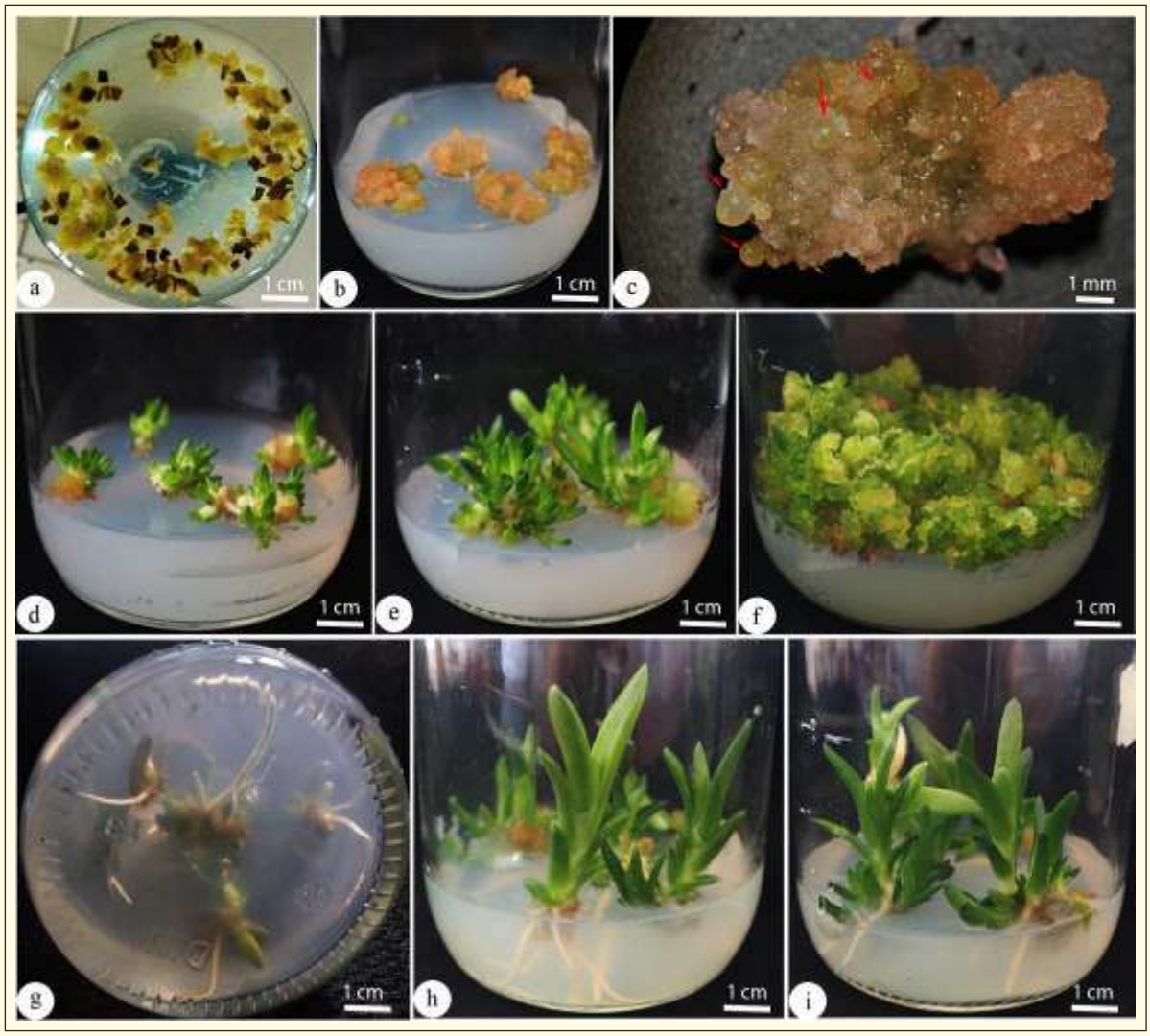

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Cover page courtesy :
Trinh and Tran, p. 185
(*in vitro* plantlet regeneration in *Haworthia retusa)*

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JOURNAL OF HORTICULTURAL SCIENCES

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In this issue…

Horticulture production has increased 13 times from 25 million tonnes in 1950-51 to 351 million tonnes during 2022-23, which is more than the food grain production. Horticulture contributes 33% to the agriculture Gross Value Added (GVA) making a very significant contribution to the Indian economy. This record increase in horticulture production was made possible mainly due to the sustained efforts by the scientific force by carrying out basic and applied research in various disciplines of horticulture and resulting in the accumulation of knowledge about horticultural crops.

Journal of Horticultural Sciences attempts to attract the knowledge gained from the scientific community and share with the peers. The number of articles has increased to thirty-two including one review published in this issue. The editorial team gratefully acknowledges the authors, subscribers as well as members of the Society for Promotion of Horticulture for their constant support.

A review on impacts of climate change on root and tuber crops by ***Saravanan and Gutam*** *highlighted the influence of climate change on the root and tuber crops with case studies and success stories; adaptation strategies for climate resilience and policy and knowledge gaps, in ensuring long-term sustainability and food security in a changing climate.*

Crop improvement ensures superior quality and enhanced yield in varieties and hybrids. In order to improve fruit and yield traits coupled with PRSV-P tolerance in papaya, ***Megha et al.*** *evaluated intergeneric F_1 hybrid progenies and found that seven progenies of Arka Prabhath x V. cauliflora and six progenies of Arka Prabhath* x *V. cundinamarcencis were found superior. Mutation breeding technique is an efficient tool to create variability.* ***Bhat et al.*** *reported that the mutagenic efficiency and effectiveness were found highest at lower dose (50 Gy) and chlorophyll deficient mutants were also observed. On evaluation of ber cv. Apple on different training systems,* ***Nikumbhe et al.*** *revealed that Y-shape training system can be a good option for yield and quality improvement.*

Athulya et al. *assessed the genetic diversity and screened genotypes for bacterial wilt in tomato, and identified diversified parents which are useful for improving the fruit size of bacterial wilt resistant varieties.* ***Kaur et al.*** *determined genetic diversity and population relationship in parthenocarpic cucumber using simple sequence repeats (SSR) markers and suggested that it could be used to estimate genetic variation within a group of elite genotypes.* ***Senthilkumar et al.*** *studied the effects of high temperature stress on the gene expression profiles and molecular responses in the leaf, fibrous root, and storage root tissues of sweet potato and provide valuable insights in identifying key genes and pathways involved in the response to high temperature stress.* ***Bharathi et al.*** *evaluated single petalled tuberose genotype IIHR 17-23SP-08, which is early flowering, having long spike, high loose flower and bulb yield, resistant to root knot nematode and tolerant to leaf burn disease.*

Singh et al. *assessed genetic diversity of Dahlia germplasm using multivariate statistics, which discriminate different dahlia accessions based on the variability in quantitative and qualitative traits Similarly,* ***Gurung et al.*** *studied the genetic variability, association and path analysis in Chrysanthemum and suggested that for yield in terms of number of flowers per plant, direct selection of traits such as days to first flower opening, number of leaves per plant, flower diameter and number of branches per plant may be effective in selection.* ***Bhargav et al.*** *analysed the genetic diversity in China aster and identified quantitative traits for its improvement. Stability analysis in fennel by* ***Lal et al.*** *revealed that the genotypes GF-12, AF-1 and RF-101 found most suitable and adopted to organic production system.*

Recent advances in production technology of horticultural crops addressed many production constraints. ***Lakshmipathi et al.*** *reported that foliar application of ethrel @ 50 ppm and NAA @ 25 ppm + GA_3 50 ppm as well as zinc sulphate (0.5%) + borax (0.1%) improved the kernel as well as apple quality traits in Cashew. In order to standardise fertigation in papaya cv. Arka Prabhat,* ***Manjunath et al.****, indicated that application of 75% RDF through drip is beneficial to get higher fruit yield, nutrient use efficiency, net returns and benefit cost ratio.* ***Nair et al.*** *standardised media for potted plant production of marigold and reported that potting media of Arka fermented cocopeat+ vermicompost with weekly application of nutrient solution of 128:24:144 ppm N: P_2O_5 : K_2O respectively, @ 50 mL per pot was found best.*

Vittal et al. *study on the impact of carbohydrate metabolism pathways on bearing habit of mango genotypes indicated selection of suitable recombinants and hybrids having regular bearing habit in early nursery stage itself overcoming the problem of long gestation periods and other economic constraints.* ***Goyary et al.*** *investigated the effect of maturity stages on the quality indices of wood apple and developed colour chart to distinct maturity stages which can used by farmers for maturity detection of wood apple.* ***Chang and Tang*** *from Taiwan reported that by preserving leaves with delayed pruning might potentially mitigate the negative impacts of warmer winters due to climate change on litchi flowering. Studies by* ***Kalaivanan et al.*** *shown variation in leaf mineral nutrient contents in Pummelo grafted on different rootstocks and suggested that Pummelo can be an ideal rootstock for commercial cultivation with better nutrient absorption capacity, reduced chlorosis, and Phytophthora incidence.* ***Nimbolkar et al.*** *reported that polyembryonic mango genotypes viz., Turpentine Deorakhio, Olour and Bappakkai performed better in response to their physio-biochemical behaviour at higher level of salinity, indicating their potential as rootstocks for salt sensitive commercial mango cultivars.*

Sidhu et al. *optimized explants, media, plant growth regulators and carbohydrates for callus induction and plant regeneration in Citrus jambhiri.* ***Mahananda et al.*** *reported that pre-treatment of immature flower bud and tepal segment with 1 drop Tween-20 + 70% ethanol + 1% sodium hypochlorite and 0.1% carbendazim + 1 drop Tween-20 + 70% ethanol + 2% sodium hypochlorite recorded contamination free culture.* ***Trinh and Tran*** *in Vietnam established an efficient plantlet regeneration system by indirect somatic embryogenesis through callus derived from the leaf*

tissues in a span of 16-18 weeks in Haworthia retusa, which can be commercially exploited for its largescale production.

Effective postharvest management of the produce can reduce the loss occurred during harvest to arrival of the produce at the end user level. ***Anand et al.*** *recommended hot water treatments of mango cv. Banganapalli as it is safe and non-chemical treatment to manage diseases, fulfil quarantine requirements, improve quality and storage life.* ***Gurjar et al.*** *reported that post-harvest melatonin application reduced browning and extend shelf life in minimally processed lettuce during low temperature storage.*

Gowda and Sriram *observed that low doses of silver and silver chloride nanoparticles exhibited significant inhibition against Colletotrichum truncatum fungal spores, hence, could be used as potential antifungal agents against to control anthracnose in chilli.* ***Dhananjaya et al.*** *screened and identified resistance source for gummy stem blight, powdery mildew and cucumber green mottle mosaic virus in bottle gourd, which will be useful in developing resistance varieties.* ***Suriya et al.*** *studied the seasonal incidence, population dynamics and morphometric traits of exotic coconut whiteflies in southern Tamil Nadu and found that the exotic whitefly species viz., Aleurodicus rugioperculatus and Paraleyrodes bondari were prevalent throughout the year in southern tracts of Tamil Nadu in coconut.* ***Mandla and Vaidya*** *studied the production function analysis for vegetable cultivation in Kullu valley of Himachal Pradesh using Cobb-Douglas production model.*

Umesha et al. *revealed that constitutive expression of anti-apoptotic gene showing enhanced tolerance against the most dreaded Fusarium oxysporum f. sp. cubense Race 1 in highly susceptible variety Rasthali.* ***Sasipriya et al.*** *induced spectrum of chlorophyll mutations and morphological variations in okra through gamma radiation and found different chlorophyll mutations such as chlorina, xantha, viridis and albino green from higher at lower dose.* ***Abhishek and Bala*** *characterized standard chrysanthemum and found that genotypes with medium sized flowers and better keeping quality were found most suitable for pot culture, cut flower and flower arrangement, whereas, the varieties with bigger sized flowers were found suitable for exhibition purpose.*

On behalf of the Editorial Team, I extend sincere thanks to all the authors, readers, reviewers and executive committee members of SPH for their continuous support and faith in the team.

Rajiv Kumar
Editor in Chief

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Review

Climate change impacts on tuber crops: Vulnerabilities and adaptation strategies

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ABSTRACT

Climate change poses significant challenges to root and tuber crops, requiring robust adaptation strategies to mitigate vulnerabilities. This review examines the impacts of climate change on root and tuber crops, including rising temperatures, altered rainfall patterns, extreme weather events, and changes in pest and disease dynamics. These changes significantly affect root and tuber crop production, leading to lower yields, compromised quality, increased susceptibility to pests and diseases, and limited access to water resources. Adaptation strategies encompass various approaches, such as agronomic practices, crop diversification, improved water management, breeding for climate resilience, and agroecological methods. However, addressing knowledge gaps and research needs is crucial for better-understanding climate change impacts and developing effective adaptation strategies for root and tuber crops. Future research should prioritize resilient cultivar identification, enhanced cropping systems, improved pest and disease management, and exploring socio-economic dimensions of adaptation. This review emphasizes the urgent need to address climate change impacts on tropical root and tuber crops. It highlights the critical role of adaptive measures in ensuring long-term sustainability and food security in a changing climate.

Keywords : Adaptation strategies, climate change, extreme events, temperature, tuber crops

INTRODUCTION

Climate change refers to long-term shifts in Earth's climate system due to human activities such as burning fossil fuels and deforestation. These activities release greenhouse gases, trapping heat and gradually raising global temperatures. This has significant implications for agriculture, which heavily relies on weather patterns and water availability. Recognizing the importance of climate change in agriculture is crucial for formulating effective strategies to mitigate its impacts and ensure long-term food security.

Temperature changes have far-reaching consequences for crop growth. Rising temperatures make plants vulnerable to heat stress, negatively affecting their productivity. The Intergovernmental Panel on Climate Change (IPCC) reports highlight the potential geographic shift of crop ranges due to increasing temperatures, impacting their suitability in specific regions (Pachauri *et al*., 2014). The Earth Observatory (2022) reports that since the inception of the Industrial Revolution, the planet's average temperature has experienced an increase of approximately 1.1°C (1.9°F), primarily attributed to human activities, particularly the combustion of fossil fuels. Recent years have been marked by record-breaking warmth, with 2022 registering an average global temperature of 14.7°C (58.5°F) (Fig. 1). This warming trend has triggered a cascade of adverse consequences on the planet, including the escalation of sea levels, a surge in the frequency and intensity of extreme weather events, and notable disruptions to both plant and animal ecosystems.

A study by Lobell *et al*. (2011) demonstrated the detrimental effects of elevated temperatures on significant crops such as maize, wheat, and rice, resulting in reduced yields. These findings underscore the vulnerability of tropical root and tuber crops to temperature shifts caused by climate change and emphasize the urgency of implementing adaptation strategies to ensure long-term sustainability.

Altered precipitation patterns: They also significantly affect tropical root and tuber crops. Changes in rainfall timing, intensity, and distribution directly influence water availability and soil moisture,

impacting plant growth and productivity. The IPCC reports emphasize the increased likelihood of extreme weather events, including droughts and heavy rainfall, due to climate change. Contingent droughts can lead to water deficit stress in root and tuber crops, affecting tuberization processes and overall yield (Pachauri *et al.,* 2014). Conversely, heavy rainfall events can result in waterlogging, causing oxygen deficiency in the soil and adversely affecting root health and tuber development. Various studies, including Easterling *et al.* (2007), indicate that changes in precipitation patterns have already influenced crop productivity in different regions worldwide. These altered precipitation patterns pose significant challenges to cultivating tropical root and tuber crops and necessitate adaptive strategies, such as improved water management and irrigation techniques, to ensure their resilience and productivity under changing climatic conditions.

Increased frequency of extreme events: Climate change is projected to intensify the occurrence and severity of extreme weather events, such as heatwaves, storms, and hurricanes (Hobday *et al.,* 2018). These events have significant implications for tropical root and tuber crops. Heatwaves, for instance, exposed root and tuber crops to prolonged high temperatures, leading to heat stress and reduced photosynthesis. This negatively impacts tuber development and ultimately decreases yields. Intense storms and hurricanes can physically damage crops, uprooting plants and causing lodging, further reducing productivity. A substantial decline in maize and soybean yields in the United States due to extreme heat (Schlenker & Roberts, 2009). Studies highlight the vulnerability of tropical root and tuber crops to the increasing frequency of extreme events associated with climate change and emphasize the urgent need for adaptive strategies, including improved infrastructure, early warning systems, and crop protection measures, to mitigate the impact of these events and ensure the resilience of root and tuber crops in a changing climate (Tylor *et al.,* 2019).

Impact on pests and diseases: Climate change can significantly affect the prevalence, distribution, and dynamics of pests and diseases that harm crops (Skendžić *et al.,* 2021). Temperature and humidity changes can influence the life cycles, geographic ranges, and population dynamics of various pests and diseases (Skendžić *et al.,* 2021; Shrestha, 2019). Warmer temperatures may facilitate the expansion of pests and diseases into new regions, impacting crop productivity. Altered rainfall patterns and increased moisture can create favorable conditions for specific pests and diseases. Higher humidity, for example, promotes the growth of fungal pathogens responsible for tuber rot and leaf blight. Chakraborty *et al.* (2018) emphasize the potential impact of climate change on crop diseases and stress the need for adaptive management strategies to mitigate risks. Integrated pest management practices, including early detection, cultural practices, and biological control methods, may require adjustments to address the changing dynamics of pests and diseases in the context of climate change.

Food security and livelihoods: Climate change poses a significant threat to global food security, particularly in regions reliant on tropical root and tuber crops for sustenance and income (FAO, 2015). The Food and

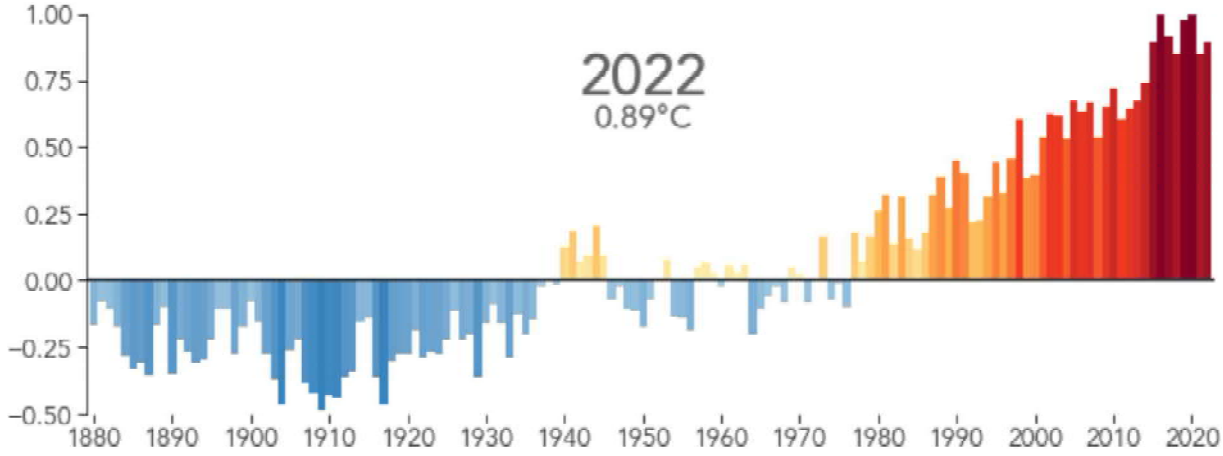

Fig. 1 : Global temperature anomaly compared to the 1950-1980 average (°C)
Source: https://earthobservatory.nasa.gov/

Agriculture Organization Report highlights climate change as a significant risk to global food security, exacerbating the challenges of ensuring access to safe and nutritious food for all (FAO, 2015). Smallholder farmers are particularly vulnerable and often engage in subsistence farming with limited resources. Reduced yields and disruptions in agricultural practices can lead to increased food prices, decreased access to nutritious food, and heightened food insecurity. Moreover, smallholder farmers' livelihoods, reliant on root and tuber crops, can be severely affected, undermining economic stability and overall well-being. Addressing climate change's challenges for root, and tuber crops is crucial for food security, poverty alleviation, and sustainable livelihoods in tropical regions. Wheeler and von Braun (2013) provide a comprehensive review of climate change's impacts on global food security, emphasizing the urgent need for effective measures to mitigate these challenges and ensure food availability for vulnerable populations. Parry *et al*. (2004) conducted extensive analysis, considering various emissions and socio-economic scenarios, highlighting the necessity of implementing adaptation strategies for food security in changing climatic conditions. Climate change significantly affects agriculture, including shifting cropping patterns, reduced yields, and increased production costs, necessitating substantial investments in adaptation measures to mitigate negative impacts and ensure long-term food security (Nelson *et al*., 2009).

Root and tuber crops are essential for food security and the livelihoods of smallholder farmers in tropical regions. Crops like cassava, sweet potato, yam, and taro are well-suited to local climates and thrive in diverse agroecological conditions. They provide nutritional value, resilience, and extended storage capabilities, making them crucial for ensuring food availability, especially in areas with limited access to other food sources. In 2021, significant areas were cultivated for various root and tuber crops. Cassava covered about 29.65 million hectares, 18.13 million hectares of potatoes, 7.41 million hectares of sweet potatoes, taro 1.79 million hectares, yams 8.69 million hectares, and yautia 0.03 million hectares (FAO, 2021). Understanding these trends is vital for sustainable agriculture and global food security. Cassava reached 314.81 million metric tons, potatoes recorded 376.12 million metric tons, sweet potatoes amounted to 88.87 million metric tons, taro contributed 12.40 million metric tons, yams yielded 75.14 million metric tons, and yautia produced 0.38 million metric tons. These substantial production quantities underline the significance of root crops in meeting global food demands and emphasize the need to manage their cultivation for future food security sustainably.

Research emphasizes the significance of tropical root and tuber crops in achieving food security and supporting smallholder farmers' livelihoods. Studies by Nanbol and Namo (2019) underline how these crops promote dietary diversity, income generation, and employment opportunities for smallholder farmers, particularly in Sub-Saharan Africa and Southeast Asia. The livelihoods of smallholder farmers are heavily dependent on tropical root and root and tuber crops (Owusu *et al.,* 2020; Nedunchezhiyan *et al.,* 2016). The world's top six root and tuber-producing countries, China, India, Nigeria, Indonesia, Brazil, and Thailand, play a critical role in the global food supply. However, climate change poses significant challenges to their root and tuber production. Rising temperatures and unpredictable weather patterns can impact crop growth and yields, decreasing productivity and potential food shortages.

Additionally, extreme weather events such as droughts and floods can disrupt cultivation practices and affect overall food security in these countries, highlighting the urgent need for sustainable agricultural strategies and adaptation measures to safeguard root and tuber production and ensure food resilience in the face of a changing climate. These crops ensure food security for farmers and their households and generate income through local market sales. Moreover, the root and tuber crops value chain provides employment opportunities in processing, transportation, and marketing, contributing to rural economies and poverty reduction.The significant impact of climate change on tuber crops is depicted in Fig. 2.

Climate change impacts on tropical root and tuber crops

Temperature shifts and their effects

Rising global mean temperatures have significant implications for the growth and productivity of tropical root and tuber crops. Increased temperatures directly affect physiological processes, altering growth patterns and overall productivity. Research indicates that

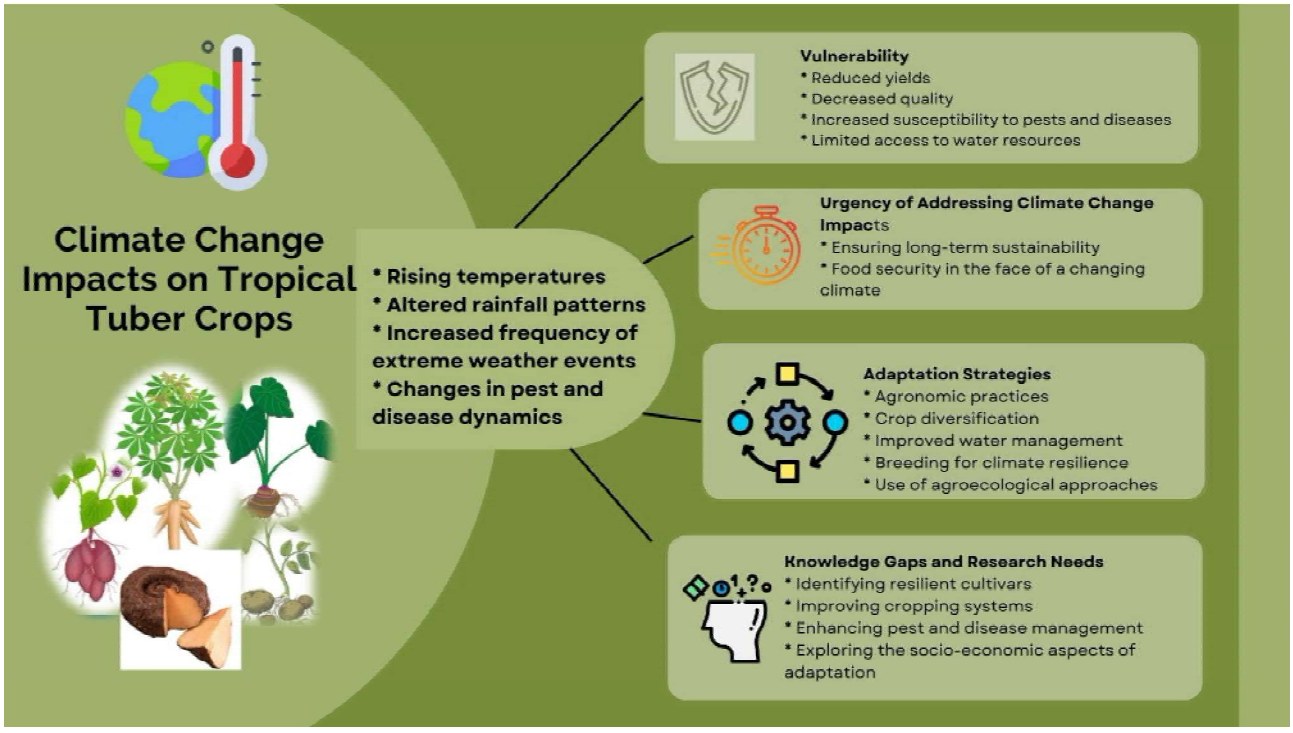

Fig. 2 : Climate change impacts on tuber crops

environmental stresses can reduce the yield of most crops by up to 50% (Reddy *et al*., 2023; Yadav *et al.,* 2018). High temperatures also compromise the tuber quality of potatoes and sweet potatoes, making them more susceptible to pests and diseases. Additionally, temperature changes can affect the timing of tuber production, posing challenges for crop planning (Reddy *et al.,* 2023).

Elevated temperatures affect root and tuber crops sprouting and establishment. High temperatures hinder seed germination, and delay sprout emergence, affecting overall crop establishment and potentially reducing yields of potatoes. Moreover, rising temperatures expedite developmental stages, resulting in shorter growth cycles and smaller tubers, reducing marketable yield and economic value (Lal *et al.,* 2022). Increased temperatures also impact photosynthesis by inducing physiological stress and reducing carbon dioxide uptake, ultimately affecting tuber growth and yield. Additionally, high temperatures contribute to water deficit stress, exacerbating negative impacts on root and tuber crops, particularly in water-limited regions. Contingent drought stress leads to smaller tubers, lower yields, and potential crop failure. The potato crop is also susceptible to the detrimental effects of rising temperatures. Elevated temperatures reduce leaf size and quantity, as well as the number of tubers (Lee *et al.,* 2020). The tuber yield of potatoes can decrease by up to 27% under elevated temperatures (Kimball, 2016). These negative consequences have significant implications for food security, potentially driving up food prices and affecting access to an adequate food supply.

Furthermore, the impacts of rising temperatures on crops may lead to shifts in cultivated crop types, impacting the entire food system. Temperature thresholds play a crucial role in determining the yield of root and tuber crops, as each crop has specific temperature requirements for optimal growth. Understanding these thresholds is vital for assessing the potential impacts of temperature fluctuations on root and tuber crop productivity (Balde *et al.,* 2018).

Various temperature stress and optimal temperatures are as follows:

- ***Optimal Temperature Range***: Cassava, for example, thrives between 25°C and 32°C, with yields declining outside of this range (Balde *et al.,* 2018). Maintaining temperatures within this optimal range is essential to maximize root and tuber crop yields.

- ***High-Temperature Stress***: Elevated temperatures beyond the optimal range can lead to heat stress, reducing yields. Sweet potato is susceptible to high-temperature stress, with yields declining when temperatures exceed 30°C (Kumar *et al.,* 2018). Mitigating the adverse effects of heat stress is crucial for temperature-sensitive crops like sweet potatoes.
- ***Cold Temperature Stress***: Cold temperatures below 10°C pose challenges for root and tuber crops. Chilling injury can significantly damage yam plants, resulting in stunted growth and decreased tuber yields (Sanginga *et al.,* 2019). Protecting yam and other root and tuber crops from chilling temperatures is essential for optimal growth and productivity.
- ***Freezing Temperatures***: Freezing temperatures significantly threaten root and tuber crops, causing frost damage and plant death. Protecting root and tuber crops from freezing temperatures, such as using coverings or frost-tolerant varieties, minimizes negative impacts and ensures successful tuber production.

Implementing appropriate measures to mitigate the effects of high and low-temperature stress is essential for sustaining root and tuber crop production and safeguarding yields (Kumar *et al.,* 2018; Sanginga *et al.,* 2019). Understanding temperature thresholds and their implications is crucial for optimizing root and tuber crop yields and mitigating the negative impacts of temperature extremes. Farmers and researchers can address the specific temperature requirements and vulnerabilities of root and tuber crops by implementing appropriate management practices and climate adaptation measures. Research has highlighted the critical role of temperature thresholds, especially potato growth, and development, affecting above-ground plant parts and tuber formation. High temperatures can stress crops, leading to a decline in potato and sweet potato yields and potential defects in tuber physiology. To ensure optimal root and tuber crop production and mitigate the adverse effects of high-temperature stress, it is essential to maintain temperatures within the optimal range and implement measures to mitigate extreme temperature events (Hatfield *et al.,* 2011). Heat stress events, intensified by climate change, can disrupt vital physiological processes, reduce yields, and alter carbohydrate balance and diurnal patterns (Challinor *et al*., 2005). Understanding the broader impact of high temperatures on crop production is crucial for developing mitigation strategies (Hatfield *et al*., 2011). Measures such as irrigation, shade structures, and heat-tolerant varieties can help mitigate the adverse effects of high temperatures and enhance root and tuber crop yields (Hatfield *et al.,* 2011).

Heat stress can significantly affect the physiological processes of root and tuber crops, including potatoes, during stages such as flowering and fruit development, leading to defects in tubers. Elevated temperatures also impact potatoes' tuberization, growth, and carbohydrate metabolism (Hastilestari *et al.,* 2018; Lal *et al.,* 2022). Understanding and managing these physiological responses are crucial for sustainable root and tuber crop production (Hastilestari *et al.,* 2018). Implementing strategies such as crop management practices, breeding for heat tolerance, and improving irrigation and water management can help mitigate the adverse effects of high temperatures and ensure food security for root and tuber crops in a changing climate (Xalxo *et al.,* 2020). Farmers and researchers can develop informed strategies to safeguard root and tuber crop production and ensure food security by considering the physiological impacts of heat stress.

Altered rainfall patterns and their consequences

Changes in rainfall patterns have significant implications for crop production and food security (Lobell *et al.,* 2008). Adapting to these changes is crucial for sustainable agriculture. Altered rainfall patterns affect water availability, planting schedules, irrigation requirements, and crop yields (FAO, 2015). Variations in rainfall intensity and distribution influence soil moisture, nutrient availability, and the occurrence of waterlogging or drought stress, impacting crop growth (FAO, 2015). Excessive rainfall and waterlogging can reduce crop yields, especially in rainfed systems (Nyakudya and S troosnijder, 2011). Prioritizing threshold planting dates and avoiding delays mitigate water logging effects (Nyakudya and Stroosnijder, 2011). Temperature stresses and uncertain precipitation patterns also impact crop growth and food security, emphasizing adaptable planting strategies (O'Brien *et al.,* 2021).

Soil management, including supplemental irrigation, optimizes tuber yield and quality, particularly for potatoes (Opena & Porter, 1999). Rainfall patterns

and irrigation practices influence soil moisture and nutrient availability, directly affecting potato tuber yield (Porter *et al.,* 1999). Moderate temperature increases during the tuber bulking stage and varying water availability affect potato yield and quality (Ávila-Valdés *et al.,* 2020). Understanding precipitation, water availability, and crop growth relationships is crucial for water management and sustainable agriculture (Ávila-Valdés *et al.,* 2020). Long-term drought stress harms root and tuber crop yield and quality, and climate change-induced temperature and precipitation fluctuations impact potato tubers (Orsák *et al.,* 2021). Water stress during different growth stages negatively affects potato plant growth, yield, and tuber quality (Wagg *et al.,* 2021). Cassava, a susceptible root and tuber crop, is affected by waterlogged soils and water deficits (Kerddee *et al.,* 2021). Implementing irrigation and water management strategies mitigates the negative impacts of drought and waterlogging on root and tuber crop productivity (Kerddee *et al.,* 2021). Considering these factors in irrigation schedules, water conservation practices, and drought-tolerant variety selection ensures sustainable root and tuber crop production in water-limited environments.

Increased incidence of pests and diseases

Climate change has significant implications for pests and diseases in root and tuber crops such as cassava (Bellotti *et al.,* 2012). Altered temperatures and rainfall patterns can affect arthropod pests in cassava agroecosystems (Bellotti *et al.,* 2012). Climate change can lead to new pests and diseases in Southeast Asian cassava production (Graziosi *et al.,* 2016). The whitefly species *Bemisia tabaci* is a cassava pest whose prevalence can increase due to climate change (Kriticos *et al.,* 2020). Co-infections of cassava viruses and pest resurgences result in severe symptoms and yield losses (Bisimwa *et al.,* 2019). Investigating climate change's impact on cassava pests is crucial for effective management (Chaya *et al.,* 2021).

Climate change influences pests and diseases in sweet potato and yam crops (Munyuli *et al.,* 2017). Vulnerability to pests like sweet potato butterflies (*Acraea terpsichore*) and African sweet potato weevils (*Cylas brunneus*) is expected to increase under climate change (Okonya & Kroschel, 2013). Models emphasize assessing sweet potato and yam responses to climate change and their interactions with pests (Raymundo *et al.,* 2014). Estimating the potential distribution of new potato pests, including those affecting sweet potato and yams, is crucial Insect pest-crop interactions lead to yield losses in sweet potato and yam cultivation (Tonnang *et al.,* 2022). Preparedness and disease-resistant cultivars are essential for managing pests and diseases in sweet potato and yam crops (Smith, 2015). The sweet potato weevil's infestation is influenced by location, altitude, and planting season. Higher temperatures can lead to a higher growth rate and severity of outbreaks (Hue and Low, 2015). In Kerala, India, upland areas experienced more severe tuber damage (4 to 50%) than lowland areas (up to 22%). However, in Kabale district, Uganda, lowland areas (up to 1814 meters above sea level) had more *Cylas* spp. Infestation (77%) compared to higher altitudes (1992–2438 meters above sea level) (23%). Weevil infestation can be reduced by strategic planting and harvesting times and by selecting well-buried root locations. Wet seasons with limited cracks in the soil show lesser damage than dry seasons, which facilitate weevil access to the roots. Understanding and managing pests like sweet potato weevils under climate change is crucial (Hue and Low, 2015). Climate change will expand potato pests' range and increase population densities (Quiroz *et al.,* 2018). Adapting potato crops to higher temperatures, diseases, pests, and water supply conditions is crucial (Haverkort and Verhagen, 2008). Integrated pest management, including cultural, biological, and chemical control methods, effectively minimizes environmental impacts (Singh *et al.,* 2013). Breeding pest-resistant potato varieties reduces reliance on pesticides (Hijmans, 2003). Estimating potential distributions of new potato pests helps anticipate challenges. Adaptation strategies like integrated pest management and breeding for pest-resistant varieties ensure sustainable potato cultivation.

Climate change affects pest life cycles, population dynamics, and disease spread through temperature and humidity changes (Legrève and Duveiller, 2010). Pest populations can be affected by changes in life cycles and temperature requirements (Van der Waals *et al.,* 2013). Climate change impacts the spread and impact of insect pests and livestock diseases Insects' shorter life cycles under warming conditions affect their abundance (Fand *et al.,* 2018). Changing climate alters insect life cycles, distribution, and fungal infections in crops. It also influences invasive pest species' dynamics and their impact on agriculture

(Dangles *et al.,* 2008). Expanded ranges of crop pests and changes in transmission dynamics challenge food production. The pests and diseases pose significant threats to root and tuber crops, including potatoes and sweet potatoes, and their potential implications are noteworthy. Here are some specific pests and diseases of concern:

- **Late blight** (*Phytophthora infestans*): A devastating disease affecting potatoes with climate change influencing its severity and distribution. The pathogen thrives in cool and moist conditions, making regions with higher humidity and rainfall more susceptible to outbreaks, and climate change can potentially create more favorable environments for its spread.
- **Potato cyst nematodes** (*Globodera* spp.): Microscopic soil-dwelling pests causing root galling and yield reduction in potatoes, influenced by changes in temperature and soil conditions. Climate change affects them by accelerating their life cycle in warmer temperatures, altering soil moisture conditions, weakening plant resistance, and potentially shifting their distribution.
- **Sweet potato weevils** (*Cylas* spp.): Major pests of sweet potatoes, causing damage to foliage and tubers, warmer temperatures, and changes in precipitation patterns can create more favorable environments for the weevils to thrive and reproduce. As these pests expand their range to new areas with suitable climates, sweet potato crops in those regions become more vulnerable to infestations, leading to increased damage to foliage and tubers.
- **Wireworms** (*Agriotes* spp.): Larvae of click beetles that damage potato tubers by tunneling into them, changes in temperature and moisture conditions can impact the behavior and lifecycle of wireworms (*Agriotes* spp.), affecting their activity levels and distribution. Warmer temperatures may accelerate their development and increase feeding rates, while alterations in soil moisture can influence their survival and movement patterns.
- **Cassava brown streak disease** (CBSD): A viral disease affecting cassava plants, causing necrotic lesions on tubers, warmer temperatures can accelerate virus replication and vector activity, promoting disease spread. Changes in rainfall patterns may also enhance the population of vector insects, increasing CBSD transmission to cassava plants. These climate-related factors contribute to the geographical expansion and severity of CBSD, posing a significant threat to cassava production and food security.
- **Cassava mosaic disease** (CMD): A viral disease-causing leaf mosaics and stunted growth in cassava, warmer temperatures can accelerate whitefly reproduction and virus multiplication, leading to increased disease transmission. Additionally, changes in rainfall patterns may affect whitefly populations, further influencing CMD's distribution and impact on cassava crops.
- **Yam anthracnose** (*Colletotrichum gloeosporioides*): A fungal disease-causing rotting of yam tubers, warmer temperatures, and increased humidity can create more favorable conditions for the fungal pathogen's growth and spread, leading to higher incidences of yam tuber rotting and potential crop losses.
- **Yam nematodes** (*Scutellonema* spp.): Microscopic soil-dwelling pests causing stunting and yield reduction in yams; warmer temperatures can accelerate their life cycle and reproduction, while alterations in soil moisture can affect their movement and survival. These factors contribute to stunting and yield reduction in yam crops, posing challenges for yam growers, especially under the influence of climate change.

These pests and diseases, among others, pose significant threats to root and tuber crops. Climate change can influence their prevalence, distribution, and severity, potentially impacting root and tuber crop production and food security. Effective pest and disease management strategies and adaptation measures are crucial to mitigate the risks associated with these threats and ensure sustainable root and tuber crop production.

Vulnerabilities of root and tuber crops to climate change

Tropical roots and tuber crops, such as cassava, sweet potatoes, yams, elephant foot yam, and taro, are vulnerable to climate change due to their unique characteristics (Yadav *et al.,* 2018). Understanding their adaptation processes and vulnerabilities is crucial for sustainable production and food security (Heider *et al.,* 2021). Researchers have studied the impact of climate change on root and tuber crops in various regions, highlighting the need for resilience-enhancing

strategies (Parker *et al.,* 2019). To address these challenges, focus is placed on seed systems, adaptation strategies, and plant growth-promoting microbes in the rhizosphere (Parker *et al.,* 2019). Like other root and tuber crops, potatoes face climate change's consequences, including temperature rise and increased disease and pest risks (Yadav *et al.,* 2018; Van der Waals *et al.,* 2013). The production of potatoes declined by more than 87% when grown under warmer temperatures in the Peruvian Andes due to the increased incidence of novel pests.

Furthermore, crop quality and value were adversely affected under simulated migration and warming scenarios (Tito and Feeley, 2018), and local farmers may face significant economic losses of up to 2,300 US$ ha^{-1} yr^{-1} due to these adverse impacts. Efforts are underway to develop climate-resilient potato varieties and implement integrated pest management practices (Yadav *et al.,* 2018; Hijmans, 2003). The rising concentration of CO_2 contributes to climate change. Research indicates that yam, taro, yam bean, and sweet potato crops have the potential to withstand elevated CO_2 levels, which could be advantageous for cultivating these crops in a changing climate (Ravi *et al.,* 2017; Ravi *et al.,* 2021; Ravi *et al.,* 2022). However, more research is needed to determine the long-term effects of elevated CO^2 on the growth and yield of these crops. It is projected that the potential impacts of climate change, specifically focusing on the mean temperature and total precipitation changes on yam (*Dioscorea* spp.) cultivation, to face growing difficulties in numerous regions across India by 2030 as a consequence of the changing climate (Remesh *et al.,* 2019). Genetic diversity is crucial in selecting stress-tolerant varieties for sweet potatoes and taro (Mukherjee *et al.,* 2015). However, root and tuber crops have limited tolerance to biotic stresses and are susceptible to pests and diseases (George *et al.,* 2017). Specific diseases, such as cassava brown streak disease and cassava mosaic disease, pose a significant threat to cassava (Yadav *et al.,* 2018) due to climate vagaries. To mitigate climate change's adverse effects on root, root, and tuber crops, the focus is placed on enhancing genetic diversity and developing climate-resilient varieties (Mukherjee *et al.,* 2015; George *et al.,* 2017). These efforts aim to cultivate varieties with improved tolerance to climatic stresses, ensuring the sustainability and resilience of root and tuber crop production (Yadav *et al.,* 2018).

Adaptation strategies for climate resilience

Development of climate-resilient varieties

Developing climate-resilient crop varieties is crucial for adapting to climate change (Acevedo *et al.,* 2020). Small-scale producers in low and middle-income countries have adopted climate-resilient root and tuber crops, enhancing resilience and food security (Acevedo *et al.,* 2020). Countries that consume tubers to a large extent, such as Ethiopia, must address climate-resilient food security (Yimer and Babege, 2018). Breeding programs are pivotal in developing climate-resilient crops with traits like root systems and drought tolerance (Banga and Kang, 2014). A multi-omics approach focusing on the root system has been employed for developing climate-resilient rice (Yoshino *et al.,* 2019) and genomics-assisted breeding aids in understanding stress responsiveness (Kole *et al.,* 2015).

The release of climate-resilient lentils and maize showcases progress in breeding for climate resilience (Gupta *et al.,* 2019; Cairns and Prasanna, 2018). Innovative breeding approaches that integrate traits and address climate challenges are essential for climate-resilient agriculture (Bakala *et al.,* 2020), ensuring sustainable food production under abnormal weather conditions (Maheswari *et al.,* 2015). Breeding techniques are essential for developing climate-resilient tropical root and root and tuber crop varieties. Traditional breeding methods and transgenic approaches offer promise in identifying adaptive traits (Singh and Bainsla, 2014). Genomics-assisted breeding aids in identifying genes associated with adaptive traits, while participatory varietal selection expedites progress (Kole *et al*., 2015; Jeeva *et al*., 2020). Innovative technologies such as in vitro techniques and artificial pollination support breeding efforts. These approaches, combined with improved breeding methods, aim to ensure food security by developing climate-resilient tropical root and root and tuber crop varieties (Banga and Kang, 2014).

Selecting resilient traits is pivotal in developing crops that withstand drought, heat, and diseases while improving nutrient use efficiency (Monneveux *et al*., 2013). Drought tolerance encompasses mechanisms like water use efficiency and root traits, with advancements in root phenotyping techniques contributing to breeding strategies

(Wasaya *et al.,* 2018). Promising outcomes are observed by incorporating drought and host plant resistance traits and combining multiple drought-tolerant traits (Low *et al*., 2020). Genetic tools such as quantitative trait loci mapping and molecular breeding aid in selecting stress-resilient traits (Kumar *et al*., 2017), enhancing adaptability under challenging conditions. Conventional breeding, molecular breeding, and biotechnological tools are crucial in developing climate-resilient crop varieties. Conventional breeding selects and crosses plants to create new varieties with improved resilience. Molecular breeding utilizes genomic information for precise trait selection (Wasaya *et al*., 2018), while biotechnological tools introduce specific genes or traits (Kumar *et al.,* 2017). Leveraging research on drought tolerance in cereals can enhance drought tolerance in potatoes (Monneveux *et al.,* 2013). Grafting techniques improve efficiency in drought resistance and water use (Kumar *et al.,* 2017). Together, these approaches develop climate-resilient varieties with traits such as drought tolerance, heat tolerance, disease resistance, and nutrient use efficiency.

Sustainable farming practices

Sustainable agricultural practices are crucial for enhancing the resilience of tropical root and root and tuber crops to climate change (Gweyi-Onyango *et al*., 2021). Investing in sustainable technologies and indigenous knowledge shows promising results in responding to climate change (Olaniyan & Govender, 2023). Ensuring sufficient food production requires actions to mitigate climate change impacts on crops (Yadav *et al*., 2018). Sustainable production systems and water management practices are necessary for smallholder agriculture and potato production (Yadav *et al*., 2018; Chartzoulakis & Bertaki, 2015). Stress-tolerant cultivars and cultivation technology change to support sustainable root and root and tuber crop production (Dahal *et al*., 2019). Local agricultural production in challenging climates promotes food security and a sustainable food system (Barbeau *et al*., 2015).

Soil and water conservation measures: Soil and water conservation measures, agroforestry, crop rotation, and integrated pest management contribute to resilient and sustainable farming (Meena *et al*., 2019; Kumawat *et al*., 2020; Delgado *et al.,* 2011). Practices like contour farming, mulching, and conservation tillage prevent soil erosion and enhance soil fertility (Delgado *et al.,* 2011). Cover cropping, agroforestry, and crop residue management conserve water and improve soil moisture (Recha *et al.,* 2014; Kumawat *et al.,* 2020). Agroforestry systems improve soil structure and nutrient cycling (Schoeneberger *et al.,* 2012). These practices support sustainable agriculture, conserve resources, reduce erosion, and manage pests and diseases, ensuring long-term viability and food security in a changing climate.

Agroforestry, crop rotation, multiple cropping systems, and integrated pest management: Conservation agriculture and organic farming practices improve soil health and water management and reduce greenhouse gas (GHG) emissions, offering sustainable alternatives (Tahat *et al.,* 2020). Techniques like cover cropping, crop rotation, reduced tillage, and ground cover maintenance enhance soil structure, water infiltration, and erosion control and minimize soil disturbance (Tahat *et al.,* 2020). Organic farming improves water use efficiency, reduces irrigation needs, and minimizes labor inputs (Tahat *et al.,* 2020). Conservation agriculture prevents soil carbon loss, reduces GHG emissions, and maintains ground cover, benefiting carbon sequestration and soil health. Studies highlight conservation agriculture's positive impacts on carbon sequestration, GHG emission reduction, and water use efficiency (Ghosh *et al.,* 2019). Integrating techniques like reduced tillage and cover cropping improves soil health, nutrient cycling, fertility, and water retention (Ghosh *et al.,* 2019). These practices promote sustainable agriculture, benefiting soil fertility, water conservation, and reducing environmental impacts. Conservation agriculture and organic farming provide sustainable alternatives, promoting long-term environmental and agricultural sustainability. They enhance soil health and water management and reduce GHG emissions, contributing to soil fertility, water conservation, and mitigating climate change (Tahat *et al.,* 2020). Implementing these practices improves agricultural productivity while minimizing negative environmental impacts. They are recognized for reducing GHG emissions, increasing carbon sinks, and promoting efficient agricultural resource use (carbon sequestration). Cassava is well adapted to multiple cropping systems, including cereals, legumes, and vegetables. A comprehensive review conducted by Ravi *et al.* (2021) explored the progress made in

multiple-cropping systems centered around cassava cultivation. They found cassava is a promising crop for successful intercropping with the above mentioned crops (Ravi *et al.,* 2021). This symbiotic approach not only aids in enhancing soil fertility but also contributes to higher yields while mitigating the vulnerability to pests and diseases.

Improved water management

Precision irrigation techniques: Efficient water management is vital for sustainable agriculture, particularly in climate change. Precision irrigation techniques like drip and micro-sprinklers optimize water use efficiency (Brar *et al.,* 2022). Drip irrigation delivers water directly to plant roots, minimizing evaporation, while micro-sprinklers provide uniform coverage, reducing wastage, evaporation, or wind drift (Brar *et al.,* 2022). Real-time soil moisture sensors enhance water management by enabling farmers to irrigate only when necessary, preventing over-irrigation.

Water-efficient cropping systems also contribute to sustainable water management. Planting drought-tolerant or low-water requirement crops significantly reduces water consumption (Ghaffar *et al.,* 2022). Crop diversification and multiple cropping optimize water resources by cultivating different crops, enhancing water productivity (Brar *et al.,* 2022). The integration of precision irrigation, rainwater harvesting, and water-efficient cropping systems holds the potential for sustainable water management in the face of climate change (Nikolaou *et al.,* 2020). These practices improve water productivity, minimize wastage, and alleviate pressure on scarce water resources in agriculture. By implementing these strategies, farmers enhance water use efficiency, mitigate water scarcity, and contribute to overall agricultural sustainability. For example, cassava requires approximately 3.0 mm of water daily during summer (Sunitha *et al.,* 2014). Climate change's impact on water availability can be addressed using precision management techniques like micro-irrigation and fertigation to enhance water use efficiency (Pushpalatha *et al.,* 2021). Modeling tools like FAO-AquaCrop help estimate yield and water needs, guiding farmers in making informed decisions for sustainable cassava production and food security amidst changing climate conditions (Sunitha *et al.,* 2023).

Rainwater harvesting and utilization of alternative water sources: Rainwater harvesting is valuable for supplementing irrigation and reducing reliance on conventional water sources. Rooftop and surface runoff harvesting collect rainwater for later use in irrigation, increasing water availability for agriculture. Wastewater recycling and drought-tolerant cultivars effectively mitigate climate change impacts on water resources. Wastewater recycling treats and reuses wastewater, reducing pressure on freshwater sources. Drought-tolerant cultivars withstand water scarcity, optimizing water use efficiency (Levy *et al.,* 2013). Implementing wastewater recycling and cultivating drought-tolerant varieties maintain agricultural productivity while minimizing dependence on limited freshwater resources (El-Nashar and Elyamany, 2023). These practices enhance the resilience of agricultural systems to water scarcity and changing climates (Patel *et al*., 2020; El-Nashar and Elyamany, 2023). Exploring alternative water sources and drought-tolerant cultivars enables farmers to adapt to climate change and ensure water resource sustainability in agriculture.

Case studies and success stories

Case studies and success stories provide valuable insights into climate change mitigation strategies and their regional applicability. A South African study emphasized neglected and underutilized crops (NUS), such as legumes and root vegetables, for climate adaptation and food security (Mabhaudhi *et al.,* 2017). Underutilized crops (NUS) such as the root vegetables cassava, sweet potato, and yam, and the legumes bambara groundnut, cowpea, faba bean, lentil, and pigeon pea, are a good source of nutrients and can be adapted to a variety of climatic conditions. They have the potential for climate adaptation and food security. These vegetables are all relatively easy to grow and can be adapted to various climatic conditions. They are also a good source of nutrients, which makes them an essential part of a healthy diet. These crops are integrated successfully into agriculture, enhancing sustainability and mitigating climate change. In Kenya, adopting resilient root crops in the agribusiness sector improved food security and livelihoods amid climate variability (Gatonye and Adam, 2022). Root crops as alternatives showcased their potential for climate change adaptation. Biofortification, enhancing crop nutrition, addressed food security challenges amidst climate change (Maqbool *et al.,* 2020). Success stories

highlighted its potential in vulnerable populations. A case study from Mizoram, India, highlighted indigenous technologies and practices for climate change adaptation (Sahoo *et al.,* 2018). Traditional farming practices sustained livelihoods in changing climates. These case studies emphasize the importance of diverse approaches, including NUS crops, resilient root crops, biofortification, and indigenous knowledge, to enhance climate resilience and food security.

Policy and knowledge gaps

Existing policies and initiatives for tropical root and tuber crop adaptation are vital but require addressing critical gaps. Specific strategies and guidelines tailored to root and tuber crops need to be improved, hindering their resilience (De Costa, 2010). Policies promoting climate-resilient varieties, sustainable farming, and improved water management are needed. Integrating root tuber crop adaptation into broader agricultural policies and research agendas is another crucial gap (Tittonell and Giller, 2013). Mainstreaming adaptation measures ensures support and effective implementation. Comprehensive data and knowledge on root and tuber crops' vulnerability and adaptive capacity need to be improved (UNDP, 2019). Research and monitoring are essential to identify suitable practices. Collaboration among stakeholders is crucial. Engagement, participatory approaches, and knowledge-sharing platforms enable the co-design and implementation of adaptation strategies. Bridging these gaps through specific strategies, policy integration, research, and collaboration is essential for the resilience and productivity of root and tuber crops in a changing climate.

Studying climate change impacts on root and root and tuber crops has revealed crucial knowledge gaps for effective adaptation strategies:

- **Understanding climate effects**: Further research is needed to examine the specific impacts of climate change on root and root tuber crops, including temperature, rainfall, and extreme events, to determine vulnerability and adaptive capacity.
- **Crop modeling**: Crop modeling is essential for developing reliable forecasting systems to predict the effects of climate change on root and tuber crops' productivity. These models provide valuable insights, inform adaptation strategies, and aid decision-making for farmers and agricultural stakeholders. With the arrival of advanced analytical tools like machine learning, combining climatic, weather, and agricultural data becomes feasible, enabling accurate predictions of annual crop yields at a country level in West African countries (Cedric *et al*., 2022). Such modeling efforts are crucial for addressing food security challenges and ensuring sustainable agricultural production in the face of climate change.
- **Genetic diversity and breeding**: Assessing root and root and tuber crops' genetic diversity and identifying traits for climate resilience, such as drought and heat tolerance, will guide breeding programs. Nutrient-rich under-utilized crop species (NUCS), including root and tuber crops, are nutrient-rich crops that can improve food security. Omics technologies can help us understand NUCS and improve their stress tolerance. Integrating omics technologies is a promising strategy for improving NUCS (Muthamilarasan *et al.,* 2019)
- Investigating efficient irrigation techniques, water storage, and water-saving practices is crucial for sustainable root and tuber crop cultivation in water-limited environments.
- Promoting agroecology, conservation agriculture, and integrated pest management can enhance root and tuber crop resilience. Research should evaluate their effectiveness in maintaining soil health, managing pests and diseases, and improving productivity.
- Understanding climate change's socio-economic implications on root and tuber crops-dependent communities and identifying supportive policy frameworks are vital. Research should explore barriers, the economic viability of adaptation strategies, and social and gender dimensions.
- Addressing these gaps supports evidence-based strategies and policies for climate-resilient root and root tuber crops, ensuring food security and livelihoods for vulnerable communities.

CONCLUSION

Climate change challenges tropical root and tuber crops, but effective adaptation strategies exist. Promoting climate-resilient varieties, efficient water management, sustainable farming, and supportive policies are vital. However, research is needed to understand climate impacts, develop crop models, assess genetic diversity, optimize water management,

study sustainable practices, and consider socio-economic aspects. By implementing these strategies and addressing research needs, we can protect root and tuber crops, ensure food security, and support vulnerable communities. Prioritizing these efforts enhances root and tuber crops' resilience and productivity in a changing climate.

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Original Research Paper

Evaluation of intergeneric F_1 hybrid progenies of papaya (Arka Prabhath x *Vasconcellea cauliflora* and Arka Prabhath x *Vasconcellea cundinamarcencis*) for morphological, fruit and yield traits coupled with PRSV tolerance

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ABSTRACT

Papaya is one of the most important fruit crops of tropical and subtropical regions of the world including India. Though India stands first in production in the world, the productivity is low as compared to other countries due to high incidence of papaya ring spot virus (PRSV-P) attack. As all the cultivated varieties under genus *Carica* are susceptible to PRSV, investigations were carried out to evaluate fifteen intergeneric hybrid progenies of Arka Prabhath x *V. cauliflora* and eighty-five progenies of Arka Prabhath x *V. cundinamarcencis* for morphological, fruit and yield traits coupled with PRSV- P tolerance. Out of fifteen, seven progenies of 'Arka Prabhath' x *V. cauliflora viz.*, $IGHF_1S4$-1, $IGHF_1S4$-12, $IGHF_1S4$-13, $IGHF_1S4$-14, $IGHF_1S4$-15, $IGHF_1S4$-17, $IGHF_1S4$-18 and out of eighty-five, six progenies of 'Arka Prabhath' x *V. cundinamarcensis viz.,* $IGHF_1S1$-17, $IGHF_1S1$-19, $IGHF_1S6$-20, $IGHF_1S2$-14, $IGHF_1S5$-12 and $IGHF_1S5$-14 recorded desirable traits such as days to first fruiting (240 to 250 days), bearing height (48 to 74 cm), plant height (175 to 200 cm), trunk circumference (37 to 48 cm), fruit weight (1133.67 to 2202.00 g), pulp thickness (2.45 to 4.05 cm), TSS (11.50 to 13.80 °Brix), fruits/tree (40 to 58) and yield (45.00 to 78.20 kg/tree) coupled with PRSV tolerance with disease score1 (only a few tiny chlorotic spots on leaves). These progenies were selected and forwarded for next generation (F_2). The hybridity was also confirmed using SSR marker (mCpCIR59).

Keywords : Evaluation, intergeneric hybrids, papaya, PRSV-P, *Vasconcellea cauliflora, Vasconcellea cundinamarcencis*

INTRODUCTION

Papaya (*Carica papaya* L; Caricaceae) is one of the most important fruit crops of the world due to its wide acceptance amongst the consumers and food processing industries. It is believed to have originated from Mexico to Panama and now has spread throughout the tropics and sub-tropics. It is a rich source of vitamins having an approximate composition of 2020 IU of vitamin A, 40 mg of vitamin B_1 and 46 mg of vitamin C per 100 g of fruit. India stands first in world production contributing 44.04 *per cent* followed by Brazil, Mexico, Indonesia, Dominican Republic and Nigeria (FAO, 2020). Though, there is an increase in area under cultivation of papaya in the recent past, the corresponding increase in production has not been realized in India due to several factors (Sharma and Tripathi, 2019). Among them, papaya ring spot virus (PRSV) type P (Litz *et al.*, 1984) a definitive poty virus species in the Potyviridae is the most devastating one in all the major papaya growing areas in the world (Sharma and Tripathi, 2019) causing yield loss upto 95 *per cent* (Babu and Banerjee, 2018). None of the control measures are successful and all the commercial cultivars of *Carica papaya* are susceptible to this disease. Hence, development of resistant/ tolerant cultivars using virus resistant wild relatives (*Vasconcellea* genus) through conventional breeding is the reliable tool for long term control of this disease (Pujar, 2019). Keeping this view, an attempt was made to evaluate the F_1 intergeneric hybrid progenies of the cross Arka Prabhath x *V. cauliflora* and Arka Prabhath x *V. cundinamarcencis* for morphological, fruit quality and yield traits coupled with PRSV tolerance.

MATERIALS AND METHODS

The field and laboratory experiments were carried out at the Division of Fruit Crops, ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake Post, Bengaluru during 2019-2021. The seeds obtained from the cross combination of Arka Prabhath x *V. cauliflora* and Arka Prabhath x *V. cundinamarcensis* were sown for raising F_1 population. A total fifteen intergeneric hybrid progenies of Arka Prabhath x *V. cauliflora* and eighty-five progenies of Arka Prabhath x *V. cundinamarcencis* were field evaluated along with its parents under the natural source of inoculum without challenge inoculation in order to know its potential for PRSV tolerance and to forward to next generation. All the individual seedlings were planted at a spacing of 2 x 2 m and standard package of practices were followed. The morphological, fruit and yield traits coupled with PRSV tolerance under natural condition were recorded as per the standards followed for papaya. All the observations pertaining to morphological and fruit traits were recorded at the time of first fruiting. Observations on yield and PRSV score were continued up to 14 months of field planting and progenies were selected based on the cumulative yield performance and average PRSV score recorded up to 14 months.

Morphological traits

The morphological traits *viz.,* days to first fruiting, bearing height (cm), plant height (cm), number of nodes, plant canopy spread (N-S & E-W in cm), number of leaves, trunk circumference (cm), number of fruits and yield per tree were recorded at first fruiting. PRSV scoring was done based on the intensity of visual symptoms on petiole, lamina and stem using the scale of 0-5 (0- resistant, 1- tolerant, 2- moderately tolerant, 3- moderately susceptible, 4- susceptible and 5- highly susceptible) as given by Dhanam (2006).

Fruit traits

The fruit traits *viz.*, fruit weight (g), fruit length (cm), fruit width (cm), pulp thickness (cm), fruit volume (ml), cavity volume (ml), fruit cavity (%), pulp colour, TSS (°Brix) and taste were recorded in 10 randomly collected ripe fruits at edible ripe stage. TSS was recorded using 'ERMA' hand refractometer and pulp colour was recorded using Royal Horticultural Society (RHS) colour chart. Fruit cavity (%) was calculated using the formula given below:

$$\text{Fruit cavity (\%)} = \frac{\text{Fruit cavity volume}}{\text{Fruit volume}} \times 100$$

Statistical analysis

The basic statistics like standard error of mean (S.E.m±), coefficient of variation (%) were performed by following the methods suggested by Singh and Chaudhary (1999).

Validation of progenies using molecular markers

Around 29 microsatellite (SSR) markers were screened to check the polymorphismin of both female (Arka Prabhath) and male parents (*V. cauliflora* and *V. cundinamarcencis*) to confirm the hybridity, out of which mcpCIR59 was selected for validating the selected hybrids. Following methodology was followed.

Isolation of DNA

DNA was extracted using CTAB method developed by Lodhi *et al*. (1994). Freshly matured leaves, free from diseases and developmental deformities were used for DNA extraction. They were brought to the laboratory in butter paper bags and cleaned using 76% ethanol to remove traces of dirt.

Polymerase chain reaction (PCR)

The PCR reaction was performed in a 15 µL reaction volume containing 10X buffer consisting 25 mM MgCl2, 1 mMdNTP's, 100 mM primers, 0.3 U of Taq DNA polymerase (Genei, Bangalore) and 20 ng template DNA in Eppendorf thermal cycler. The SSR primers were obtained from Bioserve, Hyderabad. The Primer sequence of optimized microsatellites from *Carica papaya* is as follows;

Locus name	Primer sequences (5'-3')	Ta (°C)
mCpCIR59	F: GTTGTTTGCATCCCACTGC R: CTCGCCATTCCATCTGGT	60

The PCR amplification programme for SSR was followed involving i) Initial denaturation: 94 ºC for 5 minutes, ii) Denaturation: 94 °C for 30 seconds, iii) Annealing: 60 ºC for 1 minute, iv) Extension: 72 °C for 45 seconds, v) Repeat step 2 to step 4 for 35 cycles vi) Final extension: 72 °C for 4 minutes

RESULTS AND DISCUSSION

Morphological traits

The morphological parameters varied significantly among all intergeneric hybrid progenies (Table 1 & 2). The days to first fruiting recorded in the intergeneric progenies of Arka Prabhath x *V. cauliflora* was ranged from 240 to 270 and in Arka Prabhath x *V. cundinamarcencis* it was 240 to 280 days. The early fruiting (240 to 248 days) was recorded in the selected intergeneric progenies of Arka Prabhath x *V. cauliflora* ($IGHF_1S4$-1, $IGHF_1S4$-12, $IGHF_1S4$-13, $IGHF_1S4$-15, $IGHF_1S4$-17, $IGHF_1S4$-18) and Arka Prabhath x *V. cundinamarcencis* ($IGHF_1S1$-17, $IGHF_1S1$-19, $IGHF_1S5$-12, $IGHF_1S5$-14, $IGHF_1S6$-20 and $IGHF_1S2$-14). The earliness in the fruiting observed in the progenies could be attributed to the early bearing nature of the female parent (250 days) compared to the male parents (*V. cauliflora*: 345 days and *V. cundinamarcencis*: 353 days). Early bearing progenies can escape PRSV infection and can result in quality fruits so that the farmers can get premium price in the market (Lichamo, 2015; Jayavalli, 2010).

The traits *viz.*, plant height at first fruiting, bearing height and number of nodes showed significant differences among the progenies of Arka Prabhath x *V. cauliflora* (133 to 223 cm, 56 to 86 cm and 14 to 23, respectively) and Arka Prabhath x *V. cundinamarcencis* (90 to 227 cm, 48 to 85 cm and 14 to 30, respectively). Lesser the number of nodes, lower is the bearing height. These traits are of much importance with respect to its suitability to the recent hi-tech horticultural practices like HDP to achieve higher productivity per unit area. Additionally, it was observed that lower number of nodes resulted in precocious or early bearing in papaya (Magdalita and Signabon, 2017). Hence, these traits were considered for selection of progenies.

With respect to plant canopy spread (E-W and N-S) and number of leaves, variations were observed among the progenies of Arka Prabhath x *V. cauliflora* (110 to 195 cm, 100 to 200 cm and 13 to 27, respectively) and Arka Prabhath x *V. cundinamarcencis* (107 to 220 cm, 100.00 to 290 cm and 11 to 34, respectively). All the progenies in both the combination recorded narrow leaf type (papaya type). The number of leaves play an important role in deciding the photosynthetic area of a genotype. Hence, wider canopy indicates more the number of leaves and more photosynthates for the developing fruits which helps in enhancing the final yield. In addition, source- sink relationship influences the rate

Table 1 : Morphological parameters of $IGHF_1$ progenies of Arka Prabhath x *Vasconcellea cauliflora*

Progeny	Days to first fruiting	Height to first fruiting (cm)	Plant height at first fruiting (cm)	No. of nodes to first fruiting	Plant canopy spread		No. of leaves at first fruiting	Trunk circum-ference (cm)	Number of fruits		Yield (Kg/tree)	PRSV Score
					E-W (cm)	N-S (cm)			At first fruiting	14 months after planting		
Range	240.00-270.00	56.00-86.00	133.00-223.00	14.00-23.00	110.00-195.00	100.00-200.00	13.00-27.00	26.00-48.00	18.00-35.00	20.00-50.00	11.00-58.00	Tolerant (1) to highly susceptible (5)
Mean	257.59	68.65	177.82	19.41	172.76	172.82	21.35	36.94	29.82	38.12	35.09	-
SEm±	5.85	2.26	5.22	0.51	5.41	6.23	1.03	1.34	2.50	3.51	4.07	-
CV (%)	9.37	13.59	12.10	10.78	12.91	14.87	19.87	14.99	34.55	37.96	47.82	-

Table 2 : Morphological parameters of $IGHF_1$ progenies of Arka Prabhath x *Vasconcellea cundinamarcencis*

Progeny	Days to first fruiting	Height to first fruiting (cm)	Plant height at first fruiting (cm)	No. of nodes to first fruiting	Plant canopy spread		No. of leaves at first fruiting	Trunk circum-ference (cm)	Number of fruits		Yield (Kg/tree)	PRSV Score
					E-W (cm)	N-S (cm)			At first fruiting	14 months after planting		
Range	240.00-280.00	48.00-85.00	90.00-227.00	14.00-30.00	107.00-220.00	100.00-290.00	11.00-34.00	30.00-50.00	11.00-46.00	13.00-58.00	12.75-78.20	Tolerant (1) to highly susceptible (5)
Mean	260.40	66.87	185.24	19.49	174.60	175.52	22.23	39.23	27.51	31.98	34.71	-
SEm±	1.52	0.79	2.40	0.31	2.23	2.50	0.49	0.45	0.86	1.26	1.46	-
CV (%)	5.46	11.00	12.09	15.04	11.93	13.27	20.47	10.68	29.20	36.83	39.11	-

of increase in yield, the resilience of yield and nutritional quality. It was observed that higher leaf area had a positive contribution towards higher productivity in papaya genotype Sunrise Solo (Paixao *et al*., 2019).

Stem circumference, number of fruits and yield per tree showed a wide difference among the progenies of Arka Prabhath x *V. cauliflora* (26 to 48 cm, 20 to 50 fruits/tree and 11 to 58 kg/tree, respectively) and Arka Prabhath x *V. cundinamarcencis* (30 to 50 cm, 13 to 58 fruits/tree and 12.75 to 78.20 kg/tree, respectively). As the fruiting behavior of papaya is cauliflorus in nature, thicker stem indicates the capacity of such progenies to tolerate virus infection and bear more number of fruit**s (**Magdalita and Signabon, 2017). Yield is a complex trait contributed by several factors. Hence, in the present investigation, the progenies with more number of fruits were considered as a selection criterion to advance further as it contributes to the final yield. Under the PRSV infection, it was observed that there was a considerable reduction in the growth as well as yield but the progenies which were having tolerance were able to put forth the leaves continuously and were able to yield considerable number of fruits. It was observed in the present study that the crosses involving *V. cauliflora* and *V. cundinamarcencis* as male parents were able to produce more number of fruits (80 and 85 fruits/tree, respectively in *V. cauliflora* and *V. cundinamarcencis*) which could have been inherited to the progenies resulting in the more number of fruits.

In the present study, based on the final score after 14 months of field evaluation, the male parents (*V. cauliflora* and *V. cundinamarcencis*) remained non-symptomatic, while, the female parent expressed disease symptoms after eight months of field planting (at first harvest) and was found to be highly susceptible. The intergeneric progenies *viz.,* $IGHF_1$ S4-1, $IGHF_1$S4-12, $IGHF_1$S4-13, $IGHF_1$S4-14, $IGHF_1$S4-15, $IGHF_1$S4-17 and $IGHF_1$S4-18 of Arka Prabhath x *V. cauliflora* and $IGHF_1$S1-17, $IGHF_1$S1-19, $IGHF_1$S2-14, $IGHF_1$S5-12, $IGHF_1$S5-14 and $IGHF_1$S6-20 of Arka Prabhath x *V. cundinamarcencis* developed few chlorotic spots and mild symptoms on fruits but the plants were able to tolerate the disease incidence and were vigorous with continuous growing tip. This result corroborated with the findings of Magdalita and Signabon (2017) showing that reduction in symptom severity could be attributed to the disease tolerance that could have been inherited from the resistant parents. As expected, in the present investigation also, the intergeneric progenies of *Carica* and *Vasconcellea* were found to be tolerant compared to the female parent (Jayavalli, 2010; Sudha *et al.,* 2013; Sunil, 2014; Lichamo, 2015; Pujar, 2019).

Fruit traits

Fruits are the economic part of the plant contributing to the final yield and related traits (Table 3 and 4). Wide variations were observed among the intergeneric progenies with respect to fruit shape, size and colour (Figure 1 and 2). These traits are highly heritable, hence, they are of high importance in progeny evaluation and selection. The fruit size is determined by the fruit weight which also contributes to the final yield.

Fruit weight recorded wide variations among the progenies of Arka Prabhath x *V. cauliflora* which ranged from 572.60 to 1245.00 g. With respect to Arka Prabhath x *V. cundinamarcencis,* it was ranged from 833.00 to 2267.25 g. Medium to big sized fruits are having more demand for both fresh fruit consumption as well as for processing industries (Magdalita and Signabon, 2017). The variations in fruit size could be due to the different heterotic combinations of the progenies. Wide variation in fruit size was also obtained (Jayavalli, 2010; Sudha *et al*., 2013) in intergeneric hybrids of papaya.

Table 3 : Fruit quality parameters of $IGHF_1$ progenies of Arka Prabhath x *Vasconcellea cauliflora*

Progeny	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Pulp thickness (cm)	Fruit volume (ml)	Fruit cavity (%)	Pulp colour	TSS (°Brix)
Range	572.60-1245.00	13.18-21.49	9.70-13.22	2.13-3.00	407.50-1099.00	16.38-43.37	Orange-orange red	8.20-12.80
Mean	969.40	16.48	11.35	2.53	722.42	26.28	-	11.16
SEm±	76.65	0.71	0.54	0.14	65.79	1.71	-	0.38
CV (%)	32.60	17.84	19.48	23.16	37.55	26.80	-	14.18

Table 4 : Fruit quality parameters of $IGHF_1$ progenies of Arka Prabhath x *Vasconcellea cundinamarcencis*

Progeny	Fruit weight (g)	Fruit length (cm)	Fruit width (cm)	Pulp thickness (cm)	Fruit volume (ml)	Fruit cavity (%)	Pulp colour	TSS (°Brix)
Range	833.00-2267.25	15.10-25.38	10.18-16.28	2.45-4.05	620.00-1666.67	16.76-33.85	Orange-orange red	7.00-14.85
Mean	1576.62	20.04	12.85	3.17	1138.69	22.83	-	12.97
SEm±	33.68	0.26	0.20	0.04	25.48	0.47	-	0.11
CV (%)	19.93	12.04	14.70	12.08	20.87	19.04	-	8.27

Variation in fruit shape and size | Variation in pulp colour

Fig. 1 : Variation in fruit shape, size and pulp colour of F_1 intergeneric progenies of Arka Prabhath x *V. cauliflora*

Variation in fruit shape and size | Variation in pulp colour

Fig. 2 : Variation in fruit shape, size and pulp colour of F_1 intergeneric progenies of Arka Prabhath x *V. cundinamarcencis*

A wide variation was also observed in the pulp thickness and cavity per cent recorded among the progenies of Arka Prabhath x *V. cauliflora* (2.13 to 3.00 cm and 16.38 to 43.37 %) and Arka Prabhath x *V. cundinamarcencis* (2.45 to 4.05 cm and 16.76 to 33.85 %). The pulp colour varied from orange to orange-red in both the combinations. The cavity per cent is an important parameter which determines the final pulp recovery. A thick pulp is an important trait of a genotype as it contributes considerably to the edible portion of the fruit. The reason attributed to the thick and firm pulp with lower cavity in the intergeneric progenies could be inherited from the female parent, Arka Prabhath (Jayavalli, 2010; Sudha *et al.,* 2013). Thus, the progenies with lower cavity per cent, higher pulp percentage and attractive pulp colour were given preference for further advancement. The earlier workers have reported that pulp contained rich carotene and lycopene in the intergeneric progenies of papaya involving female parent *viz.,* Arka Surya (Lichamo, 2015), 'Arka Prabhath' (Pujar, 2019), C0 7 and Pusa Nanha (Jayavalli, 2010).

Total soluble solids recorded wide variations among the progenies of Arka Prabhath x *V. cauliflora* (8.20 to 12.80 °Brix) and Arka Prabhath x *V. cundinamarcencis* (7.00 to 14.85 °Brix). Magdalita and Signabon (2017) were opined that TSS is an important trait to be considered in selection of genotypes for fresh fruit consumption. It was also observed that majority of the intergeneric progenies (99.00%) recorded excellent to intermediate taste similar to that of female parent (Arka Prabhath) which might have been inherited from the female parent. Though, the wild species used in the study is of poor quality, none of the progenies were found to have the linked trait of poor quality but were found to be superior in taste. Several workers have also reported that the intergeneric hybrids of papaya produced good quality fruits (Jayavalli, 2010; Sudha *et al*., 2013; Lichamo, 2015).

Morphological, fruit quality, yield traits and PRSV score of the selected progenies

The traits of the selected progenies are depicted in Table 4, 5 and 6. The selected progenies of Arka Prabhath x *V. cauliflora* ($IGHF_1S4$-1, $IGHF_1S4$-12, $IGHF_1S4$-13, $IGHF_1S4$-14, $IGHF_1S4$-15, $IGHF_1S4$-17 and $IGHF_1S4$-18) and Arka Prabhath x *V. cundinamarcencis* ($IGHF_1S1$-17, $IGHF_1S1$-19, $IGHF_1S6$-20, $IGHF_1S2$-14, $IGHF_1S5$-12 and $IGHF_1S5$-14) were found semi-dwarf (1 to 2 m), low bearing height (<1 m), thick stem circumference (>30 cm), medium-big sized fruit (>1 kg), thick pulp (>2.50 cm), low cavity (<25%), high TSS (>11 °Brix), more number of fruits/tree (>30) and high yielding (>45 kg/tree) coupled with PRSV-P tolerance (reduced symptom severity and

Table 5 : Morphological, fruit quality, yield traits and PRSV score of the selected $IGHF_1$ progenies of Arka Prabhath x *V. cauliflora*

Trait	Progenies								Parents	
	$IGHF_1$ S4-1	$IGHF_1$ S4-12	$IGHF_1$ S4-13	$IGHF_1$ S4-14	$IGHF_1$ S4-15	$IGHF_1$ S4-17	$IGHF_1$ S4-18	Mean	Arka Prabhath	*V. cauliflora*
Days to first fruiting	242.00	248.00	245.00	250.00	246.00	240.00	245.00	257.59	250.00	345.00
Plant height at first fruiting (cm)	188.00	178.00	180.00	175.00	182.00	182.00	184.00	177.82	190.00	160.00
Bearing height (cm)	71.00	65.00	72.00	69.00	74.00	66.00	67.00	68.65	74.00	58.00
Stem circumference at first fruiting (cm)	41.00	38.00	38.00	37.00	38.00	39.00	40.00	36.94	38.00	27.00
Number of nodes at first fruiting	19.00	19.00	21.00	20.00	22.00	20.00	19.00	19.41	20.00	17.00
Canopy spread E-W (cm)	185.00	190.00	185.00	190.00	185.00	195.00	185.00	172.76	185.00	170.00
Canopy spread N-S (cm)	190.00	200.00	190.00	195.00	190.00	190.00	180.00	172.82	180.00	145.00
Number of leaves at first fruiting	25.00	23.00	25.00	25.00	23.00	27.00	26.00	21.35	23.00	19.00
Number of fruits (14 months after planting)	45.00	45.00	42.00	45.00	40.00	50.00	48.00	38.12	38.00	80.00
Yield (kg/tree)	52.00	55.00	45.00	54.00	47.00	58.00	51.00	35.09	35.00	4.50
Fruit weight (g)	1225.00	1234.63	1165.20	1222.00	1245.00	1244.70	1133.67	969.40	1100.00	67.23
Fruit length (cm)	19.50	18.81	13.52	17.10	18.38	21.49	20.43	16.48	17.60	9.65
Fruit width (cm)	11.50	13.05	12.50	12.44	12.92	11.56	12.30	11.35	9.60	3.95
Fruit volume (ml)	970.00	1099.17	735.00	1032.50	735.00	943.57	828.33	722.42	975.40	38.83
Pulp thickness (cm)	3.00	2.78	2.45	2.50	2.78	2.76	3.00	2.53	2.50	0.51
Pulp colour (RHS colour chart)	Orange 25B	Orange 25A	Orange red 30A	Orange 28B	Orange red 30C	Orange 28B	Orange 25A	-	Orange red 30C	Yellow group 4D
Fruit cavity (%)	20.62	16.38	27.21	19.37	34.01	16.96	25.35	26.28	25.63	25.75
TSS (°Brix)	11.50	12.2	12.80	12.76	12.32	12.38	12.70	11.16	12.50	9.60
PRSV score	Tolerant (1)	Tolerant (1)	Tolerant (1)	Tolerant (1)	Tolerant (1)	Tolerant (1)	Tolerant (1)	-	Highly susceptible (5)	Resistant (0)

delayed expression of disease). The progenies were found vigorous even under the diseased condition indicating their tolerance to the disease. Hence, these progenies provide significant reductions in use of land, water, fuel and other inputs (cost of production) and thereby increasing the profitability as well as productivity. These progenies had recorded more number of fruits and yield than the female parent indicating that they are superior even under stressed condition reflecting their tolerance to the disease (Chalak and Hasbanis, 2017). These progenies performed better than the female parent due to the heterotic effect and this hybrid vigour for the traits of interest *viz.,* fruit quality and tolerance to disease can be exploited in the breeding program (Jayavalli; 2010; Sunil, 2014; Lichamo, 2015; Magdalita and Signabon, 2017). Hence, based on the morphological, fruit and yield traits coupled with PRSV tolerance theses progenies were advanced to next generation (F_2).

Validation of progenies using molecular markers

A total 29 microsatellite (SSR) markers were screened, one SSR marker (mCpCIR59) was selected which produced polymorphic banding pattern (367- 378 bp) in both female (Arka Prabhath) and male parents (*V. cauliflora* and *V. cundinamarcencis*). The selected intergeneric hybrid progenies of the combinations Arka Prabhath x *V. cauliflora* ($IGHF_1$S4-1, $IGHF_1$S4-12, $IGHF_1$S4-13, $IGHF_1$S4-14, $IGHF_1$S4-15, $IGHF_1$S4-17 and $IGHF_1$S4-18) and Arka Prabhath x *V. cundinamarcencis* ($IGHF_1$S1-17, $IGHF_1$S1-19, $IGHF_1$S6-20, $IGHF_1$S2-14, $IGHF_1$S5-12 and $IGHF_1$S5-14) screened using mCpCIR59 exhibited polymorphic bands of both the parents thereby, confirming their hybridity (Figure 3). Pujar (2019) and Naveen (2021) have also confirmed the hybridity in the intergeneric progenies of papaya using SSR markers.

Table 6 : Morphological, fruit quality, yield traits and PRSV score of the selected $IGHF_1$ progenies of Arka Prabhath x *V. cundinamarcencis*

Trait	Progenies							Parents	
	$IGHF_1$ S1-17	$IGHF_1$ S1-19	$IGHF_1$ S6-20	$IGHF_1$ S2-14	$IGHF_1$ S5-12	$IGHF_1$ S5-14	Mean	Arka Prabhath	*V. cundina-marcencis*
Days to first fruiting	240.00	245.00	245.00	246.00	245.00	245.00	260.40	250.00	353.00
Plant height at first fruiting (cm)	200.00	199.00	189.00	188.00	190.00	188.00	185.24	190.00	180.00
Bearing height (cm)	68.00	48.00	62.00	72.00	66.00	66.00	66.87	74.00	80.00
Stem circumference at first fruiting (cm)	41.00	47.00	43.00	48.00	46.00	40.00	39.23	38.00	35.00
Number of nodes at first fruiting	17.00	15.00	15.00	18.00	17.00	18.00	19.49	20.00	30.00
Canopy spread E-W (cm)	190.00	180.00	190.00	210.00	190.00	183.00	174.60	185.00	175.00
Canopy spread N-S (cm)	175.00	185.00	210.00	205.00	192.00	178.00	175.52	180.00	150.00
Number of leaves at first fruiting	27.00	27.00	25.00	29.00	27.00	26.00	22.23	23.00	20.00
Number of fruits (14 months of planting)	58.00	54.00	52.00	50.00	58.00	53.00	31.98	38.00	85.00
Yield (kg/tree)	69.00	75.33	76.00	72.00	69.83	78.20	34.71	35.00	6.87
Fruit weight (g)	1540.00	1395.00	1545.54	2070.20	1204.00	2202.00	1576.62	1100.00	124.37
Fruit length (cm)	21.83	21.23	22.06	20.82	18.06	20.10	20.04	17.60	8.40
Fruit width (cm)	11.51	11.29	11.96	14.90	12.18	14.95	12.85	9.60	4.80
Fruit volume (ml)	1114.71	939.64	1163.18	1100.00	867.50	1490.00	1138.69	975.40	63.75
Pulp thickness (cm)	3.15	3.12	3.42	3.10	2.78	4.05	3.17	2.50	0.85
Pulp colour (RHS colour chart)	Orange red 30B	Orange red 30A	Orange red 30A	Orange red 30A	Orange red 30A	Orange red 30A	-	Orange red 30C	Yellow group 12C
Fruit cavity (%)	18.39	20.75	16.76	19.55	20.75	18.79	22.83	25.63	18.82
TSS (°Brix)	12.50	13.00	13.30	13.75	13.00	13.80	12.97	12.50	8.20
PRSV score	Tolerant (1)	Tolerant (1)	Tolerant (1)	Tolerant (1)	Tolerant (1)	Tolerant (1)	-	Highly susceptible (5)	Resistant (0)

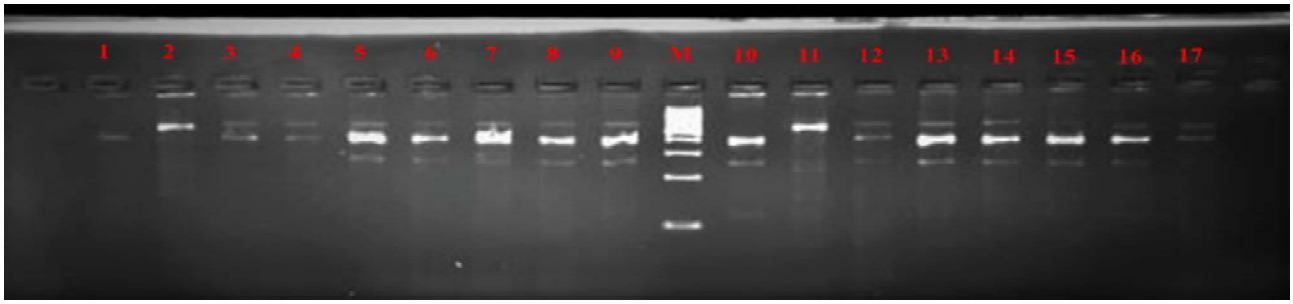

Fig. 3 : Hybridity confirmation using SSR primer (mCpCIR59): M- ladder (100 bp), Lane 1- Arka Prabhath, Lane 2- *V. cauliflora,* Lane 3 to 9- Selected progenies of Arka Prabhath x *V. cauliflora* ($IGHF_1$S4-1, $IGHF_1$S4-12, $IGHF_1$S4-13, $IGHF_1$S4-14, $IGHF_1$S4-15, $IGHF_1$S4-17 and $IGHF_1$S4-18); Lane 10- Arka Prabhath, Lane 11- *V. cundinamarcencis,* Lane 12 to 17- Selected progenies of Arka Prabhath x *V. cundinamarcencis* ($IGHF_1$S1-17, $IGHF_1$S1-19, $IGHF_1$S6-20, $IGHF_1$S2-14, $IGHF_1$S5-12 and $IGHF_1$S5-14)

CONCLUSION

In the present study, seven progenies of Arka Prabhath x *V. cauliflora* ($IGHF_1$S4-1, $IGHF_1$S4-12, $IGHF_1$S4-13, $IGHF_1$S4-14, $IGHF_1$S4-15, $IGHF_1$S4-17, $IGHF_1$S4-18) and six progenies of Arka Prabhath x *V. cundinamarcensis* ($IGHF_1$S1-17, $IGHF_1$S1-19, $IGHF_1$S6-20, $IGHF_1$S2-14, $IGHF_1$S5-12 and $IGHF_1$S5-14) were found superior in morphological, fruit and yield traits coupled with tolerance to PRSV – P. The hybridity of the selected progenies was also confirmed using SSR marker (mCpCIR59).

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Original Research Paper

Determination of mutagenic sensitivity and its manifestations on papaya (*Carica papaya* L.) cv. Arka Prabhath

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ABSTRACT

Papaya is an important fruit crop of the family Caricaceae which needs the improvement in terms of virus resistance and shelf life with dwarf stature. Mutation breeding technique has been considered as an efficient tool adopted by plant breeders to create variability in papaya. The mutation frequency and population structure of the mutants directly depend upon the type of mutagen and the time of exposure. Irrespective of the used mutagens, the ultimate induced mutations are random and therefore determination of mutagenic sensitivity is important pre-requisite. Based on this, investigation on the induction of mutation in papaya cv. Arka Prabhath was carried out with the objective of creating genetic variability through physical mutagen. In this study, papaya seeds were irradiated with five different dose of gamma rays ranging from 50 Gy to 500 Gy. The results revealed that gradual reduction in germination, survival of seedlings and delayed germination with increase in dosage of gamma rays. Based on probit analysis, LD_{50} (Lethal dose) was fixed at 186.24 Gy. Total seven types of chlorophyll mutants were observed as a result of mutation. Mutagenic efficiency and effectiveness were higher in a lower dose of gamma treatment (50 Gy).

Keywords: Arka Prabhath, gamma rays, lethal dose, mutation, papaya

INTRODUCTION

Papaya (*Carica papaya* L.) is an economically important fruit crop of the family Caricaceae. It is having commercial importance because of its high nutritive value and productivity, varied medicinal and industrial uses, round the year availability, short duration nature and its suitability for the preparation of several value-added products. It is basically a tropical fruit crop and believed to have originated in the lowlands of eastern Central America, from Mexico to Panama (Nakasone and Paul, 1998). It has been successfully cultivated throughout the world and in India. Though, there is an increase in area under cultivation of papaya in the recent past, the corresponding increase in production has not been realized in India (Sharma and Tripathi, 2019). This might be due to the losses caused by diseases incited by fungi, bacteria, phytoplasma, viruses, various pests and abiotic factors such as variations in temperature, humidity, asymmetric rainfall and soil moisture stress (Rahman and Akanda, 2008). One of the main drawbacks for papaya cultivation has been the occurrence of papaya ring spot virus (PRSV). Development of virus resistant/ tolerant cultivars through conventional breeding is the only reliable tool for long-term control of this disease (Sharma *et al*., 2017). Introgression of resistance genes from wild species into commercial cultivar background so far been unsuccessful. Among the breeding techniques, mutation is one of the important methods adopted by plant breeders to create variability in a number of species. In fruit crops, mutation breeding is advantageous over conventional breeding because it precludes segregation progenies while improving the genetic make-up during selection cycles.

The prime approach in mutation breeding has been to upgrade the well-adapted plant varieties by altering major agronomic traits, productivity and quality. The

success of mutation breeding greatly depends on the rate of mutation, the number of screened plants and the mutation efficiency. To avoid excessive loss of actual experimental materials, radio-sensitivity tests must be conducted to determine LD_{50} (the safe dose at which half of the planting material survive) doses before massive irradiation of similar materials are accepted.

The selection of effective and efficient mutagen is of paramount importance in any mutagenic experiments to obtain promising stable mutants (Kumar *et al*., 2021). Different type of mutagens can be employed to induce mutagenesis in fruit crops. Physical mutagens have been extensively used for the development of new cultivars with improved characteristics. The frequency and saturation of mutations can be regulated by varying the mutagen dose (Menda *et al.,* 2004) and mutagenic agents can induce different extensions of genomic lesions, ranging from base mutations to larger fragment insertions or deletions (Kim *et al.,* 2006). Among various radiation sources, gamma rays are very important in creating genetic variability through mutagenesis (Hong *et al*., 2022). The usefulness of mutagen depends on the spectrum of chlorophyll mutations, lethality, sterility, mutagenic efficiency and effectiveness of the mutagen. Furthermore, reports on mutagenic efficiency in fruit crops are very few and no such studies have so far been done on papaya. Various studies suggest that more desirable mutations occur at the dose, which causes the death of 50% of organisms or 50% of growth reduction (Alvarez-Holguin *et al*., 2019). Therefore, optimization of LD_{50} (lethal dose 50) is very important for any mutation breeding experiment. LD_{50} is the dosage of mutagen which causes 50 % lethality in the organism and changes with the species, the nature of the plant material and the stage of the crop. Mutagenic effectiveness measures the mutations induced per unit dose of mutagen. Gustafsson (1951) defined that identifying chlorophyll mutants are the most convenient method for evaluating the genetic effect of a mutation in plants. Papaya is an ideal and attractive crop for mutation studies as it is genomically the simplest fruit crop. Simple diploid genetics, small genome size (0.9 pg per haploid genome [Arumuganathan and Earle, 1991]), well studied genetics and a developing physical map, render papaya a better candidate for this study. Based on this, the present study was framed to determine the mutagenic effectiveness, efficiency and optimum lethal dose (LD_{50}) for gamma radiation in papaya cv. Arka Prabhath.

MATERIALS AND METHODS

This study was undertaken at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru during 2020-2021. An attempt was made to induce genetic variability in papaya cv. Arka Prabhath, an advanced generation hybrid derived from the cross of (Arka Surya x Tainung-1) x Local Dwarf released from ICAR- Indian Institute of Horticultural Research. It is gynodioecious in nature, with large sized fruits of 900-1200 g and smooth skin. The pulp is an attractive deep pink colour with good keeping quality and high TSS (13-14^{0}B). The seeds extracted from fully ripe fruits which were obtained by controlled self-pollination were used for the experiment. The seeds (80 seeds/ treatment) were irradiated with gamma rays in gamma chamber located at Indian Institute of Horticultural Research (IIHR), Bengaluru. This gamma chamber installed with the support of BRIT (Board of Radiation and Isotope Technology) and AERB with Co-60 source capacity of 518 Terabecquerel at the dosage rate of 9 KGY/hour or 0.9 Mega Rad/hour. The exposure time in the gamma chamber was 28, 57, 86, 143, 286 seconds for five different doses of gamma rays *viz*., 50 Gy (T_2), 100 Gy (T_3), 150 Gy (T_4), 250 Gy (T_5) and 500 Gy (T_6) respectively. The gamma treated seeds were soaked in GA_3 @ 100 ppm solution for 12 hours and sown in polyethylene bags containing Red soil: FYM: Sand in the ratio of 1:1:1 within 24 hours to retain the vigour of the gamma treated seeds. The gamma treated and gamma untreated (control-T_1) seeds were maintained separately in mist chamber for germination. The germination percentage, survival percentage and days taken for germination were recorded. The LD_{50} value was calculated based on probit analysis.

Probit analysis: LD_{50} values of gamma radiation was determined based on Finney's method (Finney, 1978). Probit analysis was carried out in MS excel by following procedure with some modification in log-doses (mentioned in the procedure). The dose concentration of mutagen was transformed into log10^3 value. The mortality percentage of seeds due to treatment doses were worked out and rounded to the

nearest whole number. The corrected mortality percentage was calculated using Abbott's formula given below.

Corrected mortality (%) =
[M observed - M control x 100] /
100 – M control

All the corrected values are rounded to the nearest whole number. The corrected values were converted to the probit transformation. Probit values (Y-axis) were graphed against Log concentration (X-axis) and a straight line passing through most of the plotted points is drawn; then this line was used to estimate the Log10 concentration associated with a probit of 5. Antilog to the $Log10^3$ value corresponding to the probit 5 was taken and the arrived value was divided by 10^{-3} (1000), thus LD_{50} for the particular mutagen under study was determined.

Analysis of variance: data obtained for nursery parameters were subjected to analysis of variance (ANOVA) at the significant level of 5% using Addinsoft., 2021. When statistical differences were found, the least significant difference (LSD) was used to compare means at the 5% significance level.

Further, M_1 progenies were screened for a spectrum of chlorophyll mutations. The chlorophyll mutants were classified as the scheme of Gustafson (1940) and Blixt (1972). Types of mutants found in this study explained and categorized as follows: Albina mutants were completely devoid of chlorophyll. Xantha consists of pale-yellow colored leaves due to disruption in chlorophyll. The viridis were represented by light green color in the nursery stage. This color gradually changed to the normal green color during the subsequent period of growth and found viable in nature. The chlorina mutants were yellowish green in color Xantha-viridis mutants were characterized by both viridine green color and bright yellow color occurring in the same leaf.

Mutagenic effectiveness, mutagenic efficiency and mutation rate were calculated based on the formulae proposed by Konzak *et al*. (1965) by incorporating the mutation frequency values recorded for each mutagenic treatment.

Mutagenic effectiveness = Mutagenic frequency /
Dose/Concentration of the mutagen
Mutagenic efficiency =
Mutagenic frequency / Biological damage

Mutation rate = Sum of values of efficiency or effectiveness of particular mutagen / Number of treatments of a particular mutagen

Biological damage refers to the lethality or reduction percentage over control (survival) and germination percentage reduction over control in this study. Biological damage is contributed by germination and survival of seedlings which has primary role in the establishment of crop.

RESULTS AND DISCUSSION

Gamma rays are the most widely used physical mutagen employed in mutation breeding of crop plants and are well known for bringing about morphogenetic and endomorphic changes in plants (Yasmeen *et al*., 2020). The results indicated that there was a gradual reduction in germination percent with increase in dosage of gamma irradiation with highest germination percentage being in control (92.50%). Among the treatments, highest germination percent was recorded in 50 Gy (82.19%) and higher doses above 250 Gy were lethal in papaya. Further, although lower irradiation doses did not have a marked effect, higher doses (250 Gy) delayed seed germination considerably with maximum number of days taken for germination being 23 days (T_5) and minimum being 9 days in control. Reduction in survival percentage with increased dosage with higher survival percentage was recorded in control (90 %), among the treatments, higher survival per cent was recorded in 50 Gy (80 %) (Fig. 1 and Table 1 & 2). Similar results were elicited in papaya by Aiswarya Ravi *et al*. (2022), Pujar *et al*. (2019) and Ramesh *et al*. (2019), Sahu *et al*. (2019), in mango by Parveen *et al*. (2023), pummelo (Sankaran *et al*., 2021), Rough lemon (Kaur and Rattanpal, 2010), Saini and Gill (2009) and Sharma *et al*. (2013) in Rough lemon. This might be

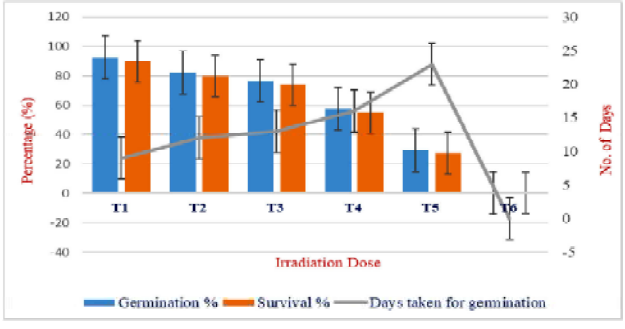

Fig. 1 : Effect of gamma irradiation on germination, survival and days taken for germination in M_1 population of papaya

Table 1 : Effect of gamma irradiation on germination percentage in papaya

Dose (Gy)	Germination count (out of 80)	Germination %	% over control	% of reduction over control
T_1 (Control)	74.00	92.50	100.00	0.00
T_2 (50Gy)	65.75	82.19	88.85	11.15
T_3 (100 Gy)	61.25	76.56	82.77	17.23
T_4 (150 Gy)	46.00	57.50	62.16	37.84
T_5 (250 Gy)	23.50	29.38	31.76	68.24
T_6 (500 Gy)	0.00	0.00	0.00	100.00

Table 2 : Effect of gamma irradiation on survival of seedlings percentage in papaya

Dose (Gy)	Survival Nos. (Out of 80)	Survival %	% over control	% reduction over control
T_1 (Control)	72.00	90.00	100.00	0.00
T_2 (50Gy)	64.00	80.00	88.89	11.11
T_3 (100 Gy)	59.00	73.75	81.94	18.06
T_4 (150 Gy)	44.00	55.00	61.11	38.89
T_5 (250 Gy)	22.00	27.50	30.56	69.44
T_6 (500 Gy)	0.00	0.00	0.00	100.00

due to the altered enzyme activity (Zou *et al*., 1999), metabolic disturbances (Ananthaswamy *et al*., 1971), inactivity of plant hormones (Sideris *et al*., 1971) and chromosomal aberrations (Nurmansyah *et al*., 2018).

In the present study, LD_{50} values were determined with the help of probit analysis based on their survival rate of the seed after treatment with different doses of gamma rays compared with untreated control. Optimum dose is the dose that cause maximum of mutation with minimum of damage to the plant. The probit curve analysis shown that the LD_{50} value for gamma rays was 186.24 Gy (Table 3 & Fig. 2). The minor difference was observed in LD_{50} doses which ranged from 300 to 350 Gy in three different varieties of papaya by Aiswarya Ravi *et al*. (2022). Lethal dose differs with biological materials, nature of treatment and subsequent environmental conditions. Several studies of mutation by gamma ray exposure in different papaya cultivars were reported. Hang and Chau (2008) exposed the seeds of the papaya variety Dai Loan Tim to gamma rays ranging from

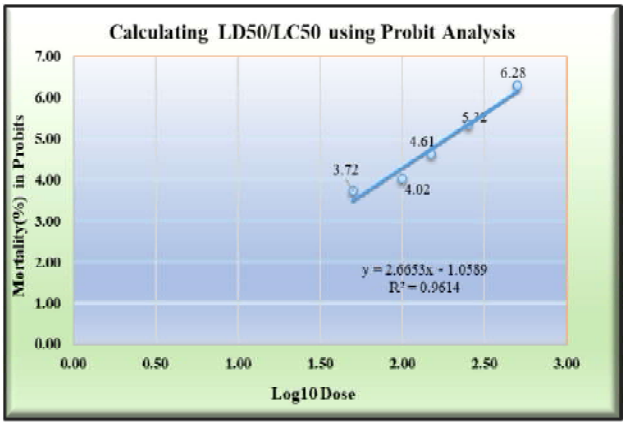

Fig. 2 : Plot of log-doses versus Probits for calculations of LD_{50} for gamma rays for papaya

Table 3 : Probit analysis for cv. Arka Prabhath irradiated with gamma radiation

Dose (Gy)	Log value of dose	Observed mortality	Corrected mortality %	Empirical value of probit unit	LD_{50} Value
T_1 (Control)	0.00	10.00	0.00	0.00	186.24 Gy
T_2 (50Gy)	1.70	20.00	11.11	3.72	
T_3 (100 Gy)	2.00	26.25	18.06	4.02	
T_4 (150 Gy)	2.18	45.00	38.89	4.61	
T_5 (250 Gy)	2.40	72.50	69.44	5.32	
T_6 (500 Gy)	2.70	100.00	100.00	6.28	

10 to 60 Gy. They indicated that LD_{50} of gamma rays in germinated papaya seeds was 30 Gy. Husselman *et al.* (2014) found that increased doses of gamma rays of 100 and 120 Gy were lethal to papaya when treated with dosages ranging from 0 to 120 Gy. Sahu *et al.* (2019) reported that LD_{50} value was 28.35 Gy and 33.13 Gy for pre-soaked seeds & 24.05 Gy and 23.78 Gy for seeds immersed in water for Ranchi local and Arka Surya varieties of papaya respectively. The lethal dose of gamma rays for different fruit crops was reported by Surakshitha *et al.* (2017) in grapes and Murti *et al.* (2013) in strawberries.

Chlorophyll mutants are not desirable in crop improvement programs but serve as an important parameter to determine the mutagenic efficiency (Eswaramoorthy *et al.*, 2021). In the present study, a wide spectrum of chlorophyll mutants such as Xantha, Viridis, Chlorina, Xantha-viridis, Maculata and Green-viridis (Fig. 3). Most of the chlorophyll deficient mutants died after a few days of germination whereas some showed retarded growth because of the deficiency of chlorophyll. The highest mutation frequency of chlorophyll phenodeviants was found in 250 Gy (30.42) and least in 50 Gy (9.22) (Table 4). The results are in accordance with Aiswarya Ravi *et al.* (2022), who observed retarded growth and mortality in chlorophyll deficient plants with wide spectrum of chlorophyll mutants such as xantha, chlorina, striata, virescent viridis and albino. The similar results were also observed by Naveena *et al.* (2020) and Seemanthini *et al.* (2022), in hibiscus. The occurrence of chlorophyll mutants might be attributed to a variety of factors, including defective chlorophyll biosynthesis, chlorophyll degradation, and carotenoid deficiency (Goyal *et al.*, 2019). Chlorophyll mutations are crucial for determining gene function as well as understanding chlorophyll metabolism and regulation in plants (Dwivedi *et al.*, 2021). Hence chlorophyll mutations can be used as the most reliable marker for assessing the genetic impact of mutagenic treatments in different crops.

Mutagenic effectiveness indicates the frequency of mutations induced by a unit dose of mutagen and mutagenic efficiency is a measure of the proportion of mutation in relation to lethality and sprouting percentage reduction. Biological damage is purely dose-dependent which increased with increased concentration/dose of gamma rays. In the present study, the mutagenic effectiveness found to be highest (18.44) at a lower dose (50 Gy) and lowest (12.17)

3a: Green-viridis

3b: Chlorina

3c: Xantha-viridis

3d: Maculata

3e: Xantha

3f: Viridis

Fig. 3 : Chlorophyll mutants obtained from gamma irradiated papaya seeds

Table 4 : Spectrum of chlorophyll mutants, Mutagenic effectiveness and efficiency in papaya

Treatment	Chlorophyll mutants						M_f	Biological damage		Mutation effectiveness	Mutation efficiency	
	Xantha	Chlorina	Viridis	Xantha-viridis	Maculata	Green-viridis		Survival reduction over control (%)	Germination reduction over control (%)		Based on survival reduction over control	Based on germination reduction over control
T_2 (50Gy)	2	1	2	0	1	0	9.22	11.11	11.15	18.44	82.99	82.69
T_3 (100 Gy)	2	1	2	2	0	1	13.12	18.06	17.23	13.12	72.64	76.15
T_4 (150 Gy)	2	3	4	1	1	1	26.07	38.89	37.84	17.38	67.04	68.89
T_5 (250 Gy)	1	2	2	2	0	0	30.42	69.44	68.24	12.17	43.81	44.58
T_6 (500 Gy)	-	-	-	-	-	-	0.00	100.00	100.00	0.00	0.00	0.00

Table 5 : Mutation rate of gamma radiations in papaya

Treatments	Mutation rate in terms of effectiveness	Mutation rate in terms of efficiency	
		Lethality	Germination reduction
Nos. 5	12.22	53.30	54.46

at higher dose (250 Gy) of gamma rays. Mutagenic efficiency determined based on the lethality of embryos recorded highest value (82.69) at 50 Gy. Likewise, mutagenic efficiency calculated based on survival reduction (Biological damage) also found in the same pattern in which mutagenic efficiency was maximum (82.99) in the lower dose of gamma radiation (Table 4). Similar results of mutation efficiency with the lower dose of gamma rays were also reported by Aiswarya Ravi *et al*. (2022) in papaya, Naveena *et al*. (2020) in hibiscus, Seemanthini (2022) in hibiscus and Padmadevi (2009) in chrysanthemum.

The overall mutation rate in terms of effectiveness and efficiency was outstanding in gamma radiation treatment in papaya cv. Arka Prabhath. The maximum mutation rate in terms of effectiveness and efficiency reflects the usefulness of mutagen (Table 5). For obtaining high efficiency, the mutagenic effect should overcome other effects in the cells such as chromosomal aberrations and toxic effects. High mutation rate accompanied by minimal deleterious effects is desirable for a successful mutation programme. But generally, the mutagen that gives the higher mutation rate also induces a high degree of lethality, sterility and other undesirable effects (Blixt *et al*., 1964).

CONCLUSION

The results indicate that induced mutation through gamma irradiation was found to be effective and efficient, which can be employed in enhancing the variability in papaya. Chlorophyll deficient mutants were observed among the irradiated population. All biological parameters showed a steady increase with increasing gamma irradiation dosages. The mutagenic efficiency and effectiveness of gamma irradiation were found highest at lower dose (T_2-50 Gy) and was decreasing with an increase in doses (T_3-100 Gy to T_6-500 Gy). Based on the current findings, the LD_{50} value (186.24 Gy) of gamma rays was optimized for papaya cv. Arka Prabhath. This information will be useful for further mutagenesis experiments for developing mutants with desirable characteristics in papaya since determination of mutagenic sensitivity is the pre-requisite for any mutation breeding programme.

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Original Research Paper

Performance of Apple ber on different training systems in hot arid condition

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ABSTRACT

Ber is an indigenous and common fruit of India. It is commercially grown under wide climatic and soil conditions. Apple ber (*Zizyphus jujube* Mill) cultivar is gaining momentum in sub-tropical and tropical climatic conditions of West Bengal, Telangana, Andhra Pradesh, Maharashtra, Rajasthan, Gujarat and in several northern parts of India. It is precocious in bearing habit with bold and crispy fruits. Many factors including training practices affect vegetative and fruit quality parameters. Due to bolder and heavier fruits, the cultivar is prone for limbs breakage or complete tilting of plants which results in uneven fruiting, reduced fruit set, and higher fruit drop. Considering its appealing fruit taste and quality and to address the above mentioned issues, different training systems were evaluated to see its performance on three to four years old apple ber orchards. Plants were trained on different training systems *viz.* Y-Shape, Espalier training system and control. Observations were recorded on vegetative and fruit quality parameters. Training systems significantly influenced various vegetative, yield and fruit quality parameters. Vegetative parameters such as leaf area, physical and quality attributes *viz.*, fruit weight and size, TSS, ascorbic acid, yield, and B: C ratio were better in Y-Shape training system. Hence, Y-Shape training system can be adopted to improve yield and fruit quality parameters in Apple ber.

Keywords : Ber, fruit quality, PAR, training system, yield

INTRODUCTION

Though ber is distributed worldwide *viz.*, Indian sub-continent, Southeast Asia, Australia, China, Africa, the Mediterranean region, and the American center, its cultivation is confined only to the drier parts of the globe. Ber (*Zizyphus mauritiana* Lamk.) is one of the most ancient and common indigenous fruit in India. It is cultivated under wide climatic and soil conditions. Though ber fruit contains 81% of moisture, it is rich in essential amino acids such as asparginine, arginine, glycine, glutamic acid, and serine (Azam-Ali, 2006). It is found that ber is rich in vitamin C and phosphorus than apples and oranges (Obeed, 2008). Ber is known as "Poor Man's Apple" as it contains all the nutritional benefits of apple and its affordable price to the poor people. Since, it is cultivated in drier regions, it is also called as 'the apple of arid zone' (Singh and Bal, 2006).

Apple ber cultivar was originated from Thailand, and is gaining momentum in sub-tropical and tropical regions of India. It is cultivated mainly in West Bengal, Telangana, Andhra Pradesh, Maharashtra, Rajasthan, and Gujarat. Unlike the Indian jujube, its name signifies the size and appearance of the fruit (green apple). Not only the shape, but the juiciness and crispiness of the fruit is also resembles to that of Apple. This cultivar has many advantages over the traditional ber varieties (Mathangi and Maran, 2020) such as fruit size, bearing potential, precocity in bearing, earliness, and crisp texture of fruit. No doubt the large size of the fruit is its prime attraction but, the total fruit yield per plant is less than normal popular varieties like Gola and Umran under irrigated condition. Ber growers of arid and semi-arid regions have started planting of Apple ber cultivar due to its bearing potential, precocity in bearing, earliness, large fruit size, etc. Some issues were noticed in Apple ber *i.e.*, breakage of branches or complete tilting of plants, poor organoleptic quality, less fruit set, more fruit drop and uneven fruiting. Among the different factors responsible for the sustainable production of quality ber fruits, the practice of training of plants in hot arid conditions is vital.

The adoption of training systems in the modern era of fruit growing is the most important factor in the areas where high yield is targeted which also leads to

better fruit quality. The training system affects the photosynthetic efficiency of plants which is directly related to bearing efficiency and fruit quality (Sansavini and Corelli, 1997). Generally, Apple ber trees have a deep tap root system but tend to develop heavy and profuse canopy under sub-tropical and arid conditions. The high-velocity winds during the winter season which coincides with the time when there is considerable fruit load on the trees results in tilting of trees or in some cases trunk/limb breakage resulting in loss of fruit trees especially in Rajasthan. Hence, there was a need to develop an ideal and strong framework for Apple ber trees by adopting training systems which can not only bear heavy fruit load but also develop proper canopy to produce quality fruits as well as remain intact under conditions of high wind velocity. The practice of adopting training systems in cultivation of Apple ber is a completely new approach. Identification of a suitable training system will able to produce quality fruits, and expected sustainability in Apple ber growing under the arid conditions where high wind velocity is a common feature.

MATERIALS AND METHODS

The present experiment was conducted at ICAR-Central Arid Zone Research Institute, Jodhpur, Rajasthan during 2018 to 2020. Three to four years old Apple ber plants were planted at a spacing of 6 m x 6 m. The soil was sandy loamy and initial soil EC (0.31 and 0.24 ds/m), pH (8.4 and 8.1), organic carbon (0.30 and 0.12 %), N (101.14 and 89.05 kg ha^{-1}) and P_2O_5 (11.51 and 10.50 kg ha^{-1}). The average temperature of 38°C and 58% relative humidity was recorded during the growth and fruit development stage in the orchard. The experiment was carried out in randomized block with three treatments replicated eight times, to standardize the training systems for obtaining quality fruits. Each replication consists of 15 uniform plants. The trees were trained according to three training systems i.e. Y-Shape (T1), Espalier (T2) and control/conventional system (T3) (Fig. 1, 2 and 3). Regular horticultural practices *i.e.*, pruning, fertilizer application, irrigation and plant protection operations were followed uniformly.

Plant growth parameters

Data was recorded on plant growth parameters such as primary shoot diameter (cm), shoot length (cm) and leaf area (cm^2) at the time of fruit maturity. The fruit size (fruit length and diameter) was recorded using digital Vernier calliper and screw gauge. The same sample was weighed using an electronic weighing scale and the mean value is registered for the analysis. The weight was measured in different ways based on the requirements for the calculations. Whole fruit weight, weight of flesh and weight of stone was measured to calculate pulp-stone ratio. The leaf area was recorded using digital leaf area meter (Biovis PSM - L3000).

Physio-biochemical parameters

The biochemical parameter such as TSS was recorded by refractometer and the acidity of the berry was estimated by potentiometric titration method. The total sugar content was measured by hydrolyzing the polysaccharides into simple sugars by acid hydrolysis and estimating the resultant monosaccharides by Anthrone method (Hedge and Hofrieter, 1962). Ascorbic acid was recorded by a spectrophotometric method. PAR was measured by a digital canopy analyzer. The yield attributes of experimental ber plants were recorded twice in both the years. Fruit yield per hectare was calculated by using following formula:

$$\text{Yield (ton/ha)} = \frac{\text{Yield per plant (kg)}}{1000} \times 278 \text{ (plant/ha)}$$

Fig. 1 : Y-Shape (T1)

Fig. 2 : Espalier (T2)

Fig. 3 : Control/Conventional system (T3)

Statistical analysis

All calculations were carried out by the standard method of analysis of variance as described by Panse and Sukhatme (1995). The critical difference at 5 *per cent* level of significance was calculated.

RESULTS AND DISCUSSION

Training systems *i.e.*, Y-Shape (T1), Espalier (T2) and control/conventional system (T3) were evaluated in the Apple ber orchard for two years. Morphological, physical, biochemical and yield parameters were influenced by training systems. Concerning growth parameters, the minimum primary shoot length (103.8 cm) and maximum primary shoot diameter (2.47 cm) and leaf area (26.33 cm^2) were recorded in T1 which is significantly different than other training systems, whereas, the least growth observations were recorded on T3. Among the fruit quality parameters, the highest fruit weight (66.88 g), fruit length and breadth (5.05 cm and 4.57 cm) and higher pulp: stone ratio (13.08) were found to be in T1 *i.e.*, Y-shape training which is significantly higher than the other two training systems (Table 1). Likewise, Gill *et al.* (2011) reported that bush-trained plants improve the productivity of pomegranate and offer the possibility to obtain high-quality fruits. The highest fruit length, breadth and weight of 6.62 cm, 6.97 cm and 283.9 g and TSS and juice percentage were recorded in fruits harvested from trained trees compared to other systems of training (Fig. 4).

Among, biochemical parameters, significantly higher TSS (15.64 °B) total sugars (11.34 %), and ascorbic acid (89.30 mg/100 g) were recorded in T1 compared to other treatments (Table 2). These results are similar to the finding of Kalkan *et al*. (2022) and Yin *et al*. (2022) who reported that vines trained to Y shaped support system had greater total soluble solids and sugar composition in berries than those trained to closing Y-shaped trellis.

Fig. 4 : Apple ber fruits

Table 1 : Effect of different training systems on the growth and fruit quality in Apple ber

Treatment	Primary shoot length (cm)	Primary breadth shoot (cm)	Leaf area (cm^2)	Fruit weight (g)	Fruit length (cm)	Fruit breadth (cm)	Pulp: Stone ratio
T1 (Y-Shape)	103.8	2.47	26.33	66.88	5.05	4.57	13.08
T2 (Espalier)	106.3	2.18	25.47	55.98	4.82	4.33	12.69
T3 (Control)	165.9	2.07	24.94	44.24	4.57	4.10	10.93
SE (m)±	2.81	0.08	0.005	0.95	0.06	0.04	0.14
CD at 5%	8.22	0.23	0.015	2.79	0.17	0.14	0.43

Table 2 : Effect of different training systems on quality of fruit, yield and B: C ratio in Apple ber

Treatment	TSS (°B)	Acidity (%)	Total sugar (%)	Ascorbic acid (mg/100g)	Fruit yield/plant (kg)	Fruit yield/ha (ton)	Increase in yield over control (%)	B:C ratio
T1 (Y-Shape)	15.64	0.12	11.34	89.30	63.44	18.27	58.04	3.52
T2 (Espalier)	13.60	0.13	10.56	87.20	46.58	13.42	16.08	2.58
T3 (Control)	13.29	0.13	9.50	71.15	40.13	11.56	-	2.21
SE (m)±	0.26	0.004	0.63	1.21	1.72	1.46	-	0.10
CD at 5%	0.54	NS	1.06	3.63	5.07	0.50	-	0.28

In addition, photosynthetically active radiation (PAR) was recorded from flowering to fruit maturity (November to February) phase and it was varied significantly according to the training system, wherein, maximum PAR ($\mu mols^{-1} \cdot m^{-2}$) was recorded in T1 (Fig. 5). Modification of vine training systems to achieve a balance between vine vigor and yield has led to divided canopy systems that might simultaneously increase yield and improve fruit composition through optimization of canopy light microclimate (Reynolds and Vanden Heuvel, 2009).

Fig. 5 : Effect of different training systems on PAR values ($\mu mol\ s^{-1} \cdot m^{-2}$) in plant canopy

With respect to fruit yield parameters, highest fruit yield was recorded in T1 (63.44 kg $plant^{-1}$ and 18.27 t ha^{-1}) followed by T2 (46.58 kg $plant^{-1}$ and 13.42 t ha^{-1}), while, T3 *i.e.*, control treatment recorded lowest (40.13 kg $plant^{-1}$ and 11.56 t ha^{-1}) yield. Increase in good quality yield over untreated control was recorded in T1 (58.04 %) followed by T2 (16.08 %). The economics of different treatments revealed significantly higher B: C ratio in T1 (3.52) (Table 2). These results are in partial agreement with the findings of Caruso *et al.* (1998) *i.e.*, trees trained to 'Y shape' yielded more and recorded highest fruit quality. Almost similar results were obtained by Palliotti (2012) who reported that vines trained to the 'Y system' recorded about 13% more fruit due to the 15% higher cluster weight and 13% berry mass compared to vertically shoot-positioned (VSP) vines over 5 years of study. Similar results were also obtained by Abrosca *et al.* (2017) who recorded the highest cumulative efficiency yield in slender spindle training system compared to the others *i.e.*, oblique palmette, free palmette, V-shaped, Tatura trellis, Bibaum, modified Bibaum, triple leader and Solaxe.

CONCLUSION

The performance of Apple ber cultivar on different training systems in hot arid conditions was studied. Based on the results, it can be concluded that Y-shape training system showed a significant positive effect on qualitative and quantitative growth parameters, light penetration into the canopy, fruit quality and yield attributes. Thus, Y-Shape training system can be a good option for yield and quality improvement in Apple ber which can fetch good market price for ber growers.

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Original Research Paper

Genetic diversity and screening for bacterial wilt in tomato (*Lycopersicon esculentum*)

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ABSTRACT

Thirty-four tomato genotypes from different geographical locations were evaluated for genetic diversity and screened for bacterial wilt (BW) caused by *Ralstonia solanacearum*. Results revealed that plant height, fruits per cluster, fruit weight, fruit diameters, locules per fruit, fruit firmness, yield per plant, and quality parameters exhibited high heritability and genetic advance. Clustering based on D^2 analysis, classified genotypes into four clusters. Maximum intra-cluster distance was recorded within cluster I and maximum inter-cluster distance between cluster II and IV followed by cluster I and IV, indicating existence of wide genetic variability. Genotypes in cluster IV (AVTO 1711, AVTO 1717 and AVTO 1718) recorded high fruit weight coupled with high yield. These may be explored as promising donors for developing large sized bacterial wilt resistant tomatoes. The large fruited genotypes in cluster IV can also contribute to the genetic improvement of existing bacterial wilt resistant varieties placed in cluster I. Out of 34 genotypes screened for BW disease, 5 genotypes were classified as resistant and 7 as moderately resistant.

Keywords : Bacterial wilt, genetic advance, heritability, humid tropics

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), the second most important vegetable in the world after potato excels as a good source of vitamin A, C, E, contains large quantity of water, calcium and niacin. The crop largely attracts farmers due to its short duration, low input costs and feasibility for cultivation throughout the year. In India, tomato has registered a production of 20.30 million tonnes from 830.75 thousand ha area (NHB, 2022). Madhya Pradesh is the leading producer of tomato with 2970.0 thousand metric tonnes from an area of 1,03,000 hectares. Successful crop breeding depends on the variability and genetic diversity in the base population. Yield and its components, with their polygenic inheritance, are vulnerable to environmental sways. Variability present in the base population could be segmented into heritable, and non-heritable, segments with genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV), heritability and genetic advance. GCV and PCV indicates the amount of variability present in the base population, while, heritability and genetic advance assist in determining environmental influences, and the degree to which improvement is achievable (Patel *et al*., 2013).

Diverse parents bring about hybrid vigor, consequently, examination of genetic diverseness is necessary to determine the breeding strategy (Harrington, 1940). According to D^2 statistics (Mahalanobis, 1936), genetic divergence helps in identifying diverse parents which on hybridization yield bumptious transgressive segregants (Naveen *et al*., 2018).

Bacterial wilt caused by *Ralstonia solanacearum* have caused havoc in the commercial cultivation of tomato leading to heavy yield losses. It causes 26% loss of fresh fruit production in hybrid tomatoes and yield losses reach up to 90.62% (Dharmatti *et al*. 2009). Development of resistant varieties can be employed as alternative to overcome bacterial wilt disease. Most of the bacterial wilt resistant sources have only small fruit size due to linkage drag of wilt resistant gene with small fruit size (Wang *et al*., 1998). Identifying a resistant genotype with better fruit size will help in easy transfer of resistance into different background. With this foreground, the present study was carried

out to analyse the diversity in tomato genotypes and screening for bacterial wilt disease.

MATERIALS AND METHODS

The present investigation was accomplished employing 34 tomato genotypes. Out of 34 genotypes, 23 were collected from the ICAR-NBPGR, New Delhi, 3 from World Vegetable Centre, Taiwan and remaining 8 genotypes (3 advanced lines and 4 varieties) from Kerala Agricultural University, Kerala. Two experiments were carried out. In first experiment, 34 genotypes were planted in pots in a completely randomized block design with 2 replications. Standards package of practices recommended by Kerala Agricultural University was followed. Data on growth, yield and quality traits were subjected to statistical analysis as per Comstock and Robinson (1952), Johnson *et al*., (1955), and Allard (1961). Mahalanobis D^2 analysis (Mahala nobis, 1936) and Euclidean clustering (Spark, 1973) was used to elucidate divergence and consequent selection of parents for hybridization.

Second experiment was laid out in completely randomized block design with 2 replications to screen the genotypes for bacterial wilt incidence under field conditions with one susceptible check variety Pusa Ruby. Prior to crop establishment, the soil was tested for pathogen load by serial dilution, which recorded an inoculum load of 61 x 10^6 cfu/ g soil. Plants were observed on daily basis during the entire crop period for bacterial wilt symptom which was confirmed by ooze test. Bacterial wilt incidence was recorded and per cent wilt incidence was calculated by the following formula.

$$\text{PDI} = \frac{\text{number of plants infected}}{\text{total number of plants observed}} \text{ X } 100$$

The genotypes were grouped into different categories based on the per cent disease incidence (PDI) and the reaction of the genotypes to bacterial wilt as described by Mew and Ho (1976).

Reaction	Per cent disease incidence
R (Resistant)	0-20
MR (Moderately resistant)	21-40
MS(Moderately susceptible)	41-60
S (Susceptible)	61-100

RESULTS AND DISCUSSION

Heritability, variance components and genetic advance

Significant variations were recorded for growth and yield traits in the base population (Table 1). The PCV was imperceptibly higher than GCV, indicating the environmental impact on the expression of these traits. Estimates of GCV and PCV were high for yield per plant, fruit weight, number of fruits per plant, secondary branches per plant, fruit firmness, ascorbic acid acidity, lycopene, and beta carotene. This designated greater magnitude of phenotypic and genotypic variability in the base population. GCV alone, cannot be depended upon to decide the magnitude of heritable variation, and hence, the knowledge on heritability also is entailed.

Heritability plays decisive role in breeding, expressing the reliability of phenotype as an indicator of its breeding values. Heritability was high (61.31% - 97.97%) for most of the traits, suggesting less influence of environment factors, and hence, effectiveness in selection. High genetic advance as percentage of mean was observed for all traits except for days to flowering, days to harvest, and total soluble solids (Ara *et al*., 2009), suggesting the predominance of additive gene action. TSS recorded high heritability with moderate genetic advance, while days to flowering and days to harvest recorded low heritability and low genetic advance implying the control by non-additive gene action.

On the basis of D^2 analysis, 34 genotypes were grouped into four highly divergent clusters (Table 2 and Fig. 1). High inter-cluster and low intra cluster values highlighted the cluster divergence. Numbers of genotypes in clusters were in the order: Cluster I >cluster III >cluster II > cluster IV. The clustering pattern showed that accessions from different geographical areas were clubbed in single cluster indicating that there existed no parallelism between genetic diversity and geographical origin (Meena and Bahadur, 2015). Similarly, accessions from same geographical origin were distributed into different clusters, indicating that these accessions must have under gone changes for characters under selection which could be attributed to selection or genetic drift, creating more diversity rather than genetic distance. This clearly explained that selection of parents for hybridization must be emphasized on genetic diversity rather than geographical diversity (Naveen *et al.,* 2018).

Table 1 : Estimates of variance for yield and yield contributing traits in tomato

Characters	Range	Mean	GV	PV	GCV (%)	PCV (%)	H^2	GA	GAM
Plant height (cm)	35.25-76.5	52.9	63.00	102.55	15.00	19.14	61.43	12.82	24.23
Days to flowering	47.5-59.5	55.38	4.82	19.08	3.96	7.89	25.27	2.27	4.11
Days to harvest	84-98.5	89.94	8.42	19.27	4.90	3.24	43.7	3.95	4.39
Primary branch	4.75-10.5	7.46	1.02	1.85	13.53	18.21	55.20	1.54	20.70
Secondary branch	7.5-25.75	12.00	11.70	13.58	28.48	30.69	86.11	6.54	54.45
Fruits per cluster	2.1-4.7	3.07	0.34	0.55	18.86	24.08	61.31	0.94	30.42
Fruits per plant	13.38-69	24.91	191.31	198.85	56.27	57.37	96.21	27.95	113.70
Fruit weight (g)	15.15-118.4	50.01	602.55	655.61	49.03	51.15	91.91	48.48	96.83
Polar diameter (cm)	11.1-21.1	14.40	4.02	4.95	13.92	15.45	81.22	3.72	25.85
Equatorial diameter (cm)	9.95-21.7	13.81	4.03	4.92	14.54	16.05	81.99	3.75	27.11
Locules per fruit	2-5	3.67	0.40	0.47	17.17	18.70	84.31	1.19	32.48
Fruit firmness	0.52-1.8	1.16	0.15	0.16	33.02	34.34	92.48	0.76	65.42
TSS (ºBrix)	4.4-7.15	6.12	0.35	0.48	9.73	11.33	73.62	1.05	17.19
Ascorbic acid (mg/100g)	8.16-26.53	13.28	26.78	27.34	38.98	39.38	97.97	10.55	79.48
Acidity (%)	0.25-1.21	0.52	0.05	0.06	43.30	47.00	84.87	0.43	82.17
Lycopene (mg/100g)	1.49-10.74	4.97	5.46	6.14	47.00	49.83	88.97	4.54	91.33
Beta carotene (mg/100g)	0.93-7.29	3.32	1.88	2.01	41.32	42.69	93.68	2.73	82.39
Total sugars (mg/100g)	1.96-3.26	2.52	0.11	0.12	13.35	13.58	96.58	0.68	27.03
Shelf life (days)	7.25-16.5	10.29	7.19	8.58	26.06	28.47	83.76	5.05	49.13
Yield (kg)	0.36-2.42	1.10	0.46	0.48	62.07	63.27	96.25	1.37	125.45

GV-genotypic variance, PV-phenotypic variance, GCV-genetic coefficient of variation, PCV-phenotypic coefficient of variation, H^2-heritability, GA-genetic advance, GAM- genetic advance as percentage of mean

Table 2 : Cluster wise distribution of tomato genotypes

Cluster No.	Total number of accessions	Name of Accessions
I	19	EC-914087, EC-914094, EC-914100, EC-914107, EC-914091, EC-914096, EC-914099, EC-914093, Sakthi, Mukthi, Anagha, Manuprabha, EC-914090, EC-914103, EC-914109, EC-914098, EC-914102, EC- 9140107, EC-914085
II	4	Sln-2 (Mukthi x IIHR 2195-F2-38-5-1),Sln-6 (Mukthi x IIHR 2195-F2-38-3-6), Sln-7 (Mukthi x IIHR 2196- F2-57-4-45),Sln-9 (LE-1-2 x H24-F2-59-3-20)
III	8	EC-914089, EC-914108, EC-914086, EC-914092, EC-914097, EC-914087, EC-914100, EC-914104
IV	3	AVTO-1718, AVTO-1711, AVTO-1717

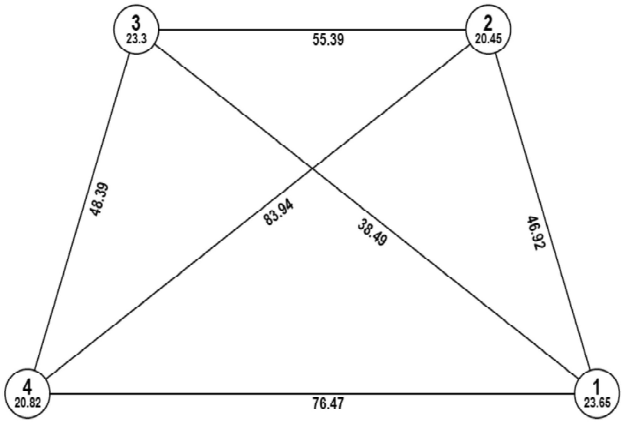

Fig. 1 : Dendrogram showing clustering of tomato genotypes

Average inter and intra cluster distance (Table 3 and Fig. 2) revealed that inter cluster distances were higher than that of intra cluster distances, suggesting homogeneous and heterogeneous nature of the germplasm within and between the clusters, respectively (Rai *et al*., 2017). Cluster I recorded the highest intra cluster distance suggesting the presence of maximum diversity among the genotypes in it. At inter cluster level, minimum distance was recorded between cluster I and cluster III, while, cluster II and cluster IV recorded the maximum inter cluster distance. Minimum inter cluster distance indicated that these genotypes are closely related, and a higher inter cluster distance indicated wider genetic diversity among the genotypes, hence, parents for hybridization must be selected from these clusters, to generate maximum heterotic progenies and for getting desirable transgressive segregants (Naveen *et al*., 2018).

Table 3 : Intra and inter cluster distance in tomato genotypes

Cluster No.	I	II	III	IV
I	23.65	46.93	38.49	76.47
II		20.45	55.39	83.94
III			23.30	48.39
IV				20.82

The cluster means of characters indicated the presence of appreciable amount of genetic variation among clusters (Table 4). Intercrossing among the genotypes with outstanding mean performance (cluster mean) gives heterotic crosses (Kumar *et al*., 2013). The genotypes in the cluster II recorded high mean values for days to harvest, fruits per cluster, fruits per plant, TSS, ascorbic acid, lycopene, and beta carotene. Cluster III showed maximum mean values for primary branches, secondary branches, locules per fruit, and fruit firmness. Genotypes from cluster III could give plants with more branches, and firm fruits when

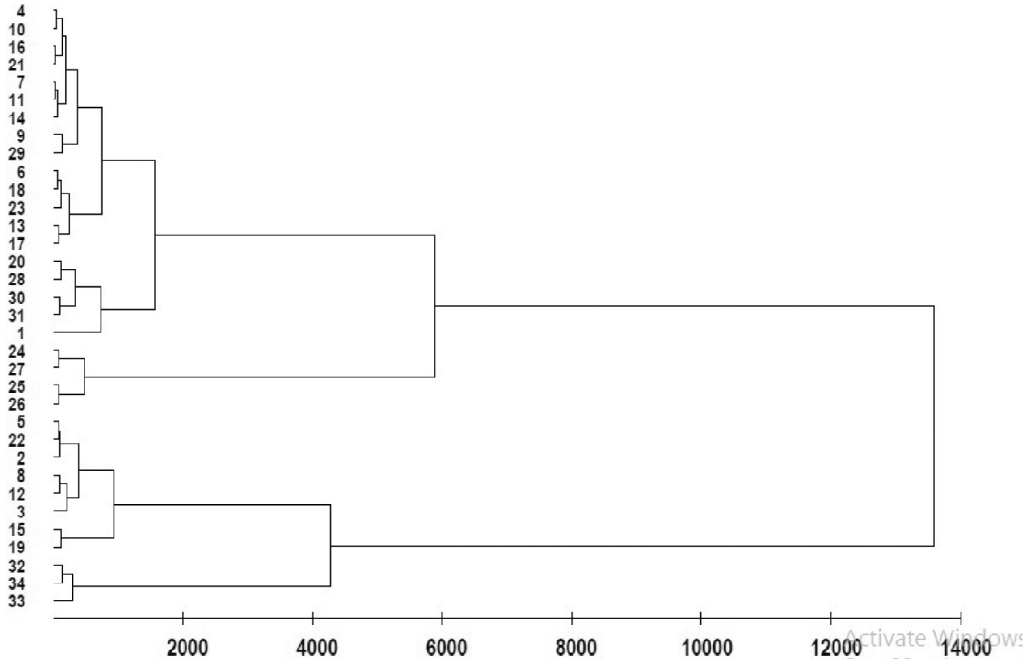

Fig. 2 : Mahalnobis Euclidean Distance (not to scale)

Table 4 : Cluster wise mean performance of tomato genotypes

Character	I	II	III	IV
Plant height (cm)	53.78	38.06	53.72	65.25
Days to flowering	55.87	54.97	55.95	51.33
Days to harvest	90.22	87.09	91.27	88.50
Primary branch	7.67	5.81	7.88	7.25
Secondary branch	11.51	8.88	15.09	11.08
Fruits per cluster	3.13	3.35	2.91	2.77
Fruits per plant	20.51	58.75	18.98	23.50
Fruit weight (g)	35.92	39.93	66.76	108.03
Polar diameter (cm)	13.67	12.19	15.86	18.14
Equatorial diameter (cm)	13.03	12.39	14.43	19.08
Locules per fruit	3.68	3.85	3.40	4.07
Yield per plant (kg)	0.64	2.11	1.23	2.28
Fruit firmness (kg/cm^2)	1.13	0.93	1.34	1.23
TSS (^{o}brix)	6.09	6.38	6.28	5.53
Ascorbic acid (mg/100g)	12.88	17.54	11.11	15.92
Acidity (%)	0.51	0.59	0.48	0.60
Lycopene content (mg/100 g)	4.66	7.16	5.21	3.40
Beta carotene content (mg/100 g)	3.24	3.97	3.50	2.46
Total sugars (%)	2.61	2.21	2.59	2.14
Shelf life (days)	10.11	9.88	10.56	11.25

used in hybridization. Plant height, fruit weight, polar diameter, equatorial diameter, yield per plant, acidity, shelf life recorded maximum cluster mean values in Cluster IV, and minimum value for days to first flowering. When breeding for earliness, high fruit weight, yield, acidity, and improved shelf life, genotypes from clusters IV, could be effectively utilized (Meena and Bahadur, 2013).

Screening for bacterial wilt resistance

Based on the PDI, the genotypes were classified into four groups (Table 5). Five genotypes *i.e.*, Sakthi, Mukthi, Anagha, Manuprabha and AVTO-1711 appeared as resistant, while, seven genotypes were categorized as moderately resistant to the bacterial wilt, however, five genotypes were rated as moderately susceptible and seventeen were susceptible.

Table 5 : Classification of tomato genotypes based on per cent disease incidence (PDI)

Disease reaction	Genotype
Susceptible (61-100 PDI)	EC-914085, EC-914087, EC-914088, EC-914089, EC-914092, EC-914093, EC-914095, EC-914096, EC-914097, EC-914098, EC-914099, EC-914101, EC-914102, EC-914103, EC-914105, EC-914107, EC-914109
Moderately susceptible (41-60 PDI)	EC-914086, EC-914100, EC-914104, EC-914108, Sln-9,
Moderately resistant (21-40 PDI)	EC-914090, EC-914091, AVTO-1718, AVTO-1717, Sln-2, Sln-6, Sln-7
Resistant (0-20 PDI)	Sakthi, Mukthi, Anagha, Manuprabha, AVTO-1711

CONCLUSION

Significant diversity among tomato genotypes could be effectively exploited in developing promising and high yielding bacterial wilt resistant hybrids. High heritability and genetic advance as percentage of mean were observed for plant height, fruits per cluster, fruit weight, polar and equatorial diameter, locules per fruit, fruit firmness, yield per plant and quality parameters, referring that these traits could be focused for developing promising high yielding tomato hybrids. Cluster analysis grouped the exotic large fruited genotypes in cluster IV, and the bacterial wilt resistant genotypes in cluster I, and small fruited bacterial wilt moderately resistant improved genotypes in cluster II. Maximum inter cluster distance was recorded between cluster II and cluster IV, followed by cluster I and cluster IV, indicated that exotic genotypes from World Vegetable Centre could be one of the promising parents and the small fruited bacterial wilt resistant improved genotypes as the counter parent for getting maximum heterotic hybrids as they are genetically diverse. The large fruited exotic lines in cluster IV can be used for improving the fruit size of bacterial wilt resistant varieties.

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Original Research Paper

SSR analysis to assess genetic diversity and population structure in parthenocarpy cucumber (*Cucumis sativus* L.)

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ABSTRACT

The genetic diversity and population relationship was determined in 14 genotypes of parthenocarpic cucumber (*Cucumis sativus* L.) using simple sequence repeats (SSR) markers. In this study, fifty-nine SSR markers comprehensively showed polymorphism among cucumber genotypes. Total 252 alleles were identified with an average of 4.27 alleles per locus, while the polymorphism information content (PIC) of the primers ranged from 0.34 to 0.84 with a mean value of 0.62. The major allele frequency and heterozygosity ranged from 0.21 to 0.75 and from 0.43 to 0.89, respectively. Maximum major allele frequency was reported with primer Cs-Female-4, whereas the maximum value of polymorphic information content was found with the primer SSR11742. The dendrogram clustered genotypes into two main groups A and B with 8 and 6 genotypes, respectively. Jaccard's similarity coefficient ranged from 0.63 to 0.86 with maximum similarity between genotypes DDPCG3 and PLP-1, whereas minimum similarity was observed between DDPCG8 and PLP Gy-1-08B. The population structure revealed three sub-populations with some admixtures. Principal coordinate analysis (PCoA) with SSR markers revealed that the genotypes were uniformly distributed across the two axes in both the plots with 41.76% of cumulative variation. The genetic divergence within indigenous genotypes allow genotypic identification, gene mapping and cloning for improvement in cucumber breeding.

Keywords : Cucumber, Genetic diversity, Polymorphism, Population structure, SSR Markers

INTRODUCTION

Cucumber is a member of the diverse and distinct Cucurbitaceae family and is widely grown for both fresh and processing purposes around the world. Primary centre of origin was India where both wild and cultivated species exist while, China and near east are secondary centre of origin (Telford and Renner, 2010). Both cultivated and wild species viz., *Cucumis sativus* var. *hardwickii* render enormous variation for various traits like growth habit, sex expression, fruit size, spines and flesh bitterness. About 70% of the cucumber world production is contributed by Asian countries, Turkey, Iran and Russia. In India, cucumber covered an area of 104 thousand hectares with 1603 thousand MT annual production (NHB 2019).

Cucumber is an ideal model crop for genetic studies due to smaller genome size of approximately 367Mb with shorter life cycle (Kaur and Sharma, 2021). Breeding cucumber for enhancing yield, quality, and biotic and abiotic stress tolerance is a major challenge for the breeders, globally (Yuan *et al*., 2008). In spite of huge variability, it has narrow genetic base with only 12% polymorphism which limits the new cultivar development by cross breeding (Pandey *et al.,* 2018). There is scope for improvement of the productivity with the use of improved varieties or hybrids of cucumber (Pandey *et al.,* 2016). Selection of suitable parents for breeding programme depends on the existence of variability in the germplasm. Identification of the suitable parents is the most imperative for hybridization. Recent progresses in plant genomic offers an opportunity for assessing genetic diversity through use of molecular markers (Yang *et al.,* 2015). Molecular markers are more advantageous than morphological characters due to more stability under variable environment conditions. Different types of molecular markers are random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCAR), amplified fragment length polymorphisms (AFLP) and simple sequence repeats (SSR) (Dar *et al.,* 2017). Among all, SSR markers are widely used in plant genomics like gene mapping, quantitative trait loci (QTL), marker assisted selection (MAS), evolutionary studies and genetic diversity

analysis (Mahajan *et al.,* 2016). SSR markers are used in cucumber for assessment of genetic diversity in cucumber (Yang *et al.,* 2015). Genetic diversity and population structure is very important for the maintenance, conservation and improvement in productivity in agriculture. Plant genetic diversity can be preserved and stored in the form of plant genetic resources in gene banks and DNA libraries for long term conservation. These plant genetic resources could be utilized in future for the crop improvement against various biotic and abiotic stresses to meet global food security (Garzon-Martinez *et al.,* 2015). Due to narrow genetic base and use of limited number of SSR markers for genetic diversity analysis, there is a dire need for studying genetic diversity using SSR markers for bridging the gap in the crop improvement by hybridization. Therefore, this study was focused to determine genetic diversity and population structure using SSR markers in cucumber. The findings of this work will aid in the selection of cucumber genotypes with a high genetic diversity of the genes used in crossbreeding, QTL mapping, gene tagging and other imperative genomic studies

MATERIALS AND METHODS

Experimental material

This study was conducted at Research Farm of Vegetable Science, Department of Vegetable Science and Floriculture (N 32° 6[1], E 76° 3[1]), CSK-HPKV, Palampur. Agro-climatically, it is located in the mid-hill regions having humid sub-temperate climate with 2,500 mm annual rainfall. The experiment material comprised of fourteen genotypes both gynoecious parthenocarpic which were collected from CSK-HPKV (Palampur), PAU (Ludhiana, Punjab) and GBPUA&T (Pant Nagar) (Table 1). The genotypes were maintained at Experimental Farm and Molecular Biology Laboratory of Vegetable Science and Floriculture department, CSK- Himachal Pradesh Krishi Vishvavidyalaya, Palampur, India during the year 2020-21 to take up genetic diversity analysis.

Genomic DNA extraction and PCR amplification using SSR markers

About 5 g of plant tissue was finely ground in liquid nitrogen. The entire genomic DNA was extracted from each genotype using the CTAB technique (Doyle and Doyle, 1987). The DNA quantification was done using Nanodrop spectrophotometer at the OD 260/280 value and 0.8% agarose Gel-electrophoresis. For PCR, DNA

Table 1 : Cucumber germplasm and their sources used for diversity analysis

Germplasm	Collection Source
DDPCG4	CSKHPKV, Palampur
HPK-1	CSKHPKV, Palampur
Punjab Kheera-1 (PK-1)	PAU, Ludhiana
PPC-2	GBPUA&T, Pant Nagar
PPC-3	GBPUA&T, Pant Nagar
DDPCW1	CSKHPKV, Palampur
DDPCG2	CSKHPKV, Palampur
DDPCG5	CSKHPKV, Palampur
DDPCG6	CSKHPKV, Palampur
DDPCG7	CSKHPKV, Palampur
PLPGy-1-08-A (green)	CSKHPKV, Palampur
PLP Gy-1-08-B (white)	CSKHPKV, Palampur
DDPCG3	CSKHPKV, Palampur
PLP-1	CSKHPKV, Palampur

was diluted to 50 ng/ul and refrigerated at 4°C, whereas concentrated DNA stocks were kept at -80°C for later use.

For amplification of genomic DNA, a reaction mixture of 15 µl volume was prepared using template DNA (50 ng/µl), forward and reverse primer (5µM each), MgCl2 (1.6 mM), 1 X PCR buffer (1 X: 10mM Tris-HCl, 50mM KCl, pH 8.3), dNTP mix (0.25 mM) and Taq polymerase (0.75 U/µl). The PCR reaction was carried out in thermal cycler with initial denaturation at 94°C at 3-5 min, 35-36 cycles of denaturation of 94°C for 30- 60 sec, Annealing of 50-60°C for 30-60 sec, extension of 72°C for 60-80 sec and followed by final extension of 72°C for 5-10 min. The amplified products were resolved in 3 per cent agarose gel with 100 bp ladder and gels were visualized using the gel-documentation unit (Bio-Rad).

Statistical analysis

For all analyzed genotypes, exclusive DNA bands were evaluated as present (1) or absent (0). In the SIMQUAL programme of the NTSYSpc package (version 2.02), the binary data were used to generate a Jaccard's similarity coefficient through UPGMA (unweighted pair-group method with arithmetic averages) method which allowed to design a dendrogram by genotype clustering. PIC value calculates the informativeness of a particular DNA marker (Spooner *et al.,* 1993). Using the software STRUCTURE version 2.3.4 (Pritchard *et al.,* 2000), model-based cluster analysis was performed to

determine the genetic structure and number of clusters in the data set. The number of hypothesized populations (K) varied between 2 and 10 and the analysis was carried out twice and the true k was determined according to the method described by Evanno *et al.* (2005). The run with maximum likelihood was used to assign individual genotypes into groups. POPGENE was used to calculate a variety of genetic variation parameters. Using the DARwin software version 5.0, a Neighbor-Joining tree (UnWeighted) was constructed from the dissimilarity matrix (Perrier and Jacquemoud, 2006). 1000 bootstraps were used to test branch robustness. Principal coordinates analysis (PCoA) in GenALEx 6.5 was used to visualize the genetic relationship patterns in the matrix. Structure analysis was done to estimate population structure (Q matrix) using STRUCTURE (Pritchard *et al.,* 2000; Falush *et al.,* 2003) and express as membership probability. To estimate the actual population substructure, ten different Ks (from K=1 to K=10, where K is the kinship matrix) were utilized.

RESULTS AND DISCUSSION

SSR and marker informativeness

The gel electrophoresis results for 14 germplasm with primer SSR11742 is presented in Fig. 1. The total molecular variability parameters such as PIC, heterozygosity, major allele frequency, number of alleles and allele size across all 14 germplasm are presented in Table 2 (Supplimentary file). Out of 61 SSR primers, 59 primers exhibited polymorphism. A total of 252 amplicons were created, with sizes ranging from 100 to 380 bp. The total number of alleles from 59 primers observed was 252 with a mean of 4.27 alleles per locus and eight alleles were identified in SSR11742 and SSR04689. Major allele frequency varied from 0.21 (SSR04689) to 0.75 (Cs-Female-4) with an average value of 0.42. The polymorphic information content (PIC), ranged from 0.34 (SSR30647) to 0.84 (SSR11742), with an average value of 0.62 per primer. Similarly, heterozygosity varied from 0.43 (Cs-Female-4) to 0.89 (SSR 11742) with an average value of 0.70.

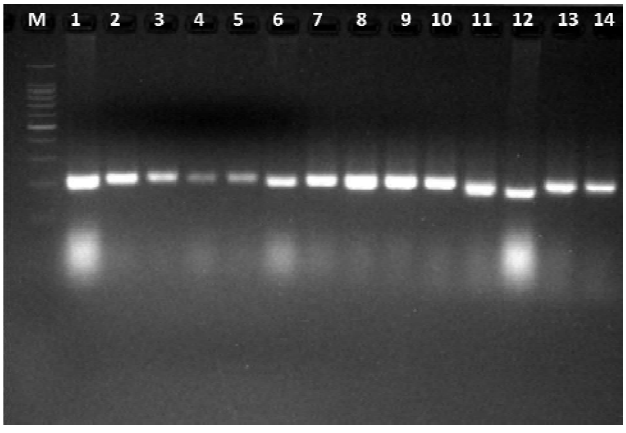


Fig. 1 : DNA profile of 14 germplasm of cucumber showing polymorphism with primer SSR11742 (M-100 bp ladder)

Genetic diversity assessment and structure analysis

Fourteen cucumber genotypes were divided into two main clusters (A and B). Cluster A was split into two sub-clusters comprising of total of 8 germplasm, while Cluster B had contained six genotypes namely, DDPCG6, DDPCG7, PLPGy-1-08A, PLPGy-1-08B, DDPCG3 and PLP-1 (Fig. 2). Based on UPGMA analysis, Jaccard's similarity coefficient varied from 0.63 to 0.84 with maximum similarity between genotype DDPCG3 and PLP-1 (0.86), whereas minimum similarity was between DDPCG8 and PLPGy-1-08B (0.59). Based on Neighbor Joining analysis, genotypes were grouped into three clusters as depicted using the color codes in Fig. 3. Cluster I (Red), Cluster II (Blue) and Cluster III (Green)

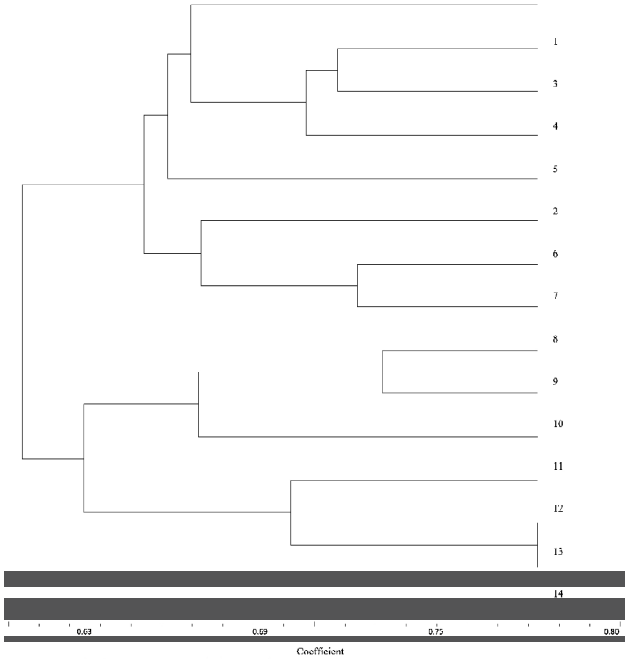


Fig. 2 : Dendrogram depicting genetic relationships among the cucumber germplasm constructed by NTSYS–PC (version 2.02) using UPGMA method

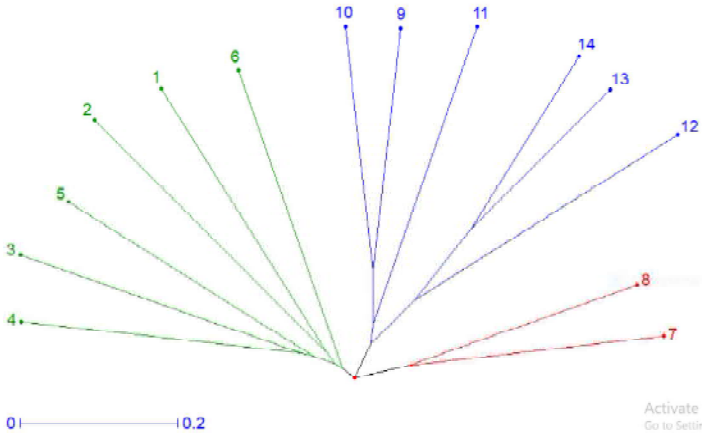
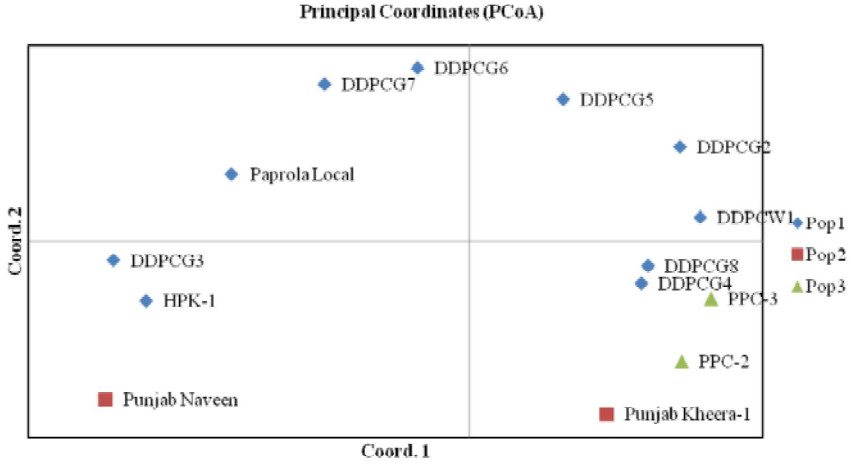


Fig. 3 : Neighbor-Joining tree of cucumber germplasm using SSR markers generated by DARwin software



Percentage of variation explained by the first 3 axes

Axis	1	2	3
%	17.59	14.10	10.08
Cum %	17.59	31.68	41.76

Fig. 4 : PCoA scatter diagram analysis showing the distribution of 14 cucumber germplasm

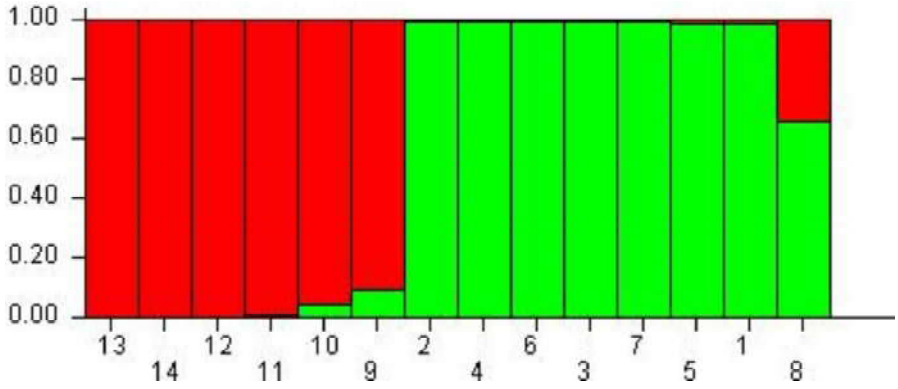


Fig. 5 : Genetic structure of 14 cucumber germplasm (red and green) represent the two groups, defined by the K value. Cucumber germplasm showing more than one color may have an admixture

comprised of two, six and six genotypes, respectively. Principal coordinate analysis (PCoA) showed that first three coordinates accounted for 41.76% cumulative variation among 14 genotypes (Fig. 4) with the first and second coordinates explaining 17.59% and 14.10% of the total variation respectively.

The STRUCTURE analysis divided the population into two groups. The differentiations at K =2 were nearly equivalent to pedigree knowledge with a few outliers. In group 1 (Red) consists of 6 genotypes and group 2 (Green) comprises 8 genotypes (Fig. 5). The germplasm generated by the NTSYS software were confirmed using STRUCTURE analysis at K = 2. As a result of this, it was established that the germplasm that were separated according to cluster analysis were almost identical to those that were divided according to structure analysis, with a few minor differences.

The genetic diversity and population structure in cucumber was investigated for improvement of various traits using crop breeding practices. A limited number of SSR molecular markers were used with Indian cucumber genotypes. It has been observed that SSR markers showed high polymorphism in cucumber. In our study, we have determined the genetic diversity using sixty-one SSR markers in 14 genotypes of cucumber comprising a wider geographical distribution of genotypes. Among 61 SSRs primers, 59 primers showed high polymorphism and a total of 252 alleles were identified with an amplicon size ranging from 100-380 bp. The number of alleles varied from 2-8 with a mean of 4.27 alleles per locus. Similarly, Dar *et al.* (2017) and Lv *et al.* (2012) observed an average number of alleles 2.9 and 13.7 per locus, respectively. The polymorphic information content (PIC), a measure related to marker discrimination, ranged from 0.34 (SSR30647) to 0.84 (SSR11742), with a mean of 0.62 per primer. Our study revealed similar results of PIC (0.62) in comparison with previous reports on cucumber *i.e.,* 0.664 and 0.69 (Hu *et al*., 2011; Normohamadi *et al.,* 2017) while, PIC was lower in Indian cucumber (0.310), Chinese cucumber (0.388) and cucumber (0.33) (Hu *et al.,* 2011; Pandey *et al.,* 2013; Dar *et al.,* 2017). A range of 0.12-0.44 was observed for PIC value for 15 primers with the mean value of 0.21 (Someh *et al.,* 2016). SSR11742 and SSR04689 markers were found more polymorphic among 59 SSR markers due to their high PIC values. The results were in agreement with earlier studies on cucumber suggesting the role of SSR markers for identification of genotypes, DNA fingerprinting and maintenance of genotypes in the gene banks. Based on UPGMA analysis with Jaccard's similarity coefficient varied from 0.63 to 0.86. Similarly, Someh *et al.* (2016) and Normohamadi *et al*. (2017) reported Jaccard's similarity coefficient ranging from 0.56 to 0.88 and 0.51 to 0.92 in cucumber, respectively. Lower range of Jaccard's similarity coefficient *viz*., 0.01-0.44 and 0.35-0.51 was reported in cucumber by Valcarcel *et al*. (2018) and Park *et al*. (2021). There was no regional distribution trend in the clustering pattern based on UPGMA and PCA. This could be due to regular gene flow through seed exchange between different places, which is most likely due to human interference (Garzon-Martinez *et al.,* 2015). Minimum Jaccard's similarity coefficient was observed in DDPCG8 and PLPGy-1-08B showing maximum diversity among genotypes. The genotypes DDPCG8 and PLPGy-1-08B were collected from different parts of Indian origin The clustering formed by the UPGMA dendrogram was moderately validated by projecting individual genotypes into a two-dimensional multivariate space in PCoA diagram. As per UPGMA method the cucumber genotypes were divided into two main clusters A (A_1-5 and A_2-3) and B (6). Similar results were reported by Dar *et al.* (2017) which grouped cucumber germplasm into two main distinct clusters. Various clustering methods were employed to assess genetic relationship of different genotypes or germplasm. Based on Neighbour Joining, fourteen genotypes were grouped into three clusters as represented by using color codes. Cluster I consists of 2 genotypes followed by 6 genotypes in cluster II and III.

PCoA is a multivariate strategy for grouping data based on similarity coefficients or variance or covariance values that provides more information about main groups, whereas cluster analysis provides higher resolution among closely related populations. PCoA explores correlations between many quantitative variables by constructing a small number of linear combinations (principal components) that retain as much information as feasible from the original data. Principal coordinate analysis (PCoA) showed that first three coordinates accounted for 41.76% cumulative variation among 14 genotypes with the first and second coordinates explaining 17.59% and 14.10% of the

total variation respectively. The population structure analysis grouped the genotypes into 2 groups including genotypes having admixtures. As a result, pedigree information was combined with cluster membership to determine the division of Red and Green groupings. Similar results were reported in cucumber (Pandey et al. 2013; Dar *et al.,* 2017) and Turkish melons (Sensoy *et al.,* 2007). The increased variance should be recorded for germplasm preservation and agricultural enhancement breeding strategies.

CONCLUSION

This study could be used to estimate genetic variation within a group of elite genotypes to employ in cucumber improvement in India. A total of 14 cucumber genotypes were assessed using 59 polymorphic SSR markers. The experiment depicted total number of 252 amplicons, with an overall average of 4.27 alleles per locus. SSR 11742 primer was recorded to have good marker informativeness. Based on UPGMA cluster analysis, maximum similarity (less diverse) was observed between genotype DDPCG3 and PLP-1 whereas minimum similarity (more diverse) between DDPCG8 and PLPGy-1-08B. The population structure depicted three main populations including admixture genotypes. It may be further utilized in future projects related to QTLs identification, genome wide association studies, DNA fingerprinting and preservation of cucumber germplasm across India and other countries.

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Original Research Paper

Transcriptome analysis and identification of leaf, tuberous root and fibrous root tissue-specific high temperature stress-responsive genes in sweet potato

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ABSTRACT

Sweet Potato is an important food crop, and its production is affected by environmental stresses, including high temperature. The gene expression patterns and molecular responses in different tissues of sweet potato under high temperature stress were studied using microarray data sets. Analysis revealed that modulation in the expression of key genes and pathways associated with various proteins including enzymes under high temperature stress in leaf, fibrous root and storage root tissues. Tissue-specific responses, with both common and unique cellular responses were observed among the tissues. Pathway analysis revealed the differential regulation of genes involved in DNA replication, metabolism, transport, signaling, and stress response during high temperature stress. Six genes viz., DnaJ-domain protein (IpDnaJ), nuclear protein (IpELF5), heat shock protein 90.1 (IpHsp90.1), ABC transporter (IpABC) hydrolase (IpNUDX1) and alternative oxidase 1a (IpAO1a), were up-regulated in the leaf, fibrous root and tuberous root tissues. These six genes might play an important role in imparting high temperature stress tolerance in the leaf, fibrous root and tuberous root tissues of sweet potato. The information generated provides valuable insights on leaf, tuberous root and fibrous root tissue-specific high temperature stress-responsive genes in sweet potato. These datasets will be helpful in selecting candidate genes and pathways for further functional and genomic analyses, facilitating the genetic improvement of sweet potato with enhanced stress tolerance.

Keywords : Fibrous root, high temperature stress, microarray, sweet potato, tuberous root

INTRODUCTION

Sweet potato [*Ipomoea batatas* (L.) Lam] holds immense importance as a staple crop in the tropical and sub-tropical regions across the globe. It is the only domesticated species in the *Ipomoea* genus (Ravi *et al*., 2014). Sweet potato is a highly nutritious crop due to its starch-rich storage root that provides a substantial amount of dietary energy and essential nutrients required to meet human nutritional requirements (van Jaarsveld and Faber, 2013). This versatile crop offers a range of benefits, including a high carbohydrate content, dietary fiber, bioactive compounds (such as proteins, vitamins, β-carotene, anthocyanins, and minerals), and nutritional composition comparable to cereals and pulses (van Jaarsveld and Faber, 2013; Ravi *et al*., 2014). Additionally, sweet potato has emerged as a "climate-resilient" and "famine-relief" crop, playing a crucial role in mitigating food shortages during natural calamities, saving numerous lives globally (Gurmu *et al.,* 2014; Ravi *et al*., 2014). Introduction of orange-fleshed sweet potatoes, rich in β-carotene, has effectively addressed vitamin A-related malnutrition disorders in pregnant women and young children in developing nations (van Jaarsveld and Faber, 2013; Gurmu *et al.,* 2014). Consequently, sweet potato holds immense potential as an essential dietary component for future human populations (Gurmu *et al.,* 2014).

High-temperature stress has detrimental effects on sweet potato growth, development, and overall yield. Studies have shown increased temperatures during early seasons result in fewer tubers and decreased yield, whereas, high temperatures during mid and late seasons promote shoot growth at the expense of root growth, ultimately affecting the final storage root yield (Gajanayake *et al.,* 2015). Moreover, elevated temperatures have been found to impact storage root growth and yield negatively, with potential reductions in sweet potato yield (Wijewardana *et al.,* 2018). It

has also been observed that high temperatures can depress photosynthetic rates, further affecting yield (Ravi *et al.,* 2014). To enhance the resilience of sweet potato crops against heat stress, it is crucial to identify and understand the genes involved explicitly in heat stress responses. By studying these genes, strategies can be developed to improve the crop's ability to with stand high temperatures and maintain optimal growth and yield.

Transcriptome analysis plays a crucial role in understanding the dynamic changes in gene expression under abiotic stress conditions (Katiyar *et al.,* 2015; Sun *et al.,* 2022). Comprehensive tissue-specific transcriptome analysis by employing techniques such as Microarray, RNA sequencing (RNA-Seq) etc., provides valuable insights into the regulatory programs that govern gene expression during organ development (Katiyar *et al.,* 2015; Ravi *et al.,* 2020). This approach is particularly relevant in the context of sweet potato, as it allows for the identification and characterization of genes specifically involved in heat stress responses, unveiling tissue-specific gene responses and regulatory networks that contribute to the crop's adaptation and resilience under high-temperature conditions (Sharma *et al.,* 2021). Tissue-specific transcriptome analysis has been employed in various plant species to uncover multiple responses to environmental stressors (Katiyar *et al.,* 2015; Tiwari *et al.,* 2023). Transcriptome analysis has provided valuable insights into tissue-specific gene expression profiles and regulatory networks involved in heat stress responses (Tao *et al.,* 2012; Sun *et al.,* 2022). These studies have revealed specific genes and pathways that are activated or suppressed in response to high temperatures in different plant tissues. Hence, in this study transcriptome analysis was performed to identify the high temperature-responsive genes in sweet potato tissues *viz*., leaf, fibrous root and tuberous root tissues using microarray. Analysis revealed that under high temperature stress, certain key genes and pathways associated with DNA replication, metabolism, transport, signaling, and stress response are modulated in leaf, fibrous root, and storage root tissues. Interestingly, tissue-specific responses were observed, with both common and unique cellular responses among the different types of tissues.

MATERIALS AND METHODS

Plant material and growth conditions

The sweet potato var. Sree Arun was grown in the earthen pots in the natural sunlight conditions with 12 hours sun light per day under 1700 µ mol m^2h^{-1}at $30^oC \pm 2^oC$ during day time and $23^oC \pm 1^oC$ during night time as described in Ravi *et al.* (2017). High temperature stress was imposed by exposing the plant to $40^oC \pm 2^oC$. High quality RNA was extracted from the leaf, storage root and fibrous root from 30 days old sweet potato plants (Ravi *et al.,* 2017).

RNA processing and cRNA synthesis

The RNA samples of leaf, fibrous root and tuberous root were labeled using Agilent Quick Amp Kit as per manufacturers protocol (Ravi *et al.,* 2017). 500 ng of RNA was reverse transcribed using oligodT primer tagged to T7 promoter sequence for synthesizing cDNA. The *in vitro* transcription step was performed to convert cDNA to cRNA using T7 RNA polymerase enzyme and Cy3 dye as per manufacturers protocol (Ravi *et al.,* 2017). The cRNA was further cleaned using Qiagen RNeasy columns (Qiagen, Cat No: 74106). The concentration and amount of dye incorporated was measured using Nano Drop Spectrophotometer (Thermo Scientific, USA).

Microarray and expression analysis

The Agilent 60-mer oligo microarray (Agilent control grid IS- 62976-8-V2-60K x 8-Gx-EQC-201000210) was used for studying the expression pattern of the genes of sweet potato (Ravi *et al.,* 2020). For these, 600 ng of labeled cRNA were hybridized on the array using the Gene Expression Hybridization kit following manufacturers instruction using Agilent Sure hybridization Chambers at 65º C for 16 hours. Hybridized slides were washed using Agilent Gene Expression wash buffers (Part No: 5188-5242). G2505C scanner (Agilent Technologies) was used to scan the slides. Sample preparation and microarray expression analysis was performed at the Genotypic Technology Pvt. Ltd., Bengaluru, India. The microarray datasets generated in this study was submitted to NCBI GEO: GSE51862. The raw data was processed and the expression of the probes was transformed into the $\log_2$ ratio. The gene expression with $\log_2$ FC >2 was considered as upregulated and gene expression with $\log_2$ FC < -2 was considered as downregulated. Differentially expressed genes (both upregulated and downregulated) were considered for further analysis. The sequence information of the sweet potato probes were used as a query and a blast search was performed against the *Arabidopsis* and rice genome database to identify the respective

Table 1 : Differentially expressed gene (DEGs) statistics under high temperature stress in sweet potato

Analysis group	Total DEGs (Log_2 FC>2 and Log_2 FC<-2)	Upregulated (Log_2 FC>2)	Downregulated (Log_2 FC<-2)
Leaf tissue	1871	967	904
Fibrous root tissue	2725	1461	1264
Tuberous root Tissue	2810	109	2701

orthologous. The gene annotated information and LOC details of *Arabidopsis* were used for predicting the gene ontology (Tian *et al.,* 2017).

RESULTS AND DISCUSSION

The present study aimed to investigate the gene expression patterns in different tissues of sweet potato (leaf, fibrous root and tuberous root) in response to high temperature stress. Transcriptome analysis using microarray technology revealed the modulation of key genes and pathways associated with various proteins and enzymes in the different tissues of sweet potato under high temperature stress.

Differential expression analysis

Differential expression analysis revealed that 967, 1461 and 109 genes were upregulated in the leaf, fibrous root and tuberous root tissues, respectively, whereas, 904, 1264 and 2701 genes were down regulated in the leaf, fibrous root and tuberous root tissues, respectively during high temperature stress (Table 1 and supplementary Table 1). The details of the probes/genes, gene expression (fold change), description, etc., are presented in supplementary Table 1.

The present findings aligned with Ravi *et al*. (2017, 2020), who demonstrated the use of microarray analysis in understanding the molecular responses of tuber development in sweet potato. Differential expression analysis identified a substantial number of upregulated and downregulated genes in each tissue, highlighting the tissue-specific response to high temperature stress. Furthermore, the study identified common and unique cellular responses among the tissues, as supported by the differential regulation of pathway genes involved in DNA replication, metabolism, transport, signaling, and stress response (Tao *et al.,* 2012; Sharma *et al*., 2021; Sun *et al.,* 2022).

High temperature stress responsive genes in the leaf, fibrous root and tuberous root tissues of sweet potato

Molecular chaperones *viz*., heat shock proteins, DnaJ-domain, etc., protects the native proteins from the stress induced damages by retaining its native structures (Muthusamy *et al.,* 2016). The movement of these proteins across cell organelles was regulated with the help of coordinated function of various transporters such as *Ran GTPase* (Choudhury *et al.,* 2021). Alternative oxidase (AOX) protects the plant mitochondria under high temperature stress (Saha et al., 2016). In this study, sixty genes were differentially regulated in all three tissues viz., leaf, fibrous root and tuberous root tissues under high temperature stress (Fig. 1 and supplementary Table 2). Out of these sixty, six genes, DnaJ-domain protein (IpDnaJ), Nuclear protein (IpELF5), heat shock protein 90.1 (IpHsp90.1), Alternative oxidase 1a (IpAO1a), ABC transporter (IpABC) and hydrolase (IpNUDX1) were upregulated (Table 2), whereas, twenty-six genes were downregulated regulated in the leaf, fibrous root and tuberous root tissues respectively during high temperature stress (Fig. 1, supplementary Fig. 1 and supplementary Table 2). Thus, these six genes might play an important functional role in protecting the cellular proteins in leaf, fibrous root and tuberous root tissues under high temperature stress in sweet potato (Muthusamy *et al*., 2017; Saha *et al*., 2016; Choudhury *et al.,* 2021). In the present study, 376 were differentially regulated in both leaf and fibrous root tissues, whereas, 148 genes were differentially regulated in both leaf and tuberous root tissues during high temperature stress (Fig. 1 and supplementary Table 1). About 250 genes were differentially regulated in both fibrous root and tuberous root tissues under high temperature stress (Fig. 1 and supplementary Fig. 1). Several studies have shown the significant modulation in the expression of transcriptome involving important genes/

Table 2 : Details of the upregulated genes in leaf, fibrous root and tuberous root tissues of sweet potato under high temperature stress

Gene	Description/ function	Sweet potato Array Probe ID	Fold Change (log_2 FC)			*Arabidopsis* ortholog (LOC ID)
			Leaf	Fibrous root	Tuberous root	
IpDnaJ	DnaJ Chaperone	JP117116	3.16	2.23	2.26	AT5G03030.1
IpNUDX 1	cytosol-localized nudix hydrolase	JP135891	3.83	3.22	2.80	AT1G68760.1
IpABCC3	ABC transporter family protein	JP140208	4.09	2.75	2.31	AT3G13080.4
IpELF5	Nuclear Protein	JP144908	2.76	2.62	2.46	AT5G62640.2
IpHsp90.1	Heat shock protein 90 (HSP90)	JP146862	3.73	3.14	2.92	AT5G56010.1
IpAO1a	Alternative oxidase 1a	JP145717	5.45	4.26	3.90	AT3G22370.1

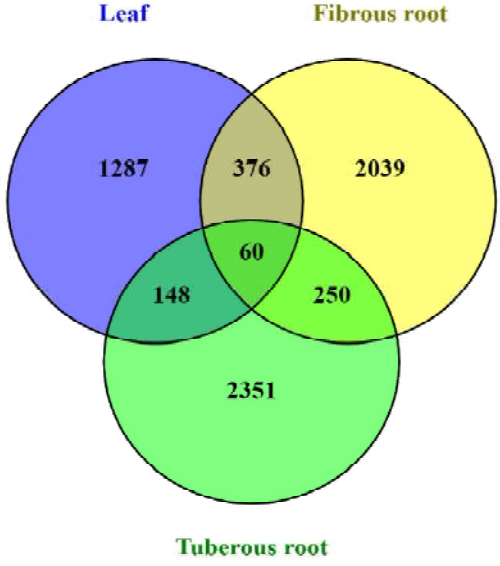
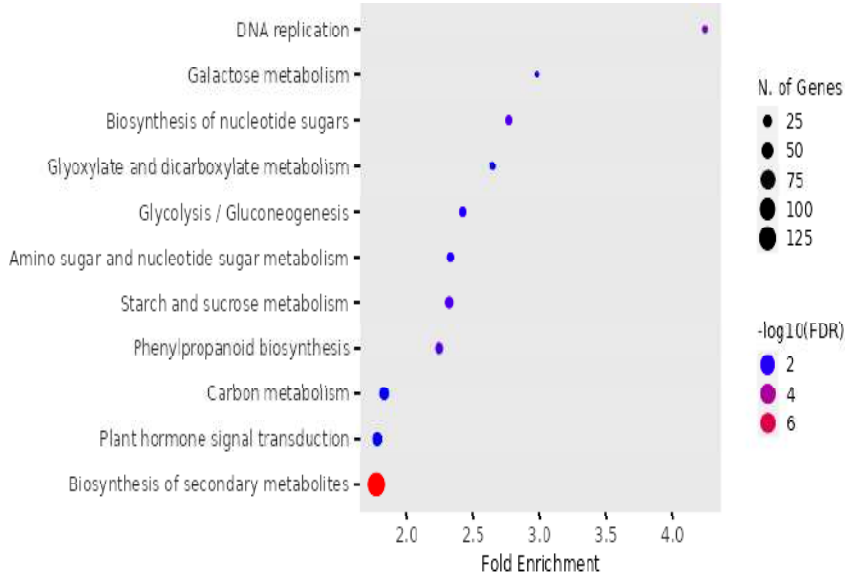


Fig. 1 : Venn diagram showing upregulated and downregulated genes in the leaf, fibrous root and tuberous root tissues of sweet potato under high temperature stress in comparison with control condition

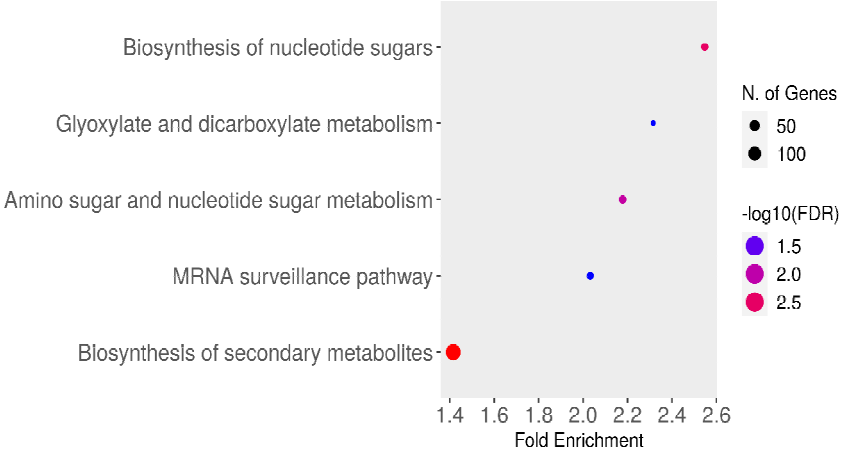
pathways during high temperature stress. The pathways/genes including phytohormones, molecular chaperones, signaling kinesis, ROS scavenging enzymes, Epigenetic modifications, transcription factors, etc., were differently expressed during high temperature stress (Sharma *et al*., 2021; Venkatesh *et al*., 2022).

Tissue specific molecular, biochemical and physiological response play important role in regulating high temperature response in plants (Muthusamy *et al.,* 2017; Sharma *et al.,* 2021; Xiang and Rathinasabapathi, 2022). Thus, in this study, under high temperature stress, the pathway genes *viz*., DNA replication, galactose metabolism, biosynthesis of nucleotide sugars, glyoxylate and dicarboxylate metabolism, glycolysis/gluconeogenesis, amino sugar and nucleotide sugar metabolism, starch and sucrose metabolism, phenylpropanoid biosynthesis, carbon metabolism, plant hormone signal transduction and biosynthesis of secondary metabolites were modulated in the leaf tissue, whereas, biosynthesis of nucleotide sugars, glyoxylate and dicarboxylate metabolism, amino sugar and nucleotide sugar metabolism, mRNA surveillance pathway and biosynthesis of secondary metabolites were modulated in the fibrous root tissues (Fig. 2).

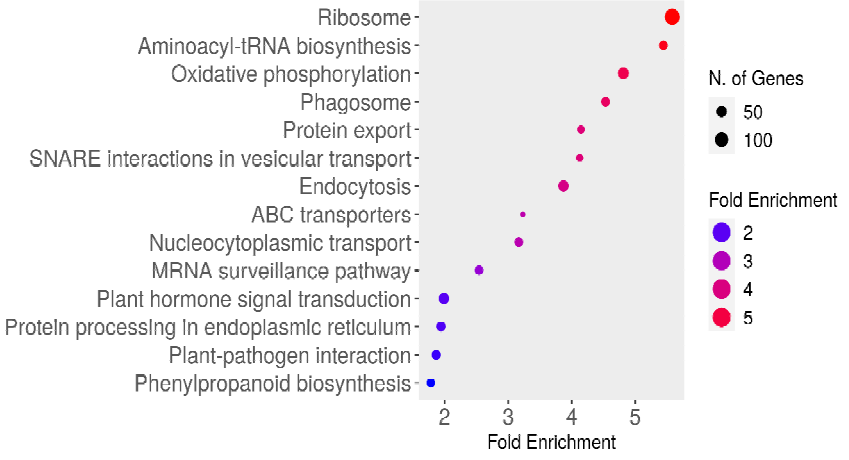
Similarly, in the tuberous root tissues, ribosome, aminoacyl-tRNA biosynthesis, oxidative phosphorylation, protein export, ABC transporters, nucleocytoplasmic transport, mRNA surveillance pathway, plant hormone signal transduction, protein processing in endoplasmic reticulum- plant-pathogen interaction and phenylpropanoid biosynthesis pathway genes were modulated. Additionally, previous research has elucidated the molecular responses of sweet potato to other stress conditions such as low temperature stress (Wijewardana *et al.,* 2018). These studies collectively contribute to understanding of the complex molecular mechanisms underlying stress responses in sweet potato (Wijewardana *et al.,* 2018; Ravi *et al.,* 2020;



A. leaf



B. fibrous root



C. tuberous root

Fig. 2 : GO categories of DEGs of sweet potato under high temperature stress.

Sun *et al.,* 2022; Xiang and Rathinasabapathi, 2022). Thus, present study demonstrates the presence of both collective and individual cellular responses to high temperature stress in the leaf, fibrous root, and tuberous root tissues of sweet potato.

CONCLUSION

The study sheds light on the effects of high temperature stress on the gene expression profiles and molecular responses in the leaf, fibrous root, and storage root tissues of sweet potato. The study identified both common and tissue-specific responses to high temperature stress among these tissues through comparative analysis. The findings provide valuable insights for identifying key genes and pathways involved in the response to high temperature stress, facilitating further functional and genomic studies aimed at enhancing the genetic improvement. By better understanding the molecular mechanisms underlying the response to high temperature stress, we can develop targeted strategies to enhance stress tolerance and improve the overall resilience of sweet potato.

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Original Research Paper

Evaluation of tuberose genotype IIHR 17-23SP-08 (IC0642158) for flower yield, quality and response to biotic stress

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ABSTRACT

Tuberose (*Agave amica*, family Asparagaceae) is an important commercial flower crop valued for its spectacular fragrant flowers. An experiment was conducted to evaluate the single petalled tuberose genotypes for growth, flowering, flower yield, concrete yield and response to biotic stress for two consecutive years from 2020 to 2022. Tuberose genotype IIHR 17-23SP-08 was found to be superior with highest plant height (55.53 cm), early flowering (94.93 days), highest number of spikes/plant (8.47), longest spikes (114.61cm) and rachis (32.11 cm) and maximum number of florets/spike (54.87). The matured bud weight of IIHR 17-23SP-08 was 1.29 g, which is preferable in the medium segment range with higher number of flower buds (725 buds per kg). It is a high yielder producing the highest number of spikes/m^2 (76.20) and loose flower yield 18.88 t/ha/year among the genotypes evaluated. The genotype IIHR 17-23SP-08 was also found to be a good multiplier with the maximum bulb production of 8.94 bulbs per clump. It was found to be resistant to root knot nematode (*Meloidogyne incognita*) and tolerant to leaf burn disease (*Alternaria polianthi*) under field conditions. It was found suitable as loose flower for garland preparation with the shelf life of 2 days under ambient conditions and for concrete extraction with the concrete yield of 0.095%. It produces white buds (RHS colour: NNI55D, white group, Fan 4) with green tinge on the tip. Thus, the genotype IIHR 17 23SP 08 was found promising and novel among the single types with better flower and bulb yield parameters.

Keywords : Concrete, evaluation, flowering, single type, shelf life, tuberose, yield.

INTRODUCTION

Tuberose, *Agave amica* (Medik.) Thiede & Govaerts (formerly *Polianthes tuberosa* Linn.) is one of the most important tropical bulbous flowering plants that belongs to the family Asparagaceae and is native to Mexico. It is an important commercial crop preferred due to its pleasant fragrance, longer keeping quality and wide adaptability. It is commercially cultivated in India in about 21,970 ha, with a loose flower production of 1,21,860 metric tonnes and cut flower production of 93,680 metric tonnes (Anon., 2021). The flowers of tuberose are highly fragrant containing 0.08 to 0.14 % of concrete and having high demand in the international market. Globally, tuberose concrete and absolute are produced and traded in India, Egypt and France. Commercial cultivation of tuberose in India is confined to West Bengal, Karnataka, Tamil Nadu, Maharashtra, Andhra Pradesh, Uttar Pradesh, Chhattisgarh and the National Capital Region (NCR).

In India, the preference of flower colour of tuberose varieties is limited to white, although some varieties show pinkish and greenish tinge in bud stage. Garland segment in tuberose prefer varieties with green tinge on the bud tip. Though, the local variety of tuberose under cultivation is with green tinge on the bud tip, but its yield potential is very low and is highly susceptible to pests and diseases. Market demand is for medium sized flowers weighing less than 1.5 g/bud which makes a greater number of flowers per unit (kg). This stipulates the development of high yielding tuberose varieties with green tinge on the bud tip and medium bud weight suitable for garland purpose. With respect to biotic stresses, crop loss of 10 to 14% was reported due to root knot nematode

infestation in tuberose (Khan and Parvatha Reddy, 1992). Leaf burn disease caused by *Alternaria polianthi* is extensive in tuberose causing significant yield losses (Mariappan *et al*., 1977; Muthukumar *et al*., 2007 and Mazumdar *et al*., 2021). Keeping the above in view, the present research work was carried out with the objective of breeding medium sized flowers with green tinge on bud tip for loose flower and garland purpose that are resistant/tolerant to root knot nematode and leaf burn disease.

MATERIALS AND METHODS

The tuberose genotype IIHR 17-23SP-08 was developed through seedling selection from GK-TC-4 during the year 2017. It was vegetatively fixed through bulbs and multiplied. Seven single petalled type of tuberose genotypes namely IIHR 17-23SP-08, GK-TC-4, Phule Rajani, Bidhan Ujwal, Calcutta Single, Arka Prajwal (commercial check) and Mexican Single (local check) were evaluated for growth, flowering, flower and concrete yield and response to biotic stress in randomized block design with three replications from 2020 to 2022 at the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, India.

Bulbs of medium size (2.5 cm diameter) were planted on raised bed of 30 cm height with a spacing of 30 cm x 30 cm with the bed size of 2.4 m^2. Standard cultural practices were followed throughout the experiment. Observations were recorded on 15 plants in total, comprising 5 plants per replication for various parameters *viz*., plant height (cm), days to spike emergence, days to opening of first floret, number of spikes per clump, spike length (cm), rachis length (cm), number of florets per spike, length of floret (cm), diameter of floret (cm), bud length (cm), matured bud weight (g), weight of 100 florets (g), number of spikes per m^2, loose flower yield per ha per year (tons), number of bulbs per clump, shelf life (days) and concrete content (%).Tuberose concrete was extracted by solvent extraction method (ASTA, 1960) with food grade hexane as solvent. The concrete content was calculated on fresh weight basis and expressed in percentage. Tuberose genotypes were screened for the resistance against root-knot nematode (*M. incognita*) for two consecutive years. Gall Index (GI) was registered in the roots in a 0-5 scale (0- immune, 1- highly resistant, 2- resistant, 3- tolerant, 4- susceptible, 5- highly susceptible) as per Taylor and Sasser (1978) at the time of bulb harvest. The per cent disease index (PDI) and host reaction of the tuberose genotypes to leaf blight (*A. polianthi*) was recorded on 0-5 disease severity scale (0- immune, 1- resistant, 2-moderately susceptible and 3- highly susceptible) under field condition at 15 days interval for three times, as per Narayanappa and Chandra (1984). The data of two years were pooled and analysed statistically (Gomez and Gomez,1984).

RESULTS AND DISCUSSION

The perusal of data presented in Table 1, revealed significant differences in the growth, flowering and yield traits among the different genotypes. Plant height was maximum in genotype IIHR 17 23SP 08 (55.53 cm), which was on par with the commercial check Arka Prajwal (54.84 cm), while, it was minimum in GK-TC-4 (36.05 cm). The variation in plant height

Table 1 : Evaluation of tuberose genotype IIHR 17 23SP 08 (IC0642158) with checks

Genotype	Plant height (cm)	Days to spike emergence	Days to first floret open	No. of spikes per clump	Spike length (cm)	Rachis length (cm)	Number of florets per spike	Single bud weight (g)
IIHR 17-23SP-08	55.53	94.93	22.17	8.47	114.61	32.11	54.87	1.29
Mexican Single	37.81	111.10	19.73	4.17	88.11	20.01	43.83	1.01
Arka Prajwal	54.84	101.03	29.00	5.18	97.39	30.90	52.50	2.04
GK-TC-4	36.05	125.03	19.77	4.00	65.25	18.29	49.67	1.22
Phule Rajani	39.02	148.17	24.10	4.00	58.14	20.15	44.10	1.09
Bidhan Ujwal	36.07	106.87	23.30	4.23	55.11	16.21	56.87	1.04
Calcutta Single	40.77	105.77	19.80	4.13	91.22	11.28	33.57	0.82
SEm±	0.79	1.77	0.35	0.12	2.10	0.88	1.25	0.03
CD (P=0.05)	2.45	5.51	1.08	0.36	6.54	2.75	3.90	0.10
CV (%)	3.18	2.70	2.67	4.15	4.47	7.20	4.52	4.75

might be due to the inherent genetic makeup of the particular genotype. Similar results on variation in plant height were also reported by Mahawer *et al*. (2013) and Dogra *et al*. (2020) in tuberose.

Days to spike emergence varied from 94.93 to 148.17 days. The genotype IIHR 17 23SP 08 was found to be early flowering (94.93 days) followed by Arka Prajwal (101.03 days) and Phule Rajani (148.17 days). Ramachandrudu and Thangam (2009) also reported early flowering in cv. Hyderabad Single. Days to opening of first floret ranged from 19.73 (Mexican Single) to 29.00 days (Arka Prajwal), however, genotype IIHR 17 23SP 08 recorded 22.17 days for first floret opening and was early as compared to commercial check Arka Prajwal. The genotypes with early flowering catch the early market and would be remunerative to the farmers. Madhumathi *et al*. (2018) also observed variation in spike emergence in different cultivars of tuberose.

The number of spikes per plant has direct influence on the yield of the tuberose. The genotypes IIHR 17 23SP 08 registered the highest number of spikes per clump (8.47), whereas, lowest was in GK-TC-4 and Phule Rajani (4.00). This variation in the production of spikes per clump might be due to the inherent genetic factor of different cultivars under prevailing environmental conditions. The results are in conformity with the findings of Dalvi *et al*. (2021) and Gandhi and Bharathi (2021) in tuberose.

The genotype IIHR 17 23SP 08 recorded the longest spike (114.61 cm), while, Bidhan Ujwal registered shortest spike (55.11 cm). The rachis length varied from 11.28 (Calcutta Single) to 32.11 cm (IIHR 17 23SP 08). Variation in spike length and rachis length might be due to the inherent genetic potential of the genotype coupled with environmental conditions during the growing period. Madhumati *et al*. (2018) also observed variation in spike length of tuberose and reported maximum rachis length in Arka Prajwal (33.40 cm), whereas, minimum rachis length was recorded in GKTC-4 (23.93 cm).

The number of florets per spike has a direct association with the flower yield of the crop. Number of florets per spike ranged from 33.57 (Calcutta Single) to 56.87 (Bidhan Ujwal). The genotype IIHR 17 23SP 08 recorded 54.87 number of florets per spike which was on par with commercial check Arka Prajwal (52.50) and was superior than the local check Mexican Single (43.83). Bharathi and Umamaheswari (2018) also reported similar results in tuberose.

Weight of matured bud is an important economical trait for loose flowers as they are sold on weight basis. Current market demand in tuberose is for the variety that produces flowers buds which weigh less than 1.5 g per bud and have a greater number of flowers per unit (kg). In the present study, matured bud weight varied from 2.04 g (Arka Prajwal) to 0.82 g (Calcutta Single). The genotype IIHR 17 23SP 08 recorded matured bud weight of 1.29 g/bud which is in the range of medium segment and is preferred in the market. Based on the individual mature bud weight, IIHR 17 23SP 08 contains approximately 725 buds per kg. Similar observations were also made by Ramachandrudu and Thangam (2009) in tuberose cv. Arka Prajwal. Hundred bud weight was recorded maximum in Arka Prajwal (219.63 g) and minimum in Calcutta Single (80.80 g). The results are in corroboration with the findings of Vijayalaxmi and Lakshmidevamma (2016) in tuberose.

The data presented in Table 2 indicates significant variation in different flower traits. The bud length varied from 5.27 cm (Bidhan Ujwal) to 6.41 cm (Mexican Single), however, the genotype IIHR 17 23SP 08 recorded the bud length of 6.20 cm, which was found to be superior to the commercial check Arka Prajwal (6.15 cm). Variation in bud length of tuberose might be due to the difference in inbuilt genetic factor of the genotypes as reported by Singh *et al*. (2018) and Bharathi and Umamaheswari (2018) in tuberose. Diameter of floret varied from 3.82 cm (Bidhan Ujwal) to 5.17 (GK-TC-4). The diversity in flower diameter is in close conformity with the findings of Singh and Dakho (2017), Singh *et al*. (2018) and Bharathi and Kirthishree (2019) in tuberose.

The highest number of spikes per m^2 was recorded in IIHR 17-23SP-08 (76.20), whereas lowest was recorded in GK-TC-4 and Phule Rajani (36.00). Loose flower yield was maximum in IIHR 17-23SP-08 (18.88 t/ha/yr) followed by Arka Prajwal (17.48 t/ha/yr), whereas the lowest loose flower yield was recorded in Calcutta Single (5.08 t/ha/yr). Number of spikes per clump and number of florets per spike were found to be the highest in the tuberose genotype IIHR 17 23SP 08 which directly related to the highest loose flower yield. The distinct variation in the flower yield may be attributed to the distinguished inherent genetic

Table 2 : Evaluation of tuberose genotype IIHR 17 23SP 08 (IC0642158) with checks

Genotype	Bud length (cm)	Hundred bud weight (g)	Diameter of floret (cm)	No. of spikes per m^2	Loose flower yield/ha/ year (tons)	No. of bulbs per clump	Shelf life (days)
IIHR 17 23SP 08	6.20	134.69	4.33	76.20	18.88	8.94	2.17
Mexican Single	6.41	108.63	4.31	37.50	8.10	7.00	2.00
Arka Prajwal	6.15	219.63	4.88	46.65	17.48	6.87	3.00
GK-TC-4	6.37	135.41	5.17	36.00	8.86	6.17	1.50
Phule Rajani	5.49	97.39	4.01	36.00	6.87	3.19	1.42
Bidhan Ujwal	5.27	116.56	3.82	38.10	8.99	5.67	1.25
Calcutta Single	5.61	80.80	4.08	37.20	5.08	6.44	2.33
SEm±	0.07	2.53	0.08	1.05	0.21	0.25	0.07
CD (P=0.05)	0.21	7.89	0.27	3.28	0.65	0.65	0.22
CV (%)	1.95	3.44	3.50	4.15	3.39	3.39	6.35

makeup of cultivars as reported by Naik *et al*. (2018) and Dalvi *et al*. (2021) in tuberose.

The multiplication efficiency of a variety is important for large scale propagation and wider spread among the farmers and ease of availability. Number of bulbs per clump ranged from 3.19 (Phule Rajani) to 8.94 (IIHR 17-23SP-08). The variations observed in the bulb parameters are due to the presence of wide genetic variability among the tested genotypes of tuberose. Similar observations were recorded by Martolia and Srivastava (2012) in tuberose.

Shelf life was found to be the highest in the commercial check Arka Prajwal (3.00 days) followed by Calcutta Single (2.33 days) and IIHR 17 23SP 08 (2.17 days). Variation among the tuberose cultivars for the shelf life may be attributable to the hereditary traits, which is further interpreted by prevailing climatic conditions. Safeena *et al*. (2019) reported the presence of genotypic variation in post-harvest life of tuberose.

Tuberose concrete and absolute are much valued in the international market which is used as powerful modifier in floral accords that blends well with other scents. Among the tuberose genotypes tested, the concrete content was found to be the highest in Calcutta Single (0.097 %) followed by IIHR 17-23SP-08 (0.095 %) (Fig 1.). The results of the study confirms that the genotype IIHR 17-23SP-08 can be exploited for concrete extraction besides use as loose flowers which can be value added and used for garland making. The existence of genetic variation among the tuberose genotypes in terms of

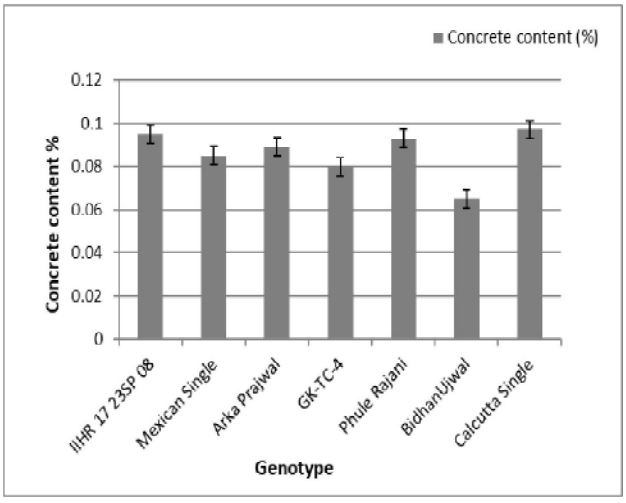

Fig 1 : Evaluation of tuberose genotypes for concrete content on fresh weight basis

concrete and absolute was reported by Chaudhary and Kumar (2017). The authors suggest that this trait may be considered as primary base for improvement programs especially for breeding tuberose varieties with high concrete content. Similar results on variation in concrete and essential oil yield among landraces were reported by Tabaei-aghdaei *et al*. (2002) in rose.

The tuberose genotype IIHR 17-23SP-08 was screened for the tolerance/resistance to root knot nematode (*M. incognita*) under field condition for two consecutive years and pooled analysis revealed that it was highly resistant under field conditions with minimal gall index of 1.31 (Table 3). Genotypic variations towards root knot nematode infestation in tuberose might be due to the genetic

Table 3 : Evaluation of tuberose genotypeIIHR-23 SP 08 with checks for leaf burn disease incidence under field condition

Genotype	Screening for leaf burn disease*	Root knot nematode Screening**
IIHR-23 SP 08	9.79 (18.24)	1.31
Mexican Single	19.20 (26.00)	2.42
Arka Prajwal	21.33 (27.52)	2.14
GK-TC-4	15.23 (22.98)	1.68
Phule Rajani	23.59 (29.06)	1.53
Bidhan Ujwal	21.10 (27.36)	1.38
Calcutta Single	13.00 (21.14)	1.51
SEm±	-	0.11
CD (P=0.05)	-	0.34
CV (%)	-	10.95

*Disease severity scale (Narayanappa and Chandra,1984); **Gall index (Taylor and Sasser,1978); Figures in parenthesis are *arc sine* transformed values

makeup of the particular genotype as reported by Gandhi *et al*. (2019) in tuberose.

The per cent disease index and host reaction of tuberose genotypes against leaf burn disease caused by *A. polianthi* under field conditions was recorded for two years. The results indicated that the tuberose genotype IIHR 17 23SP 08 was tolerant to leaf burn disease as compared to commercial check Arka Prajwal and local check Mexican Single (Table 3). The results are in line with the findings of Mazumdar *et al*. (2021) in tuberose who has observed the genetic inherent variation among the genotypes for *A. polianthi* leaf burn disease.

The quality traits of tuberose genotype IIHR 17 23SP 08 (Fig. 2) along with other genotypes have been presented in the Table 4. All the tuberose genotypes under study belong to single type. The flower/bud size was medium in IIHR 17 23SP 08 and GK-TC-4, large in Arka Prajwal and small in Mexican Single, Phule Rajani, Bidhan Ujwal and Calcutta Single. The tinge on the tip of the bud was green in all the genotypes except Arka Prajwal.

Flower spikes of IIHR 17 23SP 08

Fully opened medium size flower

Matured buds with green tinge on the tip

Fig. 2 : Tuberose genotype IIHR 17 23SP 08 (IC0642158)

Table 4 : Quality traits of tuberose genotype IIHR 17 23SP 08 (IC0642158) with checks

Genotype	Flower type	Flower/bud size	Tinge on bud	Nature of spike
IIHR 17 23SP 08	Single	Medium	Green	Straight
Mexican Single	Single	Small	Green	Slight bent
Arka Prajwal	Single	Large	Pink	Straight
GK-TC-4	Single	Medium	Green	Straight
Phule Rajani	Single	Small	Green	Straight
Bidhan Ujwal	Single	Small	Green	Crooked
Calcutta Single	Single	Small	Green	Slight bent

CONCLUSION

On the basis of two years of evaluation of seven genotypes for growth, flowering, flower, bulb, concrete yield and biotic stresses, the tuberose genotype IIHR 17-23SP-08 was found promising and novel for its single type medium size flowers having white (RHS colour: NNI55D, white group, Fan 4) flower buds with green tinge on the tip, more number of flower buds per kg (approx. 725), more number of spikes (8.47) and bulbs (8.94) per clump per year and high loose flower yield (18.88 t/ha/year). It has resistance to root knot nematode and is tolerant to leaf burn disease under field condition. Based on the study, the genotype IIHR 17-23SP-08 can be recommended as loose flower for garland purpose and for concrete extraction.

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Original Research Paper

Exploring genetic diversity of Dahlia (*Dahlia variabilis* Desf.) germplasm using multivariate statistics

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ABSTRACT

Dahlia (*Dahlia variabilis*) is a tuberous-rooted flower crop, exhibiting rich diversity in flower color and inflorescence form. The study was conducted to quantify diversity in 24 dahlia genotypes based on agronomic traits. The dahlia accessions were grouped based on their similarity for phenotypic resemblance following hierarchal clustering and principal component analysis (PCA). The hierarchical cluster analysis grouped the dahlia accessions into three distinct clusters *viz.*, C1, C2 and C3 comprising 8, 3 and 13 genotypes, respectively. The 24 dahlia genotypes were found scattered across the whole variation observed by PC1 and PC2 (explaining nearly 55.2% of the cumulative total variation). The two-dimensional PCA analysis revealed that the most appropriate traits for grouping the dahlia accessions were plant height, flower weight, stalk length, vase life and number of flowers per plant. The study signifies the importance of germplasm collection, characterization and utilization of dahlia to popularize its commercial cultivation among the flower growers.

Keywords : Characterization, dahlia, diversity index, germplasm, hierarchal clustering, principal component analysis

INTRODUCTION

Dahlia (*Dahlia variabilis* Desf.) is a tuberous-rooted herbaceous ornamental plant, cultivated for cut flowers and potted flowering plant (De Hertogh, 1996). The genus *Dahlia* comprises 35 species native to Mexico and parts of Central and South America (Slade, 2018). Recent documentation stated 35 wild species of dahlia are endemic to Mexico (Carrasco-Ortiz *et al*., 2019). However, the American Dahlia Society (ADS) has mentioned 42 species (ADS, 2020). It is believed that only four of the species namely, *D. coccinea* Cav., *D. pinnata* Cav., *D. merckii* Lehm. and *D. imperialis* Roezl have constituted the genetic basis for the evolution and development of modern-day dahlia cultivars (Gatt *et al.,* 2000; Hansen and Hjerting, 2008).

Dahlia flowers are appreciated worldwide for their long-lasting majestic blooms exhibiting diversity in flower color, inflorescence form and size, ranging from miniatures (<2.5 cm), to giant (>40 cm). Dahlia is commercially cultivated in Mexico, Japan, France, South Africa, Italy, Germany and the United States with a significant area (400 ha) of bulb production in Netherlands (Priyanka *et al.*, 2017). In India, the commercial cultivation of dahlia is limited to the plains of North-West and Central regions for the domestic flower market. Societies such as American Dahlia Society (ADS), USA; National Dahlia Society (NDS), UK; Dahlia Society of Australia and National Dahlia Society of New Zealand have been collecting and maintaining dahlia germplasm for breeding purpose (Behr and Debener, 2004).

The presence of genetic variability is a pre-requisite for a breeder to evolve varieties exhibiting novel characteristics. The source of variation includes local landraces, exotic germplasm collections, hybrids, improved varieties and mutants evolved as a result of spontaneous or induced mutations. The aesthetic quality traits representing novel flower colour, inflorescence form, uniformity and profuse flowering, longer flower duration, stem sturdiness with long vase life and floral scent hold considerable significance and forms a major breeding objective for the dahlia breeders (Marina, 2015; Dalda Şekercil and Gülşen, 2016).

The characterization of germplasm based on flower color, inflorescence form and other agronomically important traits is essential to explore the potential parentage for breeding Dahlia. The present study was carried out to analyze variability in Dahlia germplasm for inflorescence characteristics and field-based

agronomic traits through multivariate data analysis statistical tools (Rencher, 2002). The results of multivariate statistical analysis of germplasm generates a relevant and precise information on morphological diversity in different traits of dahlia germplasm, that will help in the selection of desirable accessions for utilization in future breeding programs targeting a specific trait(s). The studies on these aspects is of substantial significance and will also help in promoting the cultivation of dahlia crop with small and marginal farmers, especially in developing countries, for diversification in their traditional cropping systems and for generating additional income.

MATERIALS AND METHODS

The experiment was conducted at the Research Farm, Department of Floriculture and Landscaping, Punjab Agricultural University (PAU), Ludhiana. Twenty-four dahlia genotypes comprising of six cultivars *viz.*, Earl Haig, First lady, Hammett 96, Ice berg, Sam Hopkins and Sister Nivedita and 18 genotypes collected from secondary sources especially from local nurseries and designated as accessions numbers (D1, D2…). Six rooted cuttings each of 24 genotypes were planted at 45 cm x 30 cm in randomized block design (RBD) replicated thrice. The recommended cultural practices pertaining to the crop were followed at different growth stages. Pinching was done when plants attain 4-5 pairs of leaves. Observations were recorded for 11 quantitative traits *viz*., plant height (cm), number of primary branches, number of flowers per plant, flower diameter (cm), flower weight (g), stalk length (cm), days to first bud emergence, days to full bloom, duration of flowering (days), days to flower withering and vase life (days).

Dahlia accessions were classified for different inflorescence forms and flower size as a qualitative descriptor and were assigned codes following the guidelines proposed by ADS and NDS. Data were statistically analyzed using IBM SPSS v22 statistical software (IBM Corp, 2013) and agricolae statistical package developed by De Mendiburu and Yaseen (2020).

RESULTS AND DISCUSSION

Vegetative and floral characteristics

Dahlia genotypes revealed significant (pd”0.05) differences for vegetative and floral characteristics (Table 1). Quantifying the variability present in the traits of agronomic importance help breeders and growers to make a selection of specific genotype(s) pertaining to their objective of breeding (Carrasco-Ortiz, 2019). The plant height ranged from 39.27 cm to 103.30 cm, genotype D8 recorded the maximum number of primary branches (4.67) and minimum in D2 (1.00). The variation in number of primary branches can be attributed due to pinching of terminal growing shoots (for breaking the apical dominance) to promote sprouting of lateral buds. The uniform plant architecture in dahlia is maintained by retaining 3-5 well spaced primary branches. The greater number of branches leads to higher leaf biomass that enhances the flower and tuber yield by regulating the source-sink relationship (Manjula *et al.,* 2017). However, the results revealed that the pinching of apical shoots delayed flowering by 5-10 days and the duration of the plants were longer as compared to non-pinched plants as also reported by Miller and Filios (2011) in Dahlia. The number of flowers per plant varied from 1.67 (D7) to 12.50 (D5), flower diameter ranged 12.45 cm (Earl Haig) to 20.23 cm (Ice Berg), flower stalk length ranged 15.10 cm - 58.40 cm indicating wide diversity in dahlia genotypes for varying length of flower stalks. Dahlia variety ‘Earl Haig’ and accession D9 took maximum (92.00) number of days to bud emergence. Days to full bloom varied from 74.70 to 112.40 days. The genotypes D 15 (57.33 days) and D1 (60.90 days) were found early flowering and at par with each other. The duration for days to flower withering ranged 93.60 days (D1) to 136.33 days (D17) to and vase life from 3.20 days (D9) to 6.30 days (D20). Dahlia genotypes exhibiting both early and late emergence of buds are advantageous to get the blooms earlier and later growing season, respectively (Priyanka *et al*., 2017). Selection of accessions initiating buds deviating from the peak season are preferred by the breeders to evolve varieties that can capture the market early (Hamrick, 2003). Surprisingly, 1/3rd of the accessions revealed variation for duration of flowering, which can be attributed due to recurrent blooming habit of dahlia, that continues to flower profusely when old and withered flowers are periodically removed from the plant (Romer and Nelson, 2008).

The information on the traits such as stalk length and vase life are important for breeding dahlia to evolve novel cut flower types with longer stem length and enhanced life of cut flower (Armitage, 1993). Among the genotypes tested, 14 accessions recorded longer vase life (>4 days), signifying the identification and

Table 1 : Performance of dahlia genotypes for growth and flowering parameters

Genotype	Plant height (cm)	No. of primary branches	No. of flower per plant	Flower diameter (cm)	Single flower weight (g)	Stalk length (cm)	Days to first bud emergence	Days to full bloom	Duration of flowering (days)	Days to withering	Vase life (days)
Earl Haig	49.20	1.50	4.50	12.45	22.70	34.55	92.00	111.00	39.66	131.66	3.20
First Lady	60.23	4.33	7.33	15.67	30.73	30.00	79.66	107.50	52.34	132.00	5.50
Hammett 96	63.97	2.00	4.67	20.00	54.67	29.63	80.66	112.40	47.00	127.66	3.80
Ice Berg	90.67	2.00	9.33	20.23	65.20	32.93	70.00	95.40	52.00	122.00	5.20
Sister Nivedita	74.93	1.33	6.33	17.57	36.23	18.17	62.00	85.00	65.33	127.33	5.50
Sam Hopkins	58.23	1.33	2.67	14.23	37.23	48.87	72.66	85.90	39.34	112.00	4.20
D 1	103.30	1.70	8.80	16.60	46.70	15.30	60.90	74.70	32.70	93.60	3.70
D 2	100.70	1.00	5.90	18.20	52.50	16.80	62.30	77.00	44.80	107.10	5.20
D 3	87.90	3.00	7.40	20.50	53.30	15.10	65.30	80.60	42.30	107.70	6.20
D 4	68.53	1.33	5.67	13.77	44.60	43.30	84.66	101.20	41.67	126.33	4.80
D 5	60.80	3.30	12.50	19.90	55.90	23.60	62.70	76.30	34.20	96.90	5.70
D 6	59.33	1.33	4.33	16.17	41.97	22.17	78.33	94.00	37.67	116.00	3.50
D 7	58.83	1.67	1.67	15.93	44.47	55.67	81.00	98.20	39.00	120.00	5.20
D 8	42.57	4.67	8.00	16.27	23.43	14.57	65.33	104.12	55.00	120.33	3.50
D 9	49.20	1.50	4.50	12.45	22.70	34.55	92.00	111.00	39.66	131.66	3.20
D 13	72.36	1.67	8.33	12.50	33.80	21.06	65.33	77.78	42.44	107.78	5.83
D 14	52.63	1.67	3.00	14.90	57.30	17.97	80.00	100.40	42.33	122.33	3.50
D 15	39.27	3.00	6.67	13.23	26.23	24.57	57.33	80.20	64.67	122.00	3.50
D 17	68.97	2.67	3.33	15.67	70.30	59.30	91.00	112.40	45.33	136.33	4.80
D 18	98.77	1.33	3.67	19.73	38.07	47.33	67.00	92.40	56.33	123.33	5.20
D 19	53.70	3.00	8.00	14.03	40.73	39.47	75.00	92.40	53.66	128.66	3.50
D 20	75.37	4.00	3.67	20.00	46.30	33.53	80.66	110.40	55.34	136.00	6.30
D 22	64.70	1.67	2.67	13.93	24.27	58.40	83.33	97.00	45.33	128.66	3.80
D 24	60.60	2.33	3.33	13.23	31.37	48.07	70.00	94.10	60.00	130.00	4.20
LSD ($p<0.05$)	12.59	2.02	4.01	1.54	8.13	11.94	8.41	9.33	7.35	7.35	0.71

selection of desirable parents for the hybridization programme. The stalk length is another important post-harvest trait relevant for breeding cut flower dahlia and the variation in stalk length might due to the genetic make of the genotypes. The length of stem is considered as an oligogenic trait, governed by few genes, whereas the flower color is considered as a polygenic trait.

Dahlia genotypes revealed variation with respect to inflorescence type and days to flowering duration. The inflorescence of dahlia was classified based on the guidelines proposed by ADS and NDS classification system (Table 2) with their respective size codes. The inflorescence forms were classified as Formal Decorative (FDi), Informal Decorative (ID), Fimbriated (Fim.), Single (S), Stellar (ST), Collarette (CO), Peony (PE) and Double Orchid (DO). The DO form of inflorescence recorded highest diameter of flower (19.90 cm) followed by Fim (16.73 cm) and ID (16.62 cm) types, while CO group recorded smallest flower diameter (13.77 cm). The variation in flower diameter is important from breeding perspective as the blooms with larger and more number of petals tend to offset selfing with greater probability of out-crossing. The increase in petallage number (doubleness of flowers) and length act as a barrier for the transfer of pollen from disc florets to petal stigmas, thus increasing the chances of out-crossing (Vinanyananda, 1993; Phetpradap, 1992). The count of ray and disc florets significantly affects the weight of flower which in turn is influenced by the variation in temperature. Generally, a night temperature of 16-21^{o}C resulted in increase in the number of ray and disc florets (Moser and Hess, 1968). The inflorescence forms of dahlia were accorded *per cent* values computed against the total number of accessions evaluated. Around 41.6% accessions exhibited FDi form of inflorescence followed by 20.8% accessions revealing ID. Around 4.16% of the total accessions revealed each of the ST, PE, CO and DO forms of inflorescence. The ST form of inflorescence was not designated under NDS guidelines.

The ADS recognizes 18 types of inflorescence forms of dahlia and 9 classes for flower size ranging from 2 to over 10 inches in diameter. The type of inflorescence also determines the breeding system in dahlia that determines its potential to set seeds. Most of the dahlia are believed to be cross compatible (where bees and ants facilitate the cross pollination) with fewer (<25%) cultivars being self-compatible (Behr and Debener, 2004). The current breeding programmmes aim for evolving dahlia suitable for cut flower with novel

Table 2 : Classification of 24 dahlia accessions based on inflorescence form and size

Genotype (Nos.)	Accessions/ genotype	Classification based on ADS guidelines			Classification based on NDS guidelines		
		Inflorescence description					
		Average diameter (cm)	Form	Abbr. codes	Form	Abbr. codes	% of total accessions
10	Earl Haig, First Lady, Hammett 96, Sister Nivedita, D1, D2, D7, D8, D9, D14	16.18	Formal Decorative	FDi	Decorative, small	SD	41.6
5	Ice berg, Sam Hopkins, D4, D5, D6	16.62	Informal Decorative	ID	Decorative, medium	MD	20.8
3	D3, D15, D18	16.73	Fimbriated	FIM.	Fimbriated	FIM.	12.5
1	D17	15.93	Stellar	ST	-	-	4.16
1	D19	15.67	Peony	PE	Paeony	Pae.	4.16
1	D13	13.77	Collarette	CO	Collarette	Col.	4.16
1	D20	19.90	Double orchid	DO	Decorative, medium	MD	4.16
2	D22, D24	14.85	Single	S	Single	Sin.	8.33

ADS- American Dahlia Society, USA; NDS-National Dahlia Society, UK

flower color and inflorescence form accompanying variation in foliage architecture and foliage color as well (Hammett, 2009).

Correlation coefficients and principal component analysis

The simple correlation coefficients computed for the quantitative descriptors revealed highly significant ($p<0.05$) positive correlations (Table 3) for the growth and yield parameters. Plant height recorded significant positive correlation with flower diameter and single flower weight. Number of flowers per plant registered significant and negative association with stalk length, days to bud emergence, days to full bloom and days to withering. Flower diameter had significant positive correlation with single flower weight and stalk length had significant and positive association with days to first bud emergence, days to full bloom and days to withering. Days to first bud emergence revealed a negative correlation with number of flowers per plant followed by plant height, flower diameter and number of primary branches, but was found significantly positively correlated with stalk length. Days to full bloom was found significantly positively correlated with days to first bud emergence followed by stalk length. Negative correlation of days to full bloom was observed with number of flowers per plant followed by plant height.

Duration of flowering was found positively correlated with number of primary branches followed by days to full bloom and flower diameter. However, it was found negatively correlated with days to first bud emergence, individual flower weight and plant height. Days to flower withering was found significantly positively correlated with days to full bloom, days to first bud emergence and duration of flowering. It was found negatively correlated with number of flowers per plant, plant height, flower diameter and individual flower weight. The vase life showed significantly positive correlation with plant height, flower diameter, single flower weight, number of flowers per plant and duration of flowering, but was found negatively correlated with days to first bud emergence and days to full bloom followed by days to flower withering. Similar results were also obtained by Lal *et al.* (1982) in rose flower diameter, and Sirohi and Behera (2000) chrysanthemum vase life. Correlation analysis revealed that selection of characters that are positively correlated can lead to concomitant increase in either of the traits and is a potentially feasible tool to selection of dahlia genotypes for planned hybridization programmes.

To identify the most influential traits governing the greater proportion of variation, multivariate statistical analysis was performed to delimit the large number of variables using PCA analysis. Four PCs explained 82.4% of total variation with lowest component variance (0.104) recorded in PC4. The PC-1 accounted for 37.1% of total variation that included flower retention and longevity traits (number of flowers, plant height, flower diameter and vase life). The traits pertaining to flowering such as duration of flowering, number of flowers, and number of primary branches contributed to PC-2 revealing 18.1% of total variability. The PC-3 described 16.8% of the total variation exhibited, largely contributed by vegetative and post-harvest characters like number of primary branches, Duration of flowering and vase Life. Around 10.4% of the total variation was addressed by PC-4 primarily dominated by plant height, stalk length and duration of flowering (Table 4).

The PCA analysis revealed that the most appropriate traits for grouping dahlia genotypes were plant height, flower weight, stalk length, vase life and number of flowers (contributing for ten genotypes), days to first bud appearance was found shared by 5 genotypes and traits comprising duration of flowering and number of primary branches were governed by five genotypes. It can be inferred that the PC1 and PC2 were best PCs suggesting these as a good reference for aiding the selection of genotypes for future breeding programs.

Hierarchical cluster analysis

Hierarchical cluster analysis aided in grouping of 24 dahlia genotypes into three distinct clusters *viz*., C1, C2 and C3. Cluster C1 comprised eight dahlia genotypes namely D1, D2, D3, D5, D13, D18, Sister Nivedita and Ice berg, whereas, three genotypes (D8, D15 and D19) formed cluster C2. Cluster C3 was observed largest of the three clusters consisting of 13 genotypes (D4, D6, D7, D9, D14, D17, D20, D22, D24, First Lady, Earl

Table 3 : Pearson's correlation between 11 morphological traits of Dahlia accessions

Parameter	Plant height (cm)	No. of primary branches	No. of flower/ plant	Flower diameter (cm)	Single flower weight (g)	Stalk length (cm)	Days to first bud emergence	Days to full bloom	Duration of flowering (days)	Days to flower wither ing	Vase life (days)
Plant height (cm)	1										
No. of primary branches	-0.308	1									
No. of flower/plant	0.169	0.376	1								
Flower diameter (cm)	0.589**	0.201	0.281	1							
Single flower weight (g)	0.474*	-0.061	0.142	0.608**	1						
Stalk length (cm)	-0.129	-0.173	-0.596**	-0.268	-0.033	1					
Days to first bud emergence	-0.356	-0.118	-0.563**	-0.336	-0.026	0.552**	1				
Days to full bloom	-0.403	0.207	-0.486*	-0.125	-0.071	0.408*	0.845**	1			
Duration of flowering (days)	-0.100	0.334	-0.039	0.064	-0.249	0.015	-0.304	0.081	1		
Days to flower withering	-0.399	0.157	-0.538**	-0.252	-0.219	0.509*	0.663**	0.825**	0.513*	1	
Vase life (days)	0.533**	0.192	0.235	0.563**	0.388	-0.050	-0.282	-0.258	0.102	-0.173	1

* Significant at p=0.05, ** significant at p=0.01

Table 4 : The representation of variability from first four PCs from PCA analysis of eleven quantitative traits in dahlia accessions

Principal components (PCs)	Plant height (cm)	No. of primary branches	No. of flowers/ plant	Flower diameter (cm)	Weight of flower (g)	Stalk length (cm)	Days to 1st bud emergence	Days to full bloom	Duration of flowering (days)	Days to with-ering	Vase life (days)	Variabi-lity (%)	Cumu-lative (%)
PC1	0.317	0.022	0.344	0.300	0.205	-0.306	-0.404	-0.395	-0.054	-0.406	0.260	0.371	0.371
PC2	-0.390	0.180	0.254	-0.373	-0.497	-0.304	-0.275	-0.213	0.152	-0.129	-0.333	0.181	0.552
PC3	-0.080	0.580	0.149	0.288	0.013	-0.058	-0.094	0.226	0.541	0.341	0.280	0.168	0.720
PC4	0.279	-0.379	-0.337	-0.099	-0.271	0.256	-0.320	-0.296	0.537	0.134	0.142	0.104	0.824

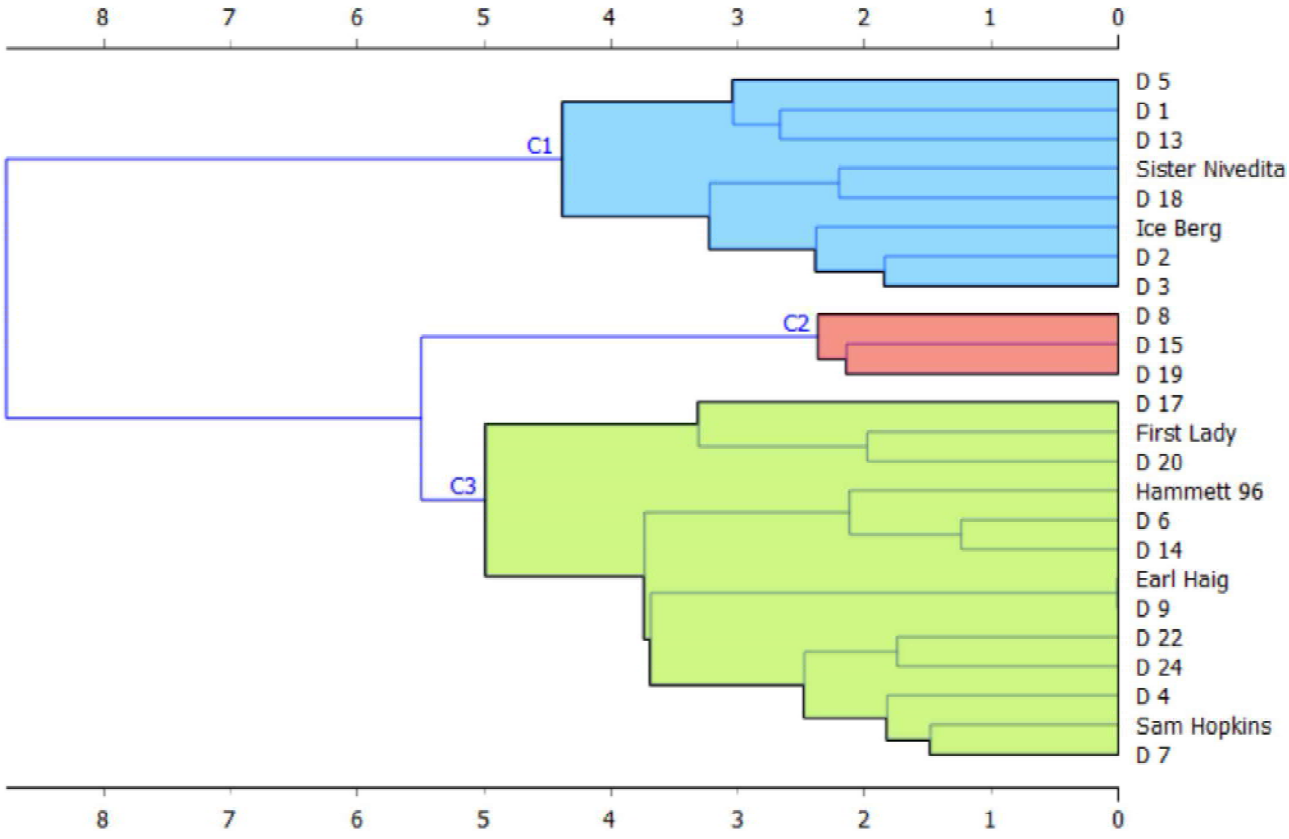

Fig. 1 : Dendrogram presenting genetic relationship among 24 Dahlia accessions

Haig, Hammett 96 and Sam Hopkins) (Fig. 1). The 3 clusters were also found in near perfect accordance with PCs, PC1 and PC2 (contributing 55.2% of the total variability) represented by 2-D plots based on performance of accessions for various quantitative traits (Fig. 2). The genotypes D4, D7, D14, D20, D22 and Hammett 96 from cluster C3 revealed no variation for both PC1 and PC2, whereas, genotypes D22 from C3 cluster reported minimal variation for both the PC components. The genotype D17 (termed as outlier) reported negative correlation for both the PCs. The genotypes D8 and D15 from cluster C2 reported variation of higher magnitude for PC2 and contributed minimal variation for PC1. The genotypes D2, D3, D18 and Ice berg from the cluster C1 reported positive variance for PC1.

The large variation was recorded by flower weight, number of flowers, days to 1st bud emergence, flower diameter and plant height as mentioned by the relative length of the vectors in the biplot diagram (Fig. 3). The biplot also revealed the trait relationship and positive association among the flower weight, plant height, flower diameter, vase life and days to withering, days to first bud emergence and stalk length as indicated by the acute angle. The biplot representing clustering of dahlia accession for different traits revealed interesting results that were found adhering to cluster analysis (Fig. 3). The genotypes from cluster C3 revealed similarity for days to flower withering, days to first bud appearance, and stalk length. The accessions from cluster C2 exhibited similarity for number of primary branches and duration of flowering. The accessions from C1 cluster were observed similarity for traits such as plant height and number of flowers. The accessions from the cluster C3 such as D4, First Lady, Sam Hopkins and D14 are similar and closer to the center and may not have environmental influence. The genotypes in the cluster C2 exhibited the highest yield and associated traits such as number of flowers per plant, flower weight, plant height and vase life. These genotypes can be selected as parent for the future breeding programme to develop high yielding cut flower varieties with better vase life.

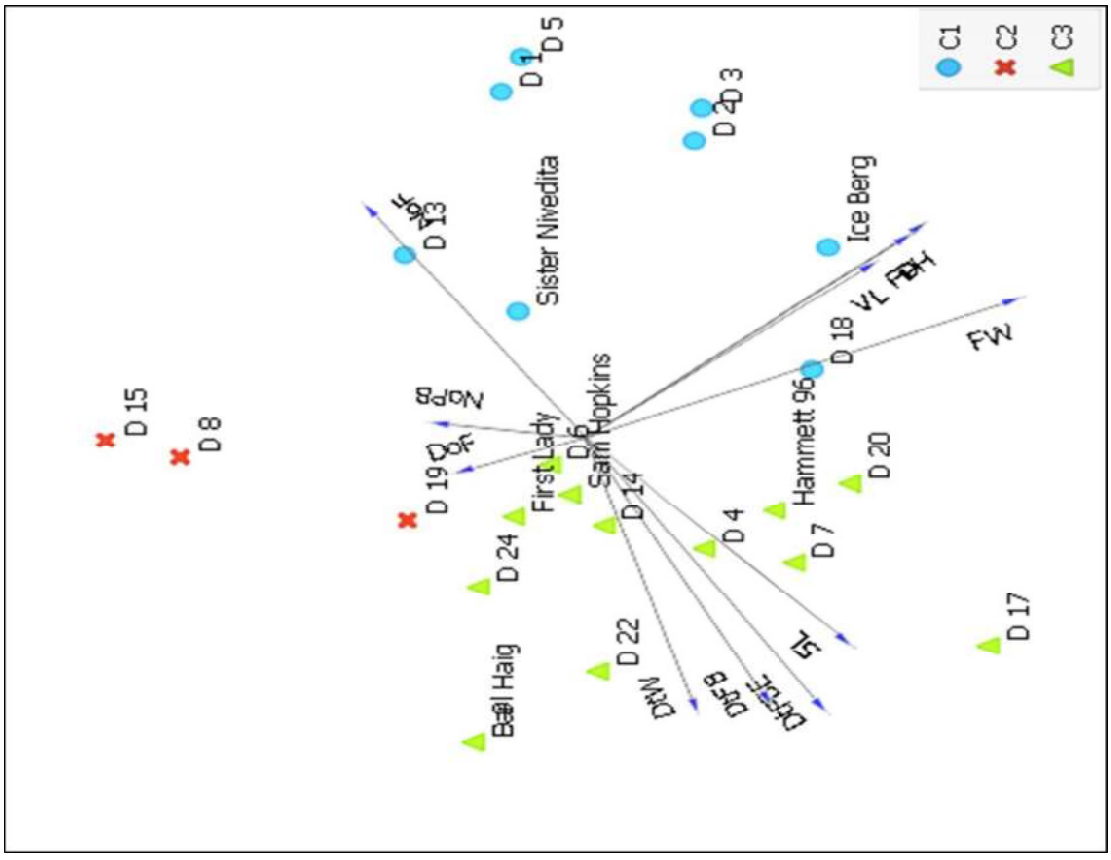

Fig. 3 : Biplot presenting the variable projection of the 24 Dahlia accessions characterized by mean plant height, number of flowers per plant, duration of flowering, number of primary branches and days to first bud appearance. [PH- plant height; NoPB- number of primary branches; NoF- number of flowers; FD- flower diameter, FW- flower weight; SL- stalk length; DtFBE- days to first bud emergence; DtFB - days to full bloom; DoF- duration of flowering; DtFW- days to flower withering; VL- vase life]

Fig. 2 : Scatter diagram for 1st and 2nd PCs for 11 morphological traits in 24 Dahlia accessions

CONCLUSION

The study undertaken describes the morphological assessment of dahlia germplasm based on multivariate statistical methods. The perusal of data presented for agronomic traits will help to characterize the germplasm collection and also aid in selection of suitable lines catering to specific breeding objectives. Our study was able to discriminate different dahlia accessions based on the variability in quantitative and qualitative traits, which would help breeders and growers to make a selection of specific genotype pertaining to their breeding objective.

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Original Research Paper

Assessment of genetic variability, character association and path coefficient analysis in Chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev)

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ABSTRACT

Thirty-one genotypes of chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev) were evaluated for nine growth and flowering related traits to assess the genetic variability, correlation and path coefficient analysis. Significant differences among genotypes for all the growth and flowering related traits were observed through analysis of variance. The range of variation was high for number of leaves plant^{-1} (66.17-164.50) followed by number of flowers plant^{-1} (30.67-116.83). The magnitude of phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the characters studied. High (>20%) PCV and GCV was recorded for plant height, number of branches plant^{-1}, number of leaves plant^{-1}, days to bud initiation, days to first flower opening and number of flowers plant^{-1}. Heritability estimates ranged from 77.72% (days to optimum flowering) to 96.93% (number of flowers plant^{-1}). High heritability coupled with high genetic advance as *per cent* of mean was recorded for all the traits studied. Number of flowers plant^{-1} exhibited positive and highly significant correlation with number of branches and leaves plant^{-1}. Path coefficient analysis using correlation coefficients revealed that days to first flower opening (1.564) exhibited positive and very high direct effect, while,number of leaves plant^{-1} (0.347) and flower diameter (0.337) showed positive and high direct effect. Hence, genotypes with superior traits may be considered for further improvement.

Keywords : Chrysanthemum, correlation and path coefficient, genetic variability, heritability

INTRODUCTION

Chrysanthemum (*Dendranthema* x *grandiflora* Tzvelev) is popularly known as '*Guldaudi*' in India and 'Glory of the East' or 'Mum' in USA. It belongs to the family Asteraceae and native to Northern Hemisphere, chiefly Europe and Asia. It is one of the important floriculture crops in the world and ranks second next to rose. It is used as a cut flower, potted plant, and herbaceous perennial, and has been grown in garden for more than 2500 years (Vijayakumari *et al*., 2019). In India, small flowered chrysanthemum is used for making garlands, *venis*, *gajaras* and in religious offerings. There has been increase in the demand for potted chrysanthemum due to its suitability as potted plant in the last few years (Abrol *et al*., 2018). It is a short-day plant; critical photoperiod is ≥13.5 h for vegetative growth and ≤12 h for reproductive development (Cockshull, 1985).

Crop improvement depends on magnitude of genetic variability and its nature and association among key traits for efficient selection. The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) helps in determining the amount of variability (Allard, 1960). Heritability estimates the relative influence of environment on expression of genotypes. Genetic advance gives an idea about the expected genetic changes, and for efficient selection, high heritability along with high genetic advance can be used (Johnson *et al*., 1955).

Studies on correlation coefficients between various desirable traits would be helpful in indirect selection of desirable traits for crop improvement. The path coefficient analysis is highly effective method to simplify the complex interactions among various traits which reveals the direct and indirect causes of such interactions. Thus, the present study was carried out to assess the genetic parameters of variability, correlation coefficients and path coefficient which would be of great significance in selection of parents for formulating appropriate breeding programme in chrysanthemum.

MATERIALS AND METHODS

The present study was carried out in the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru, during 2019-20 and 2020-21. The experimental site was geographically located at 13°58' N Latitude, 78°E Longitude and at an elevation of 890 meter above mean sea level. The experiment was carried out to evaluate thirty-one chrysanthemum genotypes for growth and flowering traits under naturally ventilated polyhouse in completely randomized design (CRD) with three replications. The 31 genotypes used as experimental material were A1 Collection, Appu, Arka Chandrakant, Arka Chankdrika, Arka Kirti, Arka Pink Star, Arka Usha Kiran, Arka Yellow Gold, Autumn Joy, Coffee, Fitonia, Flirt, Garden Beauty, Gulmohar, Heritage, Jublee, Marigold, Mayur, NBRI Little Kusum, Pachai Local, Pink Cloud, Ratlam Selection, Rekha, Shukla, Statesman, Sunil, Vasanthika, White Dolley, White Local, White Prolific and Winter Queen.

The plants of all genotypes were raised through terminal cuttings taken from healthy stock plants. After transplanting, plants were imposed with photoperiod of 15/9 hours for 30 days after transplanting and black in (dark conditions) until flower bud initiation. Uniform package of practices was followed throughout the experiment to ensure good growth. Five uniformly grown plants per replication were tagged for recording observations for various growth and flowering traits, *viz*., plant height (cm), number of branches per plant, number of leaves per plant, days to bud initiation, days to first flower opening, number of flowers per plant, optimum flowering, flower diameter (cm) and flowering duration (days). The collected data of both the years were pooled and analyzed statistically.

The analysis of variance for each character was carried out as suggested by Panse and Sukhatme (1985). The genotypic and phenotypic coefficients of variance were calculated as suggested by Burton and De vane (1953) and heritability (broad sense), genetic advance and genetic gain were calculated by the formula given by Johnson *et al*. (1955). The correlations were calculated as per Al-Jibouri *et al*. (1958) and genotypic correlation coefficient was further partitioned into direct and indirect effect with the help of path coefficient analysis as elaborated by Dewey and Lu (1959).

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences among the genotypes for various growth and flowering characters (Table 1). This infers that among the genotypes, wide range of variability exists and substantial improvement in this crop is possible through selection.

Estimation of genetic parameters for growth and flowering traits

The extent of variability *i.e*. mean, range, mean, and estimates of genetic parameters such as phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (broad sense) and genetic advance, genetic advance as *per cent* of mean for various traits present in chrysanthemum genotypes studied are presented in Table 2.

The range of variation was high for number of leaves plant^{-1} (66.17-164.50) followed by number of flowers plant^{-1}(30.67-116.83) and days to first flower opening (31.00-88.33). The magnitude of phenotypic coefficient of variation was higher than the genotypic coefficient of variation for all the characters studied, however, difference among GCV and PCV was narrow. This indicates that phenotypic expression of genotypes may be genetically controlled and environment has slight influence, implying that phenotypic variability could be a reliable measure of genotypic variability. Similar results were also reported by Kumari *et al*. (2017) in China aster and Bennurmath *et al*. (2018) in Chrysanthemum. Higher

Table 1 : Analysis of variance (ANOVA) for morphological traits in chrysanthemum

Source of variation	DF	Plant height (cm)	Number of branches plant^{-1}	Number of leaves plant^{-1}	plant^{-1} bud initiation	Days to first flower opening	Days to optimum flowering	Flower diameter (cm)	Number of flowers plant^{-1}	Flowering duration (days)
Treatment	30	8,230.95	58.67 **	13,872.46 **	471.52 **	3,867.80 **	3,852.28 **	22.18 **	9,507.31 **	9,502.86 **
Error	62	0.25	12.81	10.19	1.00	4.74	6.02	4.22	0.08	5.29

Table 2 : Genetic parameters for various growth and flowering traits in chrysanthemum

Trait	Mean	Range		Coefficient of Variation (%)	GCV (%)	PCV (%)	Heritability (%)	Genetic Advance	Genetic advance as per cent mean
		Minimum	Maximum						
Plant height (cm)	59.09	26.92	103.27	7.93	31.12	32.12	93.90	36.71	62.12
Number of branches plant^{-1}	6.35	3.33	9.67	6.92	26.27	27.17	93.51	3.33	52.33
Number of leaves plant^{-1}	99.08	66.17	164.50	7.85	24.55	25.77	90.71	47.71	48.16
Days to bud initiation	21.06	11.17	32.00	7.20	20.85	22.06	89.34	8.55	40.60
Days to first flower opening	59.4	31.00	88.33	6.98	20.45	21.61	89.57	23.68	39.87
Days to optimum flowering	76.23	51.33	104.50	8.04	15.02	17.04	77.72	20.80	27.28
Number of flowers plant^{-1}	63.95	30.67	116.83	6.39	35.92	36.48	96.93	46.58	72.85
Flower diameter (cm)	5.34	3.67	6.90	7.63	18.51	20.02	85.46	1.88	35.24
Flowering duration (days)	44.43	31.17	61.17	8.02	16.41	18.27	80.71	13.49	30.37

GCV: genotypic coefficient of variation; PCV: phenotypic coefficient of variation

phenotypic and genotypic coefficients of variation were recorded for number of flowers plant^{-1}(36.48% and 35.92%), plant height (32.12% and 31.12%), number of branches plant^{-1}(27.17% and 26.27%), number of leaves plant^{-1} (25.77% and 24.55%), days to bud initiation (22.06% and 20.85%) and days to bud first flower opening (21.61% and 20.45%), respectively. For flower diameter, PCV estimates was found to be high (20.02%), and a moderate GCV (18.51%), while, days to optimum flowering (17.04% and 15.02%) and flowering duration (18.27% and 16.41%) showed moderate estimates of PCV and GCV, respectively. Similar results of higher estimates of PCV and GCV were reported in chrysanthemum (Telem *et al*., 2017 and Henny *et al*., 2021), gaillardia (Arulmani *et al*., 2015) and China aster (Rai *et al*., 2017, Bhargav *et al*., 2019 and Nataraj *et al*., 2021).

The genotypic coefficient of variation alone is not enough to measure the heritable variations present among the genotypes. To get the best picture of the amount of advance to be expected from the selections, it should be considered in conjunction with heritability estimates (Burton, 1952). However, for more reliable conclusions, heritability estimates along with genetic gain are more meaningful in predicting the best individual for selection than the heritability value alone (Johnson *et al*. 1955). The heritability estimates for all characters were high (>80%), ranging from 77.72% (days to optimum flowering) to 96.93% (number of flowers plant^{-1}). The genetic advance ranged from 1.88 (flower diameter) to 47.71 (number of leavesplant^{-1}). All the traits had genetic advance as per cent mean estimates of more than 20%, and ranged from 27.28% (days to optimum flowering) to 72.85% (number of flowers plant^{-1}). High values of heritability estimates supplemented with greater genetic gains are indicative of additive gene effects (Narayan *et al*., 1996); therefore, this offers ample scope for efficient selection. High heritability coupled with high expected genetic advance was observed for number of flowers per plant^{-1} and number of branches plant^{-1} (Telem *et al*., 2017), and flower diameter in chrysanthemum (Henny *et al*., 2021) and days to 50% flowering in China aster (Kumari *et al*., 2017 and Nataraj *et al*., 2021).

Phenotypic and genotypic correlation coefficients for various traits

Phenotypic and genotypic correlation analysis is the biometrical technique used to find out the nature and degree of association of traits, prevailing between highly heritable with most economic characters (Khangjarakpam *et al*., 2015). It gives better understanding of the contribution of trait to the genetic make-up of a crop and helps in making indirect

selection for improvement of economically important traits. The high positive correlation between the traits shows that selection for improvement of one character results in the improvement of the other and could be useful in developing an effective selection strategy.

Correlation coefficients among different traits have been worked out and presented in Table 3. In general, the genotypic correlation coefficients were higher than phenotypic correlation coefficients, which may be due to interaction of genotypes with the environment. In the present study, number of flowers per plant has been taken as dependent variable, whereas, remaining eight characters were considered as independent variables contributing towards number of flowers per plant.

The results of correlation coefficient revealed that the number of flowers plant^{-1}exhibited genotypic positive and highly significant correlation with number of branches plant^{-1} (0.415) and number of leaves plant^{-1}(0.392), therefore, there is a scope for direct selection of these characters for improvement in number of flowers plant^{-1}. A correlation study suggests that the genotype having higher number of flowers per plant would also possess a greater number of branches and number of leaves plant^{-1}. Significant and positive correlation of number of flowers per plant with plant height and number of branches in China aster (Sreenivasulu *et al.*, 2007) and chrysanthemum (Khangjarakpam *et al*., 2015 and Telem *et al*., 2017) have been reported.

Plant height exhibited positive and highly significant association with days to bud initiation (0.580), days to first flower opening (0.674), days to optimum flowering (0.599), flower diameter (0.510) and flowering duration (0.521). Positive significant correlation of plant height with flower size in chrysanthemum (Raghava *et al*., 1992) and with days to 50% flowering in China aster (Khangjarakpam *et al*. (2015) has been reported. This leads to the conclusion that the selection of taller plants results in early bud initiation, first flower opening, optimum flowering, maximum flower diameter and longer flowering duration. Therefore, direct selection of this character results in higher flower yield.

Table 3 : Genotypic and phenotypic correlation coefficients for various growth and flowering traits in chrysanthemum

Trait		Plant height (cm)	Number of branches plant^{-1}	Number of leaves plant^{-1}	Days to bud initiation	Days to first flower opening	Days to optimum flowering	Flower diameter (cm)	Flowering duration (days)	Number of flowers plant^{-1}
Plant height (cm)	G	1.000	-0.045	-0.118	0.580**	0.674**	0.599**	0.510**	0.521**	-0.008
	P	1.000	-0.021	-0.117	0.580**	0.663**	0.588**	0.492**	0.501**	-0.008
Number of branches plant^{-1}	G		1.000	0.255*	-0.224*	0.104	0.085	-0.083	0.075	0.415**
	P		1.000	0.094	-0.106	0.072	0.056	-0.026	0.038	0.190
Number of leaves plant^{-1}	G			1.000	-0.274*	0.026	0.044	-0.249*	-0.063	0.392**
	P			1.000	-0.271*	0.022	0.039	-0.242*	-0.061	0.387**
Days to bud initiation	G				1.000	0.621**	0.628**	0.419**	0.324*	-0.209*
	P				1.000	0.612**	0.617**	0.404**	0.311*	-0.208*
Days to first flower opening	G					1.000	0.985**	0.346**	0.584**	0.087
	P					1.000	0.969**	0.332*	0.558**	0.086
Days to optimum flowering	G						1.000	0.301*	0.571**	0.057
	P						1.000	0.30*	0.542**	0.056
Flower diameter (cm)	G							1.000	0.361**	0.112
	P							1.000	0.338**	0.110
Flower duration (days)	G								1.000	-0.010
	P								1.000	-0.009
No. of flowers plant^{-1}	G									1.000
	P									1.000

Correlation r value at 5% = 0.2038; 1% = 0.3357; *Significant at 5% ; **Significant at 1%

The number of branches per plant exhibited positive significant correlation with number of leaves plant^{-1}(0.255), however, it showed negative and significant correlation with number of days to bud initiation (-0.224). Number of leaves plant^{-1}exhibited negative significant correlation with days to bud initiation (-0.274) and flower diameter (-0.249). Days to bud initiation exhibited positive and highly significant correlation with days to first flower opening (0.621), days to optimum flowering (0.628) and flower diameter (0.419), while, positive significant correlation with flowering duration (0.324). However, it showed negative and significant correlation with number of flowers plant^{-1}(-0.209). These results are in close agreement with the findings obtained by Poornima *et al*. (2007) in China aster and Panwar *et al*. (2013) in African marigold.

The days to first flower opening exhibited positive and highly significant association with days to optimum flowering (0.985), flower diameter (0.346) and flowering duration (0.584). Days to optimum flowering showed positive and highly significant association with flowering duration (0.571), while, positive significant correlation with flower diameter (0.324). Flower diameter exhibited positive and highly significant correlation with flowering duration (0.361). These results are in close agreement with the findings of Telem *et al*. (2017) in chrysanthemum and Khangjarakpam *et al*. (2015) in China aster.

Path coefficient analysis for various traits

Path coefficient analysis divides the association between two traits into direct and indirect effects. Considering number of flowers plant^{-1} to be a dependent trait, phenotypic and genotypic coefficients of correlation between number of flowers plant^{-1} and all other characters were further partitioned into direct and indirect effects (Table 4). The residual effect is 0.29, due to the characters not considered for the study.

On portioning the phenotypic correlation into direct and indirect effects, maximum positive and high direct effect on number of flowers plant^{-1}was recorded for days to first flower opening (0.625) followed by number of leaves plant^{-1}(0.353). Positive and moderate direct effect on flower diameter (0.299) and positive and low direct effect on number of branches plant^{-1}(0.111) were also recorded. Kumar *et al*. (2012) observed highest direct positive effect of number of primary branches plant^{-1} on number of flowers per plant in chrysanthemum at the phenotypic level. The high negative direct effect was recorded for number of flowers plant^{-1} through days to optimum flowering (-0.372). The negative direct effect was moderate for

Table 4 : Path coefficient analysis for various growth and flowering traits in chrysanthemum

Trait		Plant height (cm)	Number of branches plant^{-1}	Number of leaves plant^{-1}	Days to bud initiation	Days to first flower opening	Days to optimum flowering	Flower diameter (cm)	Flowering duration (days)	Number of flowers plant^{-1}
Plant height (cm)	P	**-0.084**	0.002	0.010	-0.049	-0.056	-0.050	-0.041	-0.042	-0.008
	G	**-0.234**	0.010	0.028	-0.136	-0.158	-0.140	-0.120	-0.122	-0.008
Number of branches plant^{-1}	P	-0.002	**0.111**	0.011	-0.012	0.008	0.006	-0.003	0.004	0.190
	G	-0.012	**0.267**	0.068	-0.060	0.028	0.023	-0.022	0.020	0.415
Number of leaves plant^{-1}	P	-0.041	0.033	**0.353**	-0.096	0.008	0.014	-0.085	-0.021	0.387
	G	-0.041	0.089	**0.347**	-0.095	0.009	0.015	-0.087	-0.022	0.392
Days to bud initiation	P	-0.170	0.031	0.079	**-0.293**	-0.179	-0.180	-0.118	-0.091	-0.208
	G	-0.094	0.036	0.044	**-0.162**	-0.101	-0.102	-0.068	-0.053	-0.209
Days to optimum flowering	P	0.415	0.045	0.014	0.383	**0.625**	0.606	0.207	0.349	0.086
	G	1.053	0.162	0.040	0.972	**1.564**	1.541	0.541	0.913	0.087
Number of flowers plant^{-1}	P	-0.219	-0.021	-0.014	-0.229	-0.360	**-0.372**	-0.112	-0.201	0.056
	G	-0.794	-0.113	-0.059	-0.832	-1.306	**-1.326**	-0.430	-0.757	0.057
Flower diameter (cm)	P	0.147	-0.008	-0.072	0.121	0.099	0.090	**0.299**	0.101	0.110
	G	0.172	-0.028	-0.084	0.141	0.117	0.109	**0.337**	0.122	0.112
Flowering duration (days)	P	-0.054	-0.004	0.007	-0.033	-0.060	-0.058	-0.036	**-0.107**	-0.009
	G	-0.058	-0.008	0.007	-0.036	-0.065	-0.064	-0.040	**-0.111**	-0.010

days to bud initiation (-0.293), low for flowering duration (-0.107) and negligible for plant height (-0.084). Kumar *et al*. (2012) observed highest direct negative effect on number of flowers plant^{-1} via plant height at the phenotypic level.

At the genotypic level, very high positive and direct contribution was recorded for days to first flower opening (1.564), high for number of leaves plant^{-1} (0.347), and flower diameter (0.337) and moderate for number of branches plant^{-1} (0.267). Kumar *et al*. (2012) showed positive direct effect of day to flowering on number of flowers plant^{-1} in chrysanthemum. However, days to optimum flowering (-1.326) had very high negative direct effect, while, plant height (-0.234) had moderate negative direct effect on number of flowers per plant. Days to bud initiation (-0.162) and flowering duration (-0.111) recorded low negative direct effect. Kumar *et al*. (2012) also observed highest direct negative effect on number of flowers per plant via days to flower bud initiation followed by plant height at flower bud initiation stage in chrysanthemum.

CONCLUSION

The study provides the actual information on contribution of the characters and thus forms the basis for selection of suitable characters to improve the flower yield. It may be suggested for yield in terms of number of flowers per plant, direct selection of traits such as days to first flower opening, number of leaves per plant, flower diameter and number of branches per plant may be effective in selection of Chrysanthemum.

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Original Research Paper

Assessment of genetic diversity in China aster [*Callistephus chinensis* (L.) Nees]

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ABSTRACT

China aster [*Callistephus chinensis* (L.) Nees] is a flowering annual mainly cultivated for loose flower and cut flower, bedding and pot culture. To assess the genetic diversity, 42 genotypes were evaluated for fourteen quantitative traits. The genotypes were found to be highly variable for the traits such as plant height, plant spread, flower stalk length, 100 flower weight, number of flowers per plant, weight of flowers per plant and flower yield per hectare. However, low variability was recorded for vase life and shelf life. The genotypes were broadly grouped into two clusters, which were further divided into cluster 1a, 1b and cluster 2a, 2b, respectively. All the genotypes in cluster 1a were vigorous and medium flowering, whereas, genotypes in cluster 1b were tall, erect, vigorous and late flowering. The cluster 2a comprises of the genotypes with short stature, small flower and early flowering, however, cluster 2b contains only two genotypes. In principal component analysis (PCA) PC1 was highly correlated to flower yield, weight of flowers/plant, flower stalk length and plant height and PC2 was highly positively correlated to shelf life and vase life and negatively correlated to 100 flower weight. The results suggested that the existing variation in China aster genotypes could be used for the development of trait-specific novel genotypes.

Keywords : China aster, cluster analysis, diversity, principal component analysis

INTRODUCTION

China aster [*Callistephus chinensis* (L.) Nees] is a diploid (2n=2x=18) flowering annual belonging to the family Asteraceae and is a native of China (Navalinskien *et al*., 2005). The genus *Callistephus* derives its name from two Greek words '*Kalistos*' and '*Stephos*' meaning 'most beautiful' and 'crown', respectively. In India, China aster ranks third among the flowering annuals after chrysanthemum and marigold (Chakraborty *et al*., 2019). China aster is winter season annual crop. It is commercially grown for loose andcut flower, which are used in flower decoration, preparation of bouquets and garlands. It is also used in landscape gardening as a bedding plant to provide colour break and mass effect. It is gaining popularity in India, because of ease in cultivation, array of colours and varied uses (Bhargav *et al*., 2016).

For any breeding programme, characterization and evaluation are crucial steps in developing a variety and further research. Cluster analysis and principal component analysis are two important parameters to determine the diversity of the crop. Considering the importance of the crop, the present investigation was carried out to assess the diversity among 42 genotypes of China aster based on the fourteen quantitative traits.

MATERIALS AND METHODS

The present study was conducted at the Division of Flower and Medicinal Crops, ICAR- Indian Institute of Horticultural Research, Bengaluru during 2015-16 and 2016-17. The experimental site was geographically located at 13° 58¹ N Latitude, 78°E Longitude and at an elevation of 890 m above mean sea level. The soil of experimental plot was red loamy with pH 7.35 and E.C. of 0.26 dsm^{-1}. A total of 42 genotypes including 21 varieties and 21 stabilized lines were evaluated for vegetative growth, flowering, yield and postharvest life in randomized complete block design with two replications. Twenty plants per replication were planted at a spacing of 30 x 30 cm

under open field conditions. The recommended agronomical practices were adopted to raise the crop. Five random plants were selected for recording the various quantitative traits *viz*., plant height (cm), number of leaves per plant, plant spread (cm), number of branches per plant, days to first flowering, flower stalk length (cm), flower head diameter (cm), 100 flowers weight (g), number of flowers per plant, weight of flowers per plant (g), duration of flowering (days), vase life (days) and shelf life (days).

Descriptive statistics (*e.g.*, range, standard deviation, mean, standard error of mean), clustering based on average linkage, and euclidian distance and principal component analysis (PCA) were calculated using XLSTAT (Addinsoft, 2017).

RESULTS AND DISCUSSION

The diversity among the China aster accessions for quantitative traits was high (Table 1). Wide range of variation was observed for most of the characters such as plant height (8.20-61.80 cm), plant spread (8.75-42.65 cm), flower stalk length (4.65-49.10 cm), 100 flower weight (105.00-548.25 g), number of flowers per plant (7.35-65.05), weight of flowers per plant (7.72-235.21 g), flower yield per hectare (6.48-197.57 q/ha). Highest variability was recorded for weight of flowers per plant, which directly represent the flower yield per hectare with a mean of 124.49 g and 104.57 q/ha, respectively having a C.V. of 48.65%. Minimum variability was observed for vase life (5.40-9.50 days) followed by shelf life (2.35-4.42 days) with a mean of 7.06 (C.V. 14.26%) and 3.42 days (C.V. 14.37%), respectively. Presence of such high genetic variability among the genotypes for these parameters will form the basis for effective selection of superior genotypes in China aster. Such wide variability for many quantitative traits was also reported by Gupta and Dutta (2005) and Banerji *et al*. (2012) in chrysanthemum, and Kumar *et al*. (2014) in bougainvillea.

Cluster analysis was carried out to distinguish possible groups among the populations using Ward method (Fig. 1). The Agglomerative hierarchical clustering (AHC) allows sub-division of 42 genotypes into two major clusters based on the correlation that exists between the morphological traits among the genotypes. In the present study, cluster 1 comprised of 33 populations, which was further divided into two sub-groups cluster Ia contains 18 genotypes and Ib contains 15 genotypes. Nine genotypes were classified into cluster 2, which was again divided into two sub-clusters group IIa and group IIb containing 7 and 2 genotypes, respectively.

In cluster Ia, all the genotypes were vigorous, medium flowering with big flowers, whereas, genotypes in cluster Ib were tall and erect, vigorous, late flowering with big flowers. Except genotype IIHRJ22, all the genotypes in cluster IIa belong to the Japanese

Table 1 : Descriptive statistics of quantitative traits in China aster genotypes

Trait	Range		Mean ± SE(m)	CV (%)
	Minimum	Maximum		
Plant height (cm)	8.20	61.80	45.50 ± 0.66	24.97
Number of leaves/plant	9.20	32.35	19.50 ± 0.86	22.13
Plant spread (cm)	8.75	42.65	24.54 ± 0.72	32.59
Number of branches/plant	6.65	17.60	12.08 ± 0.30	19.83
Days to first flowering	46.85	100.15	66.71 ± 0.71	16.92
Flower stalk length (cm)	4.65	49.10	35.62 ± 0.72	29.34
Flower head diameter (cm)	3.54	6.74	5.39 ± 0.07	15.96
100 flowers weight (g)	105.00	548.25	292.02 ± 2.66	31.98
Number of flowers/plant	7.35	65.05	40.92 ± 0.46	34.82
Weight of flowers/plant (g)	7.72	235.21	124.49 ± 2.06	48.65
Duration of flowering (days)	16.58	34.40	24.92 ± 0.30	19.53
Flower yield/ hectare (q/ha)	6.48	197.57	104.57 ± 1.73	48.65
Vase life (days)	5.40	9.50	7.06 ± 0.12	14.26
Shelf life (days)	2.35	4.42	3.42 ± 0.08	14.37

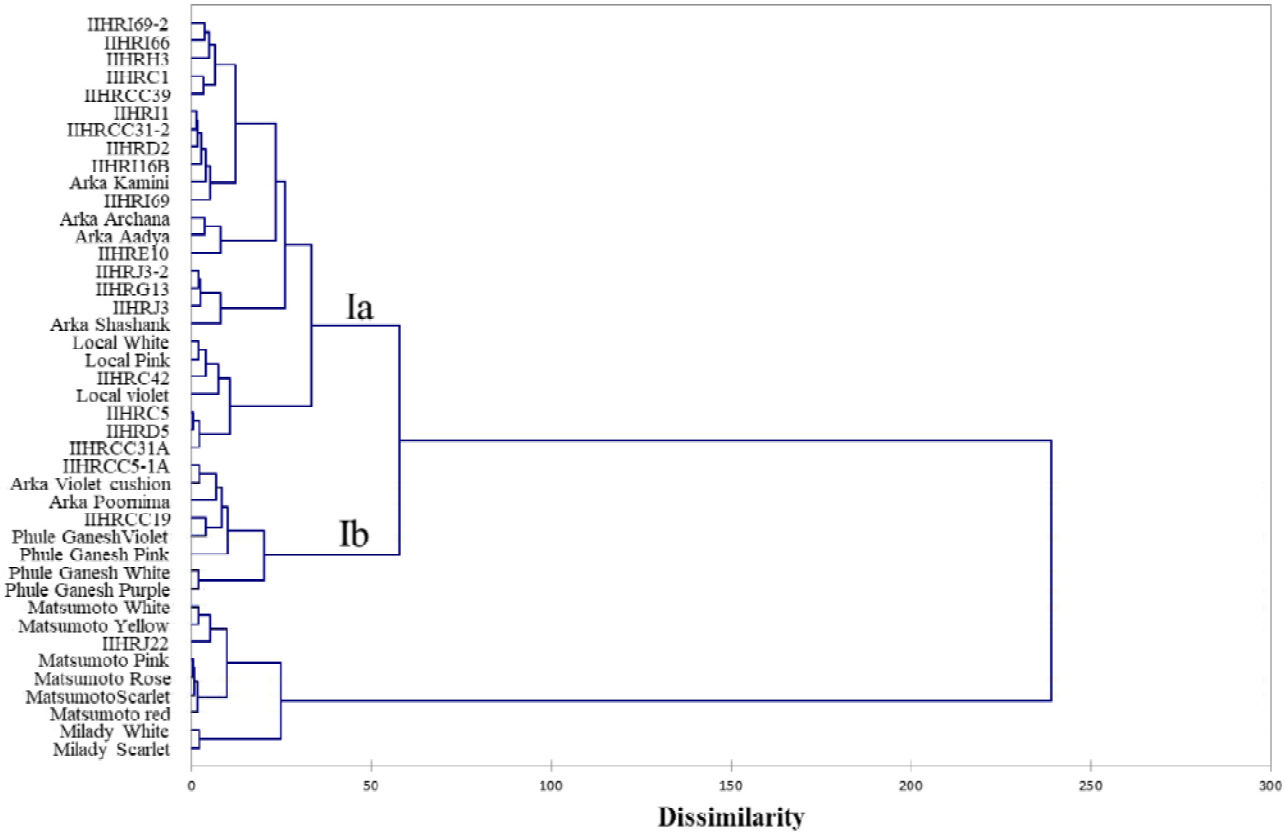

Fig. 1 : Dendrogram showing genetic relationship among 42 China aster genotypes based on morphological data

originated short stature, early flowering and small flowered genotypes, however, IIb contains only two European originated genotypes namely Milady Scarlet and Milady White. Similar results were also obtained by Kumar *et al*. (2011) in snapdragon, and Bharathi and Jawaharlal (2014) in marigold. It was observed that genotypes representing particular geographic regions were grouped together. The heterogeneous origin of genotypes, place of release, different ploidy levels often grouped together in the same cluster, suggesting the ancestral relationship between the various genotypes (Bellundagi *et al*., 2013). For further improvement in the morphological and yield parameters, genotypes may be selected on the basis of genetic divergence. Crossing between highly genetic divergent genotypes could yield better results (Singh *et al.,* 2016). Therefore, genotypes may be chosen for crossing on the basis of genetic divergence as depicted in the dendrogram. Based on the cluster distance, genotypes belonging to Matsumoto series, Milady Scarlet and Milady White, and genotypes belonging to Phule Ganesh, Arka, IIHRJ3-2 and IIHRG13 were most divergent. Therefore, crossing among the most divergent genotypes can achieve improvement in the morphological and yield attributes by getting desirable transgressive segregants.

To determine the most significant characteristics of the data set and also to determine the distances between the genotypes based on the data obtained on morphological traits, set of 42 genotypes used for cluster analysis were subjected to Principal Component Analysis (PCA) (Table 2). The analysis also helped to understand the contribution of morphological characters across the genotypes. The first two components of the seven considered accounted for most of the variation. The first principal component (PC1) explained 55.66% of the total variation and was positively correlated to all the traits; highly correlated to flower yield, weight of flowers/ plant, flower stalk length and plant height. The PC2 explained 10.55% of total variation and was highly positively correlated to shelf life and vase life, and negatively to 100 flower weight (Table 2). Because PC1 and PC2 accounting 66.21% of cumulative variance the compounds, a scatterplot was made for both (Fig. 2).

Table 2 : Principal component analysis in China aster genotypes

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Eigen value	7.79	1.48	1.19	0.95	0.71	0.54	0.46
Cumulative variance (%)	55.66	66.21	74.68	81.49	86.57	90.41	93.70
Plant height (cm)	0.82	0.29	0.22	-0.25	-0.09	-0.02	-0.22
Number of leaves/plant	0.72	-0.25	-0.41	-0.19	0.05	0.39	0.02
Plant spread (cm)	0.82	-0.05	-0.27	0.38	-0.03	-0.04	-0.12
Number of branches/plant	0.75	-0.02	-0.17	0.43	-0.15	0.20	-0.17
Days to first flowering	0.71	0.04	-0.47	-0.36	0.24	0.12	0.04
Flower stalk length (cm)	0.86	0.13	0.30	-0.12	-0.05	0.08	-0.26
Flower head diameter (cm)	0.80	-0.08	-0.11	-0.05	-0.34	-0.31	-0.17
100 flowers weight (g)	0.81	-0.33	-0.17	-0.03	-0.22	-0.23	0.25
Number of flowers/plant	0.75	-0.02	0.56	0.04	0.24	0.17	-0.01
Weight of flowers/plant (g)	0.88	-0.30	0.28	-0.01	0.05	-0.03	0.22
Duration of flowering (days)	0.61	0.27	-0.18	0.25	0.59	-0.32	-0.04
Flower yield/hectare (q)	0.88	-0.30	0.28	-0.01	0.05	-0.03	0.22
Vase life (days)	0.46	0.67	-0.10	-0.41	-0.09	-0.09	0.13
Shelf life (days)	0.38	0.70	0.02	0.39	-0.20	0.17	0.30

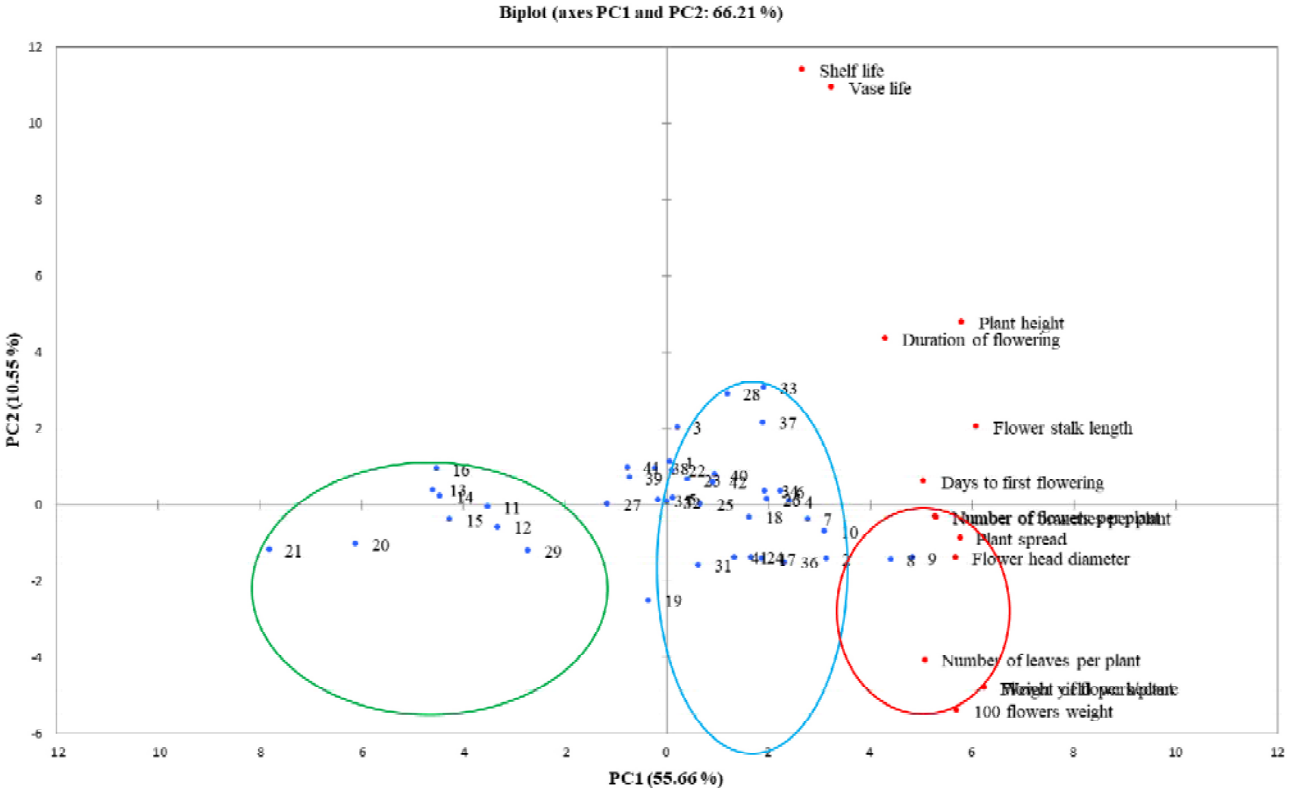

Fig. 2 : Principal Component Analysis (PCA) of China aster genotypes based on morphological characters

1. Arka Kamini 2, Arka Poornima, 3. Arka Shashank, 4. Arka Violet Cushion, 5. Arka Aadya, 6. Arka Archana, 7. Phule Ganesh Pink, 8. Phule Ganesh Purple, 9. Phule Ganesh White, 10. Phule Ganesh Violet, 11. Matsumoto Yellow, 12. Matsumoto White, 13. Matsumoto Rose, 14. Matsumoto Scarlet, 15. Matsumoto Red, 16. Matsumoto Pink, 17. Local Pink, 18. Local White, 19. Local Violet, 20. Milady Scarlet, 21. Milady White, 22. IIHRD5, 23. IIHRC5, 24. IIHRC42, 25. IIHRCC39, 26. IIHRCC5-1A, 27. IIHRCC31-2, 28. IIHRJ3, 29. IIHRJ22, 30. IIHRI1, 31. IIHRI66, 32. IIHRCC31A, 33. IIHRG13, 34. IIHRI69-2, 35. IIHRD2, 36. IIHRCC19, 37. IIHRJ3-2, 38. IIHRI69, 39. IIHRI16B, 40. IIHRH3, 41. IIHRE10, 42. IIHRC1.

Large variation was recorded in traits such as shelf life, vase life, plant height, 100 flower weight as mentioned by the relative length of the vectors in the biplot diagram. The biplot also signifies the positive correlation between the parameters *viz*., shelf life, vase life, plant height, duration of flowering, days to first flowering and 100 flower weight, flower yield per hectare, weight of flowers per plant and number of leaves per plant as indicated by the acute angle.

The genotypes like Matsumoto, Milady Scarlet and Milady White which were short and early flowering formed a group in one quadrant and all are comparatively late flowering genotypes which are, tall with big flowers formed another group and intermediate medium flowering forms the group in between. Most of the morphological traits contributed equally in grouping of genotypes except vase life and shelf life, which were distributed away from the genotypes. The result of PCA is consistent with that of the cluster analysis. A similar pattern was also observed for hips traits in *Rosa* sp. (Verma *et al*., 2013) and in pea (Esposito *et al*., 2007).

CONCLUSION

This study indicated that the quantitative traits are useful for preliminary evaluation of genetic diversity in China aster. PCA revealed that number of flowers per plant, flower yield per hectare, flower stalk length, plant height and plant spread are key traits contributing to the maximum variation among the genotypes. The cluster analysis showed significant genetic variability among the evaluated China aster genotypes, which may provide an excellent opportunity for crop improvement through hybridization between the genotypes of different clusters, to assemble desirable traits with higher heterotic potential.

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Original Research Paper

Stability analysis of yield, yield attributes and essential oil content in fennel (*Foeniculum vulgare* Mill.) evaluated under a long-term organic production system

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ABSTRACT

Eight varieties of fennel (*Foeniculum vulgare* Mill.) were evaluated under field trial for their stability of yield, yield attributes and essential oil content under the organic production system in six consecutive years from 2016 to 2021. Mean square due to environment + (variety × environment) was significant for all the traits studied indicating the existence of variety × environment interaction. Based on the mean performance, regression coefficient and deviation from regression values, it was found that stability of yield and yield components are imparted in the varieties, GF-12 and AF-1 across the years through the stable performance of characters and like numbers of primary and secondary branches, number of umbels and umbellate and seed yield. However, variety RF-101 for essential oil content can be considered as most suitable, stable and adopted to organic production system compared to other varieties. Correlation analysis revealed highly positive relationship in plant height, number of primary branches, number of umbels and umbellate per plant and seed yield. Based on the findings, fennel growers are apprised to select stable high-yielding fennel varieties for the organic production systems in semi-arid regions of India. Along with their use in hybridization programmes to converge the stability characteristics of seed yield for the development of a stable variety adapted to a wider range of environments under organic production systems.

Keywords : Correlation, essential oil, fennel, organic production system, seed yield, stability

INTRODUCTION

Fennel (*Foeniculum vulgare* Mill.), chromosome number 2n=2x= 22, is one of the most eminent medicinal and aromatic plant belonging to the family Apiaceae (Umbelliferae). Fennel is widely cultivated throughout the temperate and sub-tropical regions (Sheet *et al*., 2020). Gujarat ranks first in the area, production and productivity of fennel. It is used in a wide range of curry powder, curries flavoured soups, such as mulligatawny and shorbas, and is often used with fish. Fennel seeds are also used in pickles, chicken casseroles, salad dressings, fish liver and pork sauces and cucumber, sauerkraut lentils and pickled beef. Powdered fennel goes into biscuits, cakes and cooked apple dishes. The volatile oil in Indian fennel seed ranged from 0.7-1.2% (Saharkhiz and Tarakeme, 2013), and 4 to 6% in East European fennel seeds. Its seeds are found to be a good source of minerals like Ca, Fe, Mg, K, Na, and Zn as well as Vitamin A, and Niacin and phytate (11.35-13.10 mg/g.).

In recent times, due to awareness of health and food safety concerns, organic farming and natural cultivation practices are gaining momentum. The organic farming practices are compatible with the environment and able to sustain soil microflora, fauna and fertility in the long term. Although organic farming is still a small industry (1%–2% of global food sales), its importance is rapidly growing worldwide. In the domestic as well as the international market, there is a great demand for organic products which shows a greater potential source of income for small producers/ stakeholders. Despite the potential benefits of organic farming in terms of higher soil health and quality of produce, maintenance of high yields is one of the major challenges under organic farming systems (Tilman *et al*., 2002; Patel *et al*., 2014).

Modern cultivars are selected by plant breeders under conventional systems and they may not perform well under organic farming systems where they are grown in a stressed environment without the addition of

external inputs that are entirely different to those in which they were selected (Murphy *et al*., 2007; Singh *et at*., 2017). So, there's a need to select varieties for the organic production system which is believed as a stressed environment as crops are not supplied with chemicals for either supplying nutrients or to protect the crop from pests and diseases. Very limited scientific information is available regarding the evaluation of different varieties under organic production system, especially in seed spices for yield and quality attributes especially genetic studies on the performance of a diverse variety of fennel grown under the organic system are lacking. In recent times, improved released fennel varieties AF-1, RF-101, CO-01, Rajendra Saurabha, GF-12, RF-281, RF-125 and GF-02 are cultivated widely under different arid and semi-arid regions for high yields and quality, therefore, these varieties were chosen to investigate their performance and stability for growth and yield under an organic production system.

MATERIALS AND METHODS

A field experiment under AI-NPOE project was carried out at ICAR-National Research Centre on Seed Spices, Tabiji, Ajmer, Rajasthan for six consecutive years from 2016 to 2021 to identify suitable and stable fennel varieties for the organic production system. Eight released varieties *viz.*, AF-1, RF-101, CO-01, Rajendra Saurabha, GF-12, RF-281, RF-125 and GF-02 of fennel were tested under an organic production management system for growth, yield and quality attributes. The experiment was laid out in a randomized block design with three replications in a plot size of 12 m^2. Soil of the experimental site was sandy loam in nature and the experimental block was maintained as per the organic production requirements since 2011. Soil fertility status of an experimental site shows organic carbon (0.26%), available nitrogen (130.4 kg ha^{-1}), available phosphorus (12.06 kg ha^{-1}) and available potassium (359.07 kg ha^{-1}). The recommended dose of nutrients for fennel is 100:50:30 kg ha^{-1} and manures were applied on a nitrogen equivalent basis through organic sources (50% by FYM, 25% by vermicompost and 25% by castor cake). Nitrogen content of farmyard manure, vermicompost and castor cake is 0.50, 1.0 and 5.0%, respectively.

Every year seeds were sown during the second week of October by maintaining the row-to-row spacing of 50 cm. Fennel seeds were sown at the rate of 10 kg ha^{-1} after treating seeds with *Trichoderma virdae*, phosphate solubilising bacteria and *Azotobacter* at the rate of 10 g kg^{-1}. Irrigation and intercultural operations were followed as per recommended package of practice. The biometrical observations were recorded for ten different characters *viz*., initiation of flowering, days to 50% flowering, plant height at harvest, number of primary branches, number of secondary branches, number of umbels per plant, number of umbellate per umbel, seed yield and essential oil per cent in a seed. Observations on days to 50% flowering and days to maturity were recorded on a plot basis, whereas, data on the rest of the characters were recorded on five randomly selected plants in all three replications. For extraction of essential oil, a thirty-gram fresh seed sample in three replications was drawn from each treatment and used for essential oil extraction by hydro-distillation for 7 hrs using a Clevenger apparatus.

In the present study, stability analysis was carried out using pooled data of six *rabi* seasons over six years following the model proposed by Eberhart and Russell (1966). The model was used to find out G × E interaction and both linear (bi) and non-linear (S^2di) components of G × E interaction were considered for the indication of the performance of the individual variety. A different year of study was considered as different environment. The linear regression coefficient (bi) was considered as a measure of responsiveness and deviation from regression (S^2di) as a measure of stability. According to the model, stable variety has regression coefficient (bi) equal to unity (bi=1.0) and deviation from regression does not significantly differ from zero (S^2di=0). In general bi=1 implies average stability, bi>1 (1.01-1.30) implies below average stability and bi<1 (0.81-0.99) implies above average stability of a varieties. The desirability of a variety is judged based on stability criteria together with the mean value of the character. Stability analysis carried out using online OPSTAT (Sheron *et al*., 1998). Correlations were calculated on a genotypes mean basis, according to Pearson's test using SAS 9.3 software.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA) for the stability of different traits was analysed in selected varieties across six years (Table 1). Results of ANOVA revealed that

Table 1 : Analysis of variance for stability performance for seed yield, its components and essential oil content in fennel

Source	DF	Plant height at harvest (cm)	Days to anthesis	Days to 50% flowering	No. of primary branches	No. of secondary branches	No. of umbels/ plant	No.of umbellate/ plant	Yield (q/ha)
Variety	7	1,961.08**	52.05**	50.42**	116.43**	241.80**	564.96**	224.13**	87.91**
Environment	6	25,644.04**	1,546.61**	953.02**	67.98	108.96	1,015.50	943.18**	2,065.10**
Var. x Env	42	1,223.23**	53.50**	82.94**	20.79**	42.92**	89.15**	69.96**	52.03**
Env.+ Var. x Env.	48	26,867.26**	1,600.16**	1,035.97**	88.78**	151.88**	1,104.6**	1,013.10**	2,117.20**
Env. (Linear)	1	25,644.04**	1,546.66**	953.02**	67.98**	108.96	1,015.5**	943.18**	2,065.10**
Env. X Var. (Linear)	7	240.03**	1.808**	4.43*	16.07**	8.11*	52.36**	56.56**	10.821*
Pooled Deviation	40	983.195**	51.69**	78.51**	4.72*	34.80	36.78**	13.40*	41.20*
Pooled Error	98	6,124.55	190.58	217.50	57.68	158.50	917.2	342.14	473.71

DF- degree of freedom, * significant at p- 0.05, Var-variety, Env-Environment

the mean squares due to varieties were highly significant for all the traits *viz*., plant height at harvest, days to initiation of flowering, number of primary branches, number of secondary branches, number of umbels/plant, number of umbellate/plant, seed yield and essential oil content. It indicates that selected varieties were divergent and possess significant genetic variation for traits studied. The mean squares due to environments and interaction of variety with the environment (G x E) and environment + (variety x environment), were found highly significant for all the selected traits indicating the selected environments (years) were random and differ in climatic conditions. The partitioning of variance into components likes environments, environments (linear), variety x environment (linear) and pooled deviation (non-linear) showed that mean squares due to environment (linear) were also found significant. The significant mean squares value confirmed that the environments (years) were random and distinct, and they employed influence on the expression of a trait having significant mean squares and this variation could be attributed to have arisen due to the linear response of the expression of the variety to the environment. The significant value of mean squares for all the attributes studied revealed that the behaviour of the variety could be predicted for environments (years) more precisely for most of the traits and the G x E interaction was the result of the linear function of the environmental factors. The non-linear component arising due to heterogeneity, measured as mean squares due to pooled deviation, these significant mean squares revealed the presence of a non-linear response of the variety to the changing environments (stability performance). The significant mean squares for the pooled deviation confirmed the contribution of the non-linear component to the total G x E interaction. The variety differed in the stability of these traits making its prediction more difficult. Similar kind of results was earlier reported by Sastry *et al*. (1989), Verma and Solanki (2015), Lal (2014), Sawargaonkar & Sahu (2018) and Mangat (1986) where fennel genotypes and varieties were evaluated under a traditional production system. The results based on the stability parameters are discussed character-wise based on the model proposed by Eberhart and Russell (1966). According to Eberhart and Russell model, genotypes are grouped based on their variance of the regression deviation (either equal

or not to zero). A genotype with variance in regression deviation equal to zero is highly predictable, whilst a genotype with regression deviation more than zero has less predictable response (Scapim *et al*., 2010). A correlation coefficient was estimated according to Johnson *et al*. (1955) using SAS 9.3 software and only significant correlation discussed.

Initiation of flowering (days) averaged 87.85 across the study years, while, the mean values ranged from 85.91 to 89.00 (Table 2). In GF-12, flowering took place early, whereas, CO-01 experienced late flowering over the course of the study's years. Out of the eight varieties, AF-1 depicted regression coefficient that was unity and did not significantly deviate from the regression line, indicating good stability and favourable all environmental conditions (years) for this trait. Rajendra Saurabha, RF-125 and RF-125 had high mean values and regression coefficient that was above than unity implies above average stability. In contrast, CO-01, RF-101, and GF-02 had high mean values and regression coefficient that was less than one, indicating above average stability that was suitable for an unfavourable or poor environment (organic production system) for this trait.

The average plant height (cm) years was recorded 169.21, and the mean values ranged from 163.92 to 180.61. GF-12 had the tallest plants, whereas (RF-281) 163.92 had the shortest plants. Only three of the eight varieties, AF-1 and GF-12 had high mean, regression coefficient above unity and non-significant departures from the regression line, indicating that these two varieties have below average stability for plant height.

The average days to 50% flowering ranged from 94.76 to 97.86 against an average of 96.80 days. The earliest 50% flowering was recorded in variety (GF-12), while the late variety was Rajendra Saurabha. The variety *viz*., AF-1 and CO-01, has showed above average stability as they had higher mean, regression coefficient of more than one and non-significant deviation from regression line, hence, this variety was considered as suitable for a favourable environment for this trait and has below average stability for this trait.

The mean value for numbers of primary branches varied from 7.07 to 11.10 as against the average of 8.54 across the years under study. The maximum number of primary branches was recorded in variety GF-12 while the lowest was in CO-01. Variety GF-12 and AF-1 has high mean value with regression coefficient above unity and non-significant deviation from regression line considered as stable variety. However, variety AF-1 has high mean value with regression coefficient below unity and non-significant deviation from regression has above average stability. The findings suggested that these varieties are well adapted to suitable for poor and favourable environmental conditions respectively for this trait. RF-125 was specially adapted to the favourable environment for this trait as it had a regression coefficient above unity and a high mean with a small non-significant deviation from regression.

The average numbers of secondary branches varied from 15.45 to 21.21 as against the average of 17.82 across the years under study. The maximum numbers of secondary branches were recorded in variety GF-12 while, the lowest was in variety RF-101. Variety RF-281 has a high mean value with regression coefficient above unity and non-significant deviation from regression line considered as below average stable variety for the trait. Variety AF-1 and GF-12 have high mean values with regression coefficient below unity and non-significant deviation from regression explaining its suitability in poor (unfavourable) environment.

Across the study years, the average numbers of umbels per plants were varied from 28.95 to 37.92, with a mean value of 31.92 (Table 3). GF-12 had the highest numbers of umbels per plants, whereas CO-01 had the lowest ones during the course of the study's years. Rajendra Saurabha and RF-281varieties demonstrated regression coefficient that was unity and non-significant deviations from the regression line, indicating average stability. In contrast, AF-1, GF-12 and GF-125 had high mean values and regression coefficient that was higher than one, indicating below average stability that was suitable for an unfavourable or poor environment (organic production system) for this trait.

The average number of umbellate per plant varied from 22.21 to 28.49, whereas, across the study years, it averaged 25.22. The variety RF-281 recorded lowest number of umbellate per plant, while variety GF-12 had the highest number of umbellate per plant. Variety GF-12, AF-1, RF-125 and GF-02 had high mean

Table 2 : Stability parameters of growth, flowering and number of branches attributes in fennel

Variety	Day to initiation of flowering			Plant height (cm)			Days to 50% flowering			No. of primary branches			No. of secondary branches		
	Xi	bi	S^2di	Xi	bi	S^2di	Xi	bi	S^2di	Xi	bi	S^2di	Xi	bi	S^2di
AF-1	87.91	1.00	2.60	177.58	1.01	-8.56	96.33	1.05	6.18	10.68	0.93	-0.17	20.71	0.88	-0.39
RF-101	88.29	0.98	0.92	164.89	0.96	-20.19	96.57	1.06	0.53	7.54	0.34	-0.09	15.45	0.60	-0.40
CO-01	89.00	0.97	0.82	167.21	0.81	-9.12	97.71	1.08	-0.29	7.07	0.41	0.04	15.84	0.74	1.66
Rajendra Saurabha	88.76	1.02	-0.11	166.89	0.94	-11.60	97.86	0.93	-0.15	7.25	0.98	-0.09	16.27	1.26	-0.02
GF-12	85.91	0.96	0.12	180.61	1.02	7.61	94.76	1.07	0.13	11.10	1.02	-0.13	21.21	0.84	-0.53
RF-281	87.57	1.07	0.08	163.92	1.16	29.01	96.95	0.91	0.05	7.81	0.91	-0.13	18.28	1.01	-0.27
RF-125	88.43	1.01	0.88	164.42	1.08	22.78	97.67	0.99	1.02	8.70	1.48	-0.09	18.44	1.21	1.02
GF-02	86.91	0.97	-0.18	168.14	1.03	20.07	96.57	0.92	2.32	8.14	1.93	0.02	16.33	1.47	1.57
	Pooled Mean: 87.85			Pooled Mean: 169.21			Pooled Mean: 96.80			Pooled Mean:8.54			Pooled Mean: 17.82		
	SE(mean): 0.46			SE(mean): 2.02			SE(mean): 0.57			SE(mean): 0.14			SE(mean): 0.38		
	SE (b):0.082			SE (b):0.088			SE (b):0.128			SE (b):0.118			SE (b): 0.253		

Xi- mean, bi-regression coefficient and S^2di-deviation from the regression

values and regression coefficient that was higher than one indicating below average stability suitable in unfavourable/poor environment (organic production system) for this trait.

The average seed yield (q/ha) across the study's years was 25.75 which was ranged from 23.95 to 27.82. Variety RF-101 had the lowest seed yield per hectare, whereas, variety GF-12 had the highest. For seed yield, the regression coefficient (bi) varied from -0.88 to 1.10. This wide variation in regression coefficients suggests that different varieties have responded differently over the course of six years and in the context of an organic production system. A regression coefficient for GF-12 is unity, so GF-12 would be adaptable to all environments. Similar results was also reported in the genotypes RF-101, FNL-72 and FNL-71 had above average mean, seed yield, regression coefficient of bi=1 but non-significant deviation from regression line ($S^2di=0$). Hence, indicating its specific adaptability under good agronomic management practices (Sawargaonkar *et al*., 2018). For AF-1, it is less than unity and hence, it is adaptable across poor environments. This demonstrates its particular adaptability for yield component traits under good agronomic management. The higher seed yield per hectare that was observed for RF-125 and GF-02 and are attributed to the regression coefficient value being less than unity and the non-significant deviation from regression that explains its suitability in a harsh environment with above-average stability. Rajendra Saurabha and RF-281, regression coefficient value is more than unity that depicts above average stability, hence it performs well in favourable environments. Lal (2008) and Verma & Solanki (2015) also reported above average stability of fennel genotypes RF-205 for seed yield. Gangopadhyay *et al*. (2012) also reported similar results trait in stability analysis for seed yield of fenugreek genotypes.

The range of seed essential oil content (%) was estimated from 1.14-1.25 as against the average of 1.197 obtained across the years under study. The maximum essential oil content was recorded in variety AF-1, while, the minimum was in variety GF-02. The variety AF-1 and RF-101 had recorded higher essential oil content above average value and regression coefficient value is near unity with non-significant deviation from regression indicates that the variety is more responsive for input rich conditions.

Table 3 : Stability parameters of umbel and umbellate/plant and yield contributing traits in fennel

Variety	No. of umbels/plant			No. of umbellate/plant			Seed yield (q/ha)			Essential oil content (%)		
	Xi	bi	S^2di	Xi	bi	S^2di	Xi	bi	S^2di	Xi	bi	S^2di
AF-1	36.13	1.15	-2.18	27.86	1.16	-1.12	27.51	0.98	-1.20	1.25	1.081	0.001
RF-101	29.14	0.63	-2.71	22.21	0.80	-0.91	23.95	0.99	0.64	1.24	1.071	-0.001
Co-01	28.95	0.63	-2.22	24.10	0.56	-0.86	24.50	1.08	-0.81	1.20	0.610	0.000
Rajendra Saurabha	29.69	1.00	-2.67	24.81	0.73	-1.05	25.27	1.10	-0.82	1.21	0.810	0.001
GF-12	37.92	1.11	-1.72	28.49	1.11	-1.00	27.82	1.00	-1.16	1.19	0.730	-0.000
RF-281	31.26	0.99	-3.09	23.30	1.26	-0.34	25.75	1.02	-1.20	1.18	0.691	0.001
RF-125	32.50	1.21	-2.48	25.51	1.20	-0.39	25.43	0.88	-0.22	1.17	0.642	-0.000
GF-02	29.75	1.25	-0.49	25.51	1.19	-0.97	25.74	0.91	0.15	1.14	1.050	-0.000
	Pooled Mean: 31.92 SE(mean): 0.39 SE (b): 0.085			Pooled Mean: 25.22 SE(mean): 0.24 SE (b): 0.053			Pooled Mean: 25.75 SE(mean): 0.41 SE (b):0.063			Pooled Mean: 1.197 SE(mean): 0.98 SE (b): 0.184		

Xi- mean, bi-regression coefficient and S^2di-deviation from the regression

Table 4 : Correlation analysis among seed yield, its components and essential oil content in fennel

Characteristics	Day to initiation of flowering	Plant height (cm)	Days to 50% flowering	No. of primary branches	No. of secondary branches	No. of umbels/ plant	No. of umbellate/ plant	Seed yield (q/ha)	Essential oil content (%)
Day to initiation of flowering	1.000	-0.619	0.891*	-0.672	-0.593	-0.634	-0.567	-0.701	0.360
Plant height (cm)		1.000	-0.783	0.891*	0.780	0.863*	0.887*	0.866*	0.264
Days to 50% flowering			1.000	-0.782	-0.647	-0.735	-0.577	-0.690	-0.066
Number of primary branches				1.000	0.937**	0.977**	0.889**	0.919**	0.159
Number of secondary branches					1.000	0.979**	0.828**	0.932**	0.110
Number of umbels/plant						1.000	0.869**	0.927**	0.166
Number of umbellate/plant							1.000	0.916**	-0.024
Seed yield (q/ha)								1.000	0.002
Essential oil content (%)									1.000

*Significant at p-0.05; ** Highly significant at P 0.05

Correlation analysis among plant, yield and quality traits

The correlation study (Table 4) determines interrelationships among the studied traits showed that the days to initiation of flowering was found significant relation with days to 50% flowering, plant height showed positive association with number of primary branches, number of umbels per plant, number of umbellate per plant and seed yield. Number of primary branches revealed highly significant positive correlation with number of secondary branches, number of umbels per plant, number of umbellate per plant and seed yield similarly number of secondary branches showed positive correlation with number of umbels per plant, number of umbellate per plant and seed yield. Number of umbels per plant revealed positive highly significant correlation with number of umbellate per plant and seed yield. Similar kind of results were also reported by Dashora and Sastry, (2011), Yogi, (2013) and Abou *et al*. (2013) in fennel.

CONCLUSION

The two varieties GF-12 and AF-1 had high mean performance, non-significant regression coefficient deviation from unity (bi=1) and non-significant deviation from zero ($S^2di=0$) in terms of numbers of umbels and seed yield per hectare and RF-101 had recorded higher essential oil content. Hence, in terms of yield, yield components GF-12 and AF-1 and RF-101 for essential oil content can be considered as the most suitable, average stable over the environments (years) and adopted to organic production system compared to other varieties besides further utilization in stable varietal development programme in fennel.

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Original Research Paper

Effect of growth regulators and micronutrients on quality parameters in cashew (*Anacardium occidentale* L.)

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ABSTRACT

Cashew (*Anacardium occidentale* L.) is an important tropical nut crop of social and economic importance worldwide. However, the crop is threatened with the low yield. In the present study, an attempt was made to test the effects of plant growth hormones as well as micronutrients on nut and apple quality of cashew var. Bhaskara. Significant differences in kernel weight, shelling percentage, carbohydrates and starch content of cashew kernel and juice content of cashew apple were observed with the foliar application of growth hormones and micronutrients. The foliar application of ethrel @ 50 ppm increased shelling percentage (35.8%), carbohydrate content (21.63%), sugar content (6.26%), protein content (32.4%), starch content (31.42%), juice content (78.3%) and total soluble solids (12^0 Brix). Further, the foliar spray of zinc sulphate (0.5%) + borax (0.1%) increased shelling (36.13%), protein content (32.15%), starch content (32.03%) among all the treatments tested. Furthermore, higher cashew apple juice content (78%) and total soluble solids (12^0Brix) was also recorded with the foliar spray of zinc sulphate (0.5%) + borax (0.1%).

Keywords : Cashew, micronutrients, nut and apple quality, plant growth hormones

INTRODUCTION

Cashew (*Anacardium occidentale* L) is an important commercial plantation crop of the country, grown in an area of 10.27 lakh ha with a production of 7.25 lakh metric tonnes of raw cashew nuts (Anon. 2019). World's total area under the cultivation of cashew is around 35100 km^2 with India sharing 20 per cent and 16 per cent of cashew area and production globally, respectively. However, productivity of Indian cashew is very low (772 kg/ha). The present low productivity is attributed to several factors such as establishment of plantation with seedling of nondescript origin, due to poor and irregular flowering because of adverse environmental conditions (Parameswaran *et al.,* 1984), poor fruit set and excessive premature fruit drop (Patnaik *et al.,* 1985), low hermaphrodite flowers (Parameswaran *et al.,* 1984), nutritional deficiency (Subbaiah 1983), inefficient pollination (Heard *et al.,* 1990) and irregular and prolonged flowering (Aliyu, 2005).

Cashew is an evergreen dicotyledonous woody tropical tree with medium canopy size. On an average the plant attains 5-8 m height. The leaves are alternate, simple, globous, oblong, leathery, often notched at the apex. The size of leaf varies from 6-24 cm in length and 4-15 cm in width based on species and variety. The root system of complete grown cashew tree consists of a taproot surrounded by a well-developed and extensive network of lateral roots, 90% which lie on the 15-32 cm soil depth. The pattern of growth of cashew tree alternates with vegetative and reproductive phases. There are two types of branching in cashew intensive and extensive type (Damodaran, 1965). Intensive type of growth pattern tends to give bushy appearance to tree whereas extensive type results in spreading tree habit. Annually, two or three peak periods of growth are observed in bearing cashew tree with development of stray shoot growth. In bearing trees, from flower flush many shoots develop that give rise to terminal inflorescence/ panicle. The other vegetative flush gives rise to lateral shoots that develop soon after main crop has matured.

The cashew flowers are pentamerous, white or light green at the time of opening, later turn to pink. Two kinds of flowers *viz*. hermaphrodite (bisexual/perfect)

and male (staminate) are present in the panicle. The perfect flowers are larger than staminate flowers (Damodaran *et al.,* 1965). Cashew is considered as andromonoecious species due to presence of both male (staminate) and hermaphrodite (perfect) flowers in the same terminal panicle usually called as inflorescence of cashew. Number of panicles per plant, flowers per panicle and distribution of male and hermaphrodite flowers (sex ratio) in each panicle vary among varieties. In flowering panicle, abundance of male flowers is reported higher than perfect flowers (Rao and Hassan, 1957; Bigger, 1990 and Damodaran *et al.,* 1965). The yield of cashew is very low owing to the production of low percentage of hermaphrodite flowers, poor fruit set, immature fruit drop and low fruit retention (Haribabu, 1982).

The cashew produces abundant flowers but only less than 10 per cent of which are hermaphrodite, about 85 per cent of the hermaphrodite flowers are fertilized and only 4-6 per cent of them reach maturity to give fruits, the remaining shed away at different stages of development. The fruit drop in cashew during the early stages of development is attributed to physiological reasons (Nothwood, 1966). Immature fruit drop is one of the major reasons for reducing yield potential of cashew. The formative effects of growth hormones are gaining its importance for managing canopy, ensuring uniform flowering and enhancing fruit retention and yield under commercial cultivation for perennial fruit trees including cashew (Olivier *et al.,* 1990).

The application of exogenous plant growth hormones has been reported to induce better root and shoot development, to break seed and bud dormancy and improve flowering and fruiting in many crop plants. Foliar spray of gibberellic acid and auxin increased shoot and root growth and total shoot and root biomass in treated cashew seedlings (Shanmugavelu, 1985). The better seed germination induced by GA in cashew has also been reported by Khan *et al.,* (1957). Shanmugavelu *et al.* (1985) suggested that the natural auxin contained in seeds of tree species might probably regulate the seed germination. The use of cytokinin and auxin improved flowering and fruit set in mango (Chen, 1983) and cashew (Kumar, 1994). Therefore, growth hormones are gaining importance in cashew cultivation for overcoming problems associated with rooting, flowering, fruit set, fruit retention and poor yield. Hence, it is evident from studies that the economic importance of hormones is due to their ability to increase nut yield. There have been numerous reports considering increased yield due to the use of hormones especially in the horticultural sector but the use of plant growth regulators on cashew in particular is in its infancy. Hence, it is of utmost importance to address this research gap and it is also essential to understand how the endogenous hormones affect or regulate the stages of plant growth in order to make exogenously applied plant growth hormones to play an important role in maximizing cashew nut yield. The productivity of cashew can also be enhanced by adopting proper nutrient managementin addition to application of plant growth hormones. Numerous nutritional trials on the crop especially on the major nutrients have been attempted in India as well as in other tropical countries. And, response to applied nutrients has been very favorable. However, the information on role of micronutrients on cashew is limited. Further, no attempt has been made so far to study the influence of foliar spray of growth regulators and micronutrient in enhancing the quality parameters of raw cashew nut. In the light of aforementioned, the present study was undertaken to evaluate the effect of growth hormones and micronutrients on quality parameters of raw cashew nut.

MATERIALS AND METHODS

Site of experiment and plant material

The experiment was conducted at experimental farms of ICAR- Directorate of Cashew Research, Puttur, Dakshina Kannada district, Karnataka during 2009-10 to 2011-12 (latitude: 12.250 North, Longitude: 75.40 East), which is situated at 90 meter above mean sea level). The study was carried out on 10 years old plantation (var. Bhaskara) by adopting randomized block design (RBD) with 9 treatments and 3 replications for plant growth hormones and for micronutrient spray with 6 treatment and 4 replication. The plant growth regulators were sprayed during flushing, flowering and fruiting stages using foot pump paddle sprayer covering the entire canopy (Table 1). The growth regulator treatments consits of control (T_1), ethrel 50 ppm (T_2), 2,4-D 10 ppm (T_3), NAA 25 ppm (T_4), IAA 10 ppm (T_5), BA 1000 ppm (T_6), GA_3 50 ppm (T_7), NAA 25ppm + GA_3 50 ppm (T_8) and IAA 100 ppm + GA_3 50 ppm (T_9). However, micronutrient treatment constitute T_1 (Control), T_2 (borax 0.1%), T_3 (borax 0.2%), T_4 (Zinc sulphate 0.5%), T_5 (Zinc sulphate 0.5% + Borax 0.1%) and T_6 (Zinc sulphate

0.5% + Borax 0.2%). Observations on kernel weight (g), shelling (%), CHO (%), Sugar (%), Protein (%), Starch (%), Juice (%), TSS (^{0} Brix) were recorded. The micronutrients were sprayed during flushing, flowering and fruiting stages using a foot pump paddle sprayer covering the entire canopy (Table 2). Observations on kernel weight (g), shelling (%), CHO (%), Sugar (%), Protein (%), Starch (%), Juice (%), TSS (^{0} Brix) were recorded in all the treatments.

Fifty whole kernels were weighed and recorded in grams. Mean weight of one kernel was calculated by dividing the total weight of kernels by number of kernels. Fifty sun dried raw cashew nuts were weighed and weight was recorded in grams. These 50 nuts were shelled by using shelling machine. Weight of kernels with testa and shells obtained after shelling these nuts were recorded separately. The weight of kernel with testa and weight of shell of each sample was added up totally with the original weight of 50 nuts. Weight of kernel with testa was divided by the weight of nut (weight kernel with testa + weight of shell) and expressed as percentage, which gave the shelling percentage.

Nuts were shelled and kernels were extracted after removal of testa and the defatted cashew kernel flour was used for the estimation of carbohydrate, protein, starch, and sugar content by using the method suggested by Sadasivam and Balasubramanian (1987). Total soluble solids (TSS) of cashew apple were estimated using hand refractometer.

Statistical analysis

The data obtained from three successive seasons were pooled and analyzed using SAS 9.3 version. ANOVA was applied to evaluate the significant difference in the parameters studied in the different treatments. Least significant difference (Fisher's protected LSD) was calculated, following significant F-test (p=0.05).

RESULTS AND DISCUSSION

Effect of growth regulators

In the present study, significant increase in kernel weight, shelling percentage, carbohydrates and starch content of kernels and juice content of apple were recorded by the application of ethrel @ 50 ppm followed by NAA @ 25 ppm, NAA @ 25 ppm + GA_3 50 ppm, GA_3@ 50 ppm, IAA @100 ppm + GA_3 50 ppm, BA @ 1000 ppm, IAA @ 100 ppm and 2,4-D @ 10 ppm (Table 1). Spraying of ethrel @ 50 ppm also increased the protein percentage (32.20 %) followed by NAA @ 25 ppm (32.1 %), NAA @ 25 ppm + GA_3 50 ppm (32.00 %), GA_3 @ 50 ppm (31.4 %), IAA @ 100 ppm + GA3 50 ppm (31.5 %), BA @ 1000 ppm (31.20 %), IAA @ 100 ppm (30.50 %) and 2,4-D @ 10 ppm (30.30 %) compared to untreated trees (29.89 %). Kumar *et al.* (1996) reported that the combined application of ethrel @

Table 1 : Effect of growth regulators on quality parameters of cashew variety Bhaskara

Treatment	Kernel weight (g)	Shelling (%)	Carbohydrate (%)	Sugar (%)	Protein (%)	Starch (%)	Juice (%)	TSS (^{o} Brix)
Control	2.17^{C}	30.00^{D}	20.70^{B}	6.21	29.63^{C}	25.52^{E}	67.40^{F}	11.00
Ethrel@50 ppm	2.55^{A}	35.87^{A}	21.63^{A}	6.26	32.40^{A}	31.42^{A}	78.37^{A}	12.00
2,4-D@10 ppm	2.40^{B}	31.50^{BC}	21.47^{A}	6.21	30.43^{BC}	28.50^{D}	68.47^{E}	11.00
NAA@25ppm	2.55^{A}	35.58^{A}	21.60^{A}	6.25	32.37^{A}	31.40^{A}	76.40^{B}	11.00
IAA@100 ppm	2.40^{B}	31.17^{CD}	21.47^{A}	6.22	30.50^{BC}	29.00^{CD}	68.53^{E}	11.00
BA @1000 ppm	2.45^{B}	32.50^{B}	21.50^{A}	6.23	31.40^{AB}	29.37^{CD}	70.50^{D}	11.00
GA_3@50 ppm	2.54^{A}	35.50^{A}	21.53^{A}	6.24	31.47^{AB}	30.40^{B}	76.00^{BC}	12.00
NAA @25 ppm + GA_3 50 ppm	2.54^{A}	35.58^{A}	21.57^{A}	6.25	31.33^{AB}	31.00^{AB}	76.37^{B}	12.00
IAA @100 ppm + GA_3 50 ppm	2.54^{A}	35.50^{A}	21.53^{A}	6.24	31.50^{AB}	29.47^{C}	75.50^{C}	12.00
Mean	2.46	33.69	21.44	6.23	31.23	29.56	73.06	11.44
SE(d)	0.037	0.559	0.089	0.026	0.582	0.416	0.332	0.544
LSD at 5%	0.078	1.186	0.189	NS	1.235	0.882	0.704	NS

50 ppm and 500: 250: 250 g NPK/plant/year enhanced yield, nut and apple quality in cashew varieties. They also further opined that the increase in total soluble solids and apple yield might be attributed to the positive interaction between growth regulators and nutrients.

Spraying of growth regulators in all the treatments have given higher nut yield compared to control. It may be because of ethrel and auxin could induce better flowering in cashew (Aliyu *et al.,* 2011). Auxin is known to induce flowering via ethylene production and also independently (Li and Xu, 2014). Other reasons for more nut yield compared to control are growth regulators/hormone sprayed leaf area mobilizes all the photosynthates and nutrients which will be utilized for flower production and fruit growth (Li and Xu, 2014). And other reasons might be increased stomatal number increases inflow of carbon dioxide into the mesophyll tissue resulting more photosynthates, latter partitioned towards nut resulted in more nut yield (Aliyu *et al.,* 2011).

In the present study, gibberellic acid, GA_3 @ 50 ppm resulted in better nut and kernel quality in cashew variety Bhaskara. Application of GA_3 @ 50 ppm increased protein content (31.4%), carbohydrate content (21.5%), sugar content (6.4%) and starch content (30.4%) in cashew kernel (Table 1). Similar results were also reported by Murthy *et al.,* (1975) where they studied the free amino acid and total protein content in three developmental stages of kernel in cashew after foliar treatment with 40 ppm and 50 ppm gibberellic acid. Treatment with GA_3 resulted in a marked increase in protein content of kernel at all stages of development. In GA_3 treated cashew kernels, the amino acid contents showed progressive decrease with the growth and maturation of the nut and greater accumulation of protein.Similar results were also reported in mango where GA_3 treatment enhanced fruit quality of mango trees (Muarya and Singh, 1981; Saski and Utsunomiya, 2002 and Anila and Radha 2003.

Effect of micronutrients

Micronutrients perform an essential role in the production of fruit crops, and their deficiencies largely affect the quality of fruits. In the present study, the influence of micronutrient application on nut and apple quality was studied and presented in Table 2. Kernel weight was not significantly influenced by foliar application of micronutrients. This indicates that kernel weight is more of a genetical factor and least influenced by external factors. Higher shelling (36.13%) was found with the spray of zinc sulphate (0.5%) + borax (0.1%) compared to untreated trees (30.13%). Micronutrient spray did not significantly influence the carbohydrate and sugar content in kernel. Protein content was higher (32.15%) with the spray of zinc sulphate (0.5%) + borax (0.1%) followed by zinc sulphate (0.5%) (31.5%) and borax (0.1%) (31.3%), while it was lower in unsprayed trees (29.92%). Starch content was highest (32.03%) with the spray of zinc sulphate (0.5%) + borax (0.1%) followed by borax (0.1%) (31.38%), while unsprayed trees recorded lower starch content (25.26%).

Table 2 : Effect of foliar spray of micronutrients on quality parameters of cashew variety Bhaskara

Treatment	Kernel weight (g)	Shelling (%)	Carbohydrate (%)	Sugar (%)	Protein (%)	Starch (%)	Juice (%)	TSS (° Brix)
Control	2.43	30.13^{D}	20.85	6.20	30.10^{C}	25.26^{D}	67.38^{E}	11.00
Borax (0.1%)	2.47	34.13^{B}	21.58	6.24	31.33^{AB}	31.38^{AB}	74.00^{B}	12.00
Borax (0.2%)	2.35	31.58^{C}	21.53	6.22	30.15^{C}	28.38^{C}	69.00^{D}	11.00
$ZnSO_4$ (0.5%)	2.40	33.70^{B}	21.28	6.23	31.50^{AB}	30.35^{B}	70.75^{C}	11.00
$ZnSO_4$ (0.5%) + borax (0.1%)	2.45	36.13^{A}	22.10	6.25	32.15^{A}	32.03^{A}	78.00^{A}	12.00
$ZnSO_4$ (0.5%) + borax (0.2%)	2.41	33.38^{B}	21.48	6.22	30.60^{BC}	28.90^{C}	68.00^{DE}	11.00
Mean	2.42	33.17	21.47	6.22	30.97	29.38	71.19	11.33
SE(d)	0.25	0.52	0.49	0.02	0.48	0.49	0.59	0.60
LSD at 5%	NS	1.11	NS	NS	1.025	1.06	1.26	NS

The effect of micronutrient spray on apple quality was also studied (Table 2). Higher juice content in cashew apple (78%) was found with the spray of zinc sulphate (0.5%) + borax (0.1%) compared to unsprayed trees (67.38%). The role of micronutrients in improving the vegetative growth, fruit quality and yield has been reported in several fruit crops (Abdollahi *et al.* 2012; Farid *et al.,* 2020, Sanjeela *et al.,* 2021). The present study is in consistent with findings of Shafeek *et al.* (2014), Singh *et al.* (2015) and Mishra *et al.* (2006) where application of micronutrients mainly zinc, boron and iron improved reproductive traits mainly fruiting parameters and quality traits. Significant increase in TSS was also observed with the foliar spray of zinc sulphate (0.5%) + borax (0.1%) and Borax (0.1%). Similar results were also reported by Sanjeela *et al.* (2021) where application of boron @ 0.2% increased the TSS (8.3^0Brix) in strawberry. The role of boron in sugar translocation helps to improve fruit quality parameters (Gauch and Dugger, 1953; Sathya *et al.,* 2010). Moreover, the deficiency of boron aggravatesthe quality of fruit by increasing titrable acidity and its application improves the quality of fruit (Haider *et al.,* 2019).

CONCLUSION

Significant differences in nut and apple quality were observed with the foliar application of plant growth hormones and micronutrients. The foliar application of Ethrel @ 50 ppm and NAA @ 25 ppm + GA_3 50 ppm as well as zinc sulphate (0.5%) + borax (0.1%) improved the kernel as well as apple quality traits.The present study represents the first preliminary study on the influence of growth hormones and micronutrients on the nutritional qualities of cashew nuts and apples in the variety Bhaskara. This study can be extended to screen other cashew varieties in terms of the nutritional quality of cashew nuts and apples.

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Original Research Paper

Standardisation of fertigation in papaya for higher productivity and profitability

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ABSTRACT

A field experiment conducted to standardize the fertigation in papaya (*Carica papaya* L.) variety Arka Prabhat with 12 treatments in split plot design, indicated that fertigation with 75% recommended fertilizers (250:250:500 g NPK/plant/year) through water soluble fertilizers recorded significantly higher fruit yield (47.34 t/ha), fertilizer use efficiency (20.45 kg fruit yield/kg of nutrient applied) and increase in 31% higher yield over soil application. The TSS of papaya fruit was although not significantly influenced by both doses and sources of fertigation, significantly lower cavity index (3.12%) was observed when RDF was supplied with organics to the soil. Fertigation with 100% RDF through water soluble fertilizers recorded significantly higher soil organic carbon (1.16%). However, fertigation of 75% RDF with inorganic fertilizers was found more economical with higher gross returns (Rs.7.10 lakh/ha), net returns (Rs.4.7 lakh/ha) and benefit cost ratio (2.96).

Keywords : Benefit cost ratio, fertigation, papaya, productivity, profitability

INTRODUCTION

Papaya (*Carica papaya* L.) is a common fruit crop grown in the Southern region of India. The crop is being cultivated in an area of 1,49,000 ha with a production of 57,44,000 MT (Anonymous, 2022). Fertigation combines the application of water and nutrient required for plant growth and development and allows an accurate and uniform application of nutrients to the wetted area in the root zone. Through fertigation, it is possible to supply an adequate quantity and concentration of nutrients to meet the demand of the crop throughout growing season. Further, fertigation is the most efficient method of fertilizers application, as it ensures application of the fertilizers directly to the plant roots (Rajput and Patel, 2002). The scheduling of fertigation for crops will benefit the farmers to increase the yield and improve the quality of produce through efficient use of water and fertilizers. Use of fertigation in fruit crops was reported to save 30-50% of fertilizer doses as well as irrigation (Shirgure *et al*., 2001; Shirgure and Srivastava 2014). Further, it is imperative to achieve the high nutrient use efficiency and reducing the requirement of bulk fertilizers to 25% (Malhotra, 2016).

Fertigation has been substantiated for many crops throughout world. It has been reported that efficiency of nitrogenous fertilizers is 95% under drip-fertigation compared to 30-50% under soil application. When a fertilizer is applied to a soil, nearby water begins to move very gradually toward the area where the fertilizer has been applied. Fertilizer salts begin to diffuse, or move away from the place where they were applied. This dilutes the fertilizer and distributes it throughout a much larger area. If tender plant roots are close to the placement of a fertilizer, water is drawn from these roots, as well as from surrounding soil (Rajput and Patel, 2002). Further, Sathya *et al*. (2008) observed that the availability of N, P and K nutrient was found to be higher in root zone area of drip fertigated plot, while nitrogen and potassium moved laterally from point source up to 15 cm and vertically up to 15-25 cm and P moved 5 cm both laterally and vertically and thereafter dwindled.

Nitrogen promotes vegetative growth, flower and fruit set. High level of phosphorus throughout root zone is essential for rapid root development and good utilization of water and other nutrients by plant. Phosphorous has pronounced effect on the flowering,

in combination with N and K improves peel colour, taste, hardiness and vitamin C content and hastens maturity. Potassium tends to increase fruit size, fruit quality and rectifies many disorders. It also helps in decreasing incidence of irregular shaped fruits. However, standardisation of the schedules of fertigation is crucial to decide both the doses and in coinciding the crop nutrient requirement with different stages of the crop. Keeping this in view, a field experiment was carried out to standardize the fertigation in papaya.

MATERIALS AND METHODS

The experiment was conducted during 2020-21 at ICAR-Indian Institute of Horticultural Research, Bengaluru, Karnataka, which is located at an altitude of 890 m above mean sea level and lies between coordinates of 13° 8' N latitude and 77° 29' E longitude. Soil of experimental field was sandy loam with 6.27 pH, 0.16 dS m^{-1} EC, and 0.78% organic carbon. The soil had an initial nutrient content of 283 kg available N/ha, 42.0 kg available phosphorus/ ha and 246.4 kg available potassium/ha.

Uniform and well-developed 45 days old seedlings of papaya var. Arka Prabhat were planted at a spacing of 1.8 m x 1.8 m on raised beds during July 2020 and the treatments were imposed with the crop establishment. The crop was managed with recommended package of practices except for irrigation. The experiment was carried out in split plot design with 12 treatment combinations consisting of three doses of fertilizers, *viz*., M_0: 100% RDF (250 g N + 250 g P_2O_5 + 500 g K_2O per plant/year), M_1: 125% RDF, and M_2: 75% RDF as main plot and four sources of nutrients, *viz*., S_0: fertigation through inorganic sources (urea, MKP and SOP) S_1: fertigation through organic sources (humic acid and vermiwash), S_2: soil application of only organic sources (FYM, vermicompost, neem cake, *Sesbania* and *Glyricidia* loppings), and S_3: soil application of FYM+ RDF (urea, SSP and MOP) as control as sub-plot treatments. Each treatment was replicated four times and each replication had five plants.

Observations were recorded on various parameters of plant growth and physiology, root growth, soil fertility, yield, and TSS and fruit cavity index after 240 days after planting. The physiological parameters were measured using IRGA portable photosynthesis system. The horizontal and vertical root growth was measured for the longest spread, and the root volume was calculated based on the displacement of water technique at the end of the crop season on a destructive mode. The dry weight of roots was calculated by carefully uprooting the roots with soil, washing with water and drying with hot air oven. Soil samples were collected at the end of the crop from 0-30 cm at 30-40 cm away from the base of the plant. Soil chemical and fertility parameters such as pH, organic carbon, available phosphorus (P) and potassium (K) were analysed as per standard procedures described by Jackson (1973). The fruit cavity index (%) was calculated by fruit cavity volume divided by fruit volume and multiplied by 100. Plant canopy volume was calculated using the formula 2/3πH (A/2 x B/2)], where H stands for plant height, A and B stands for EW and NS plant canopy spread (Thome *et al*., 2002). Fertilizer use efficiency was calculated based on the fruit yield obtained and the fertilizer nutrient used in each of the treatment. All the experimental data were statistically analysed as per Panse and Sukhatme (1985), and the differences in means were compared at 5% level of significance.

RESULTS AND DISCUSSION

Plant growth parameters

The plant height in papaya was significantly influenced by fertilizer doses and fertigation sources (Table 1). Significantly higher plant height (1.19 m) was recorded with 125 % RDF and among the sources, fertigation with RDF through inorganics recorded higher plant height (1.20 m).

Number of leaves were significantly higher with 125% RDF (20.63/plant) as compared to other sources, and further application of water soluble fertilizers recorded significantly higher number of leaves (20.6/plant) differing from other sources. Among the interactions, soil application of organic sources meeting 125 % RDF recorded significantly more number of leaves (21.5/plant). The plant girth in papaya differed significantly both due to doses and sources of nutrients although their interactions were non-significant. Significantly higher plant girth (26.28 cm) was recorded with application of 75 % of RDF, and among the sources, fertigation with inorganic sources recorded more plant girth (26.92 cm). Canopy volume in papaya was not significantly influenced by the fertilizer doses and their interaction with various sources. However, fertigation with water soluble fertilizers recorded significantly higher (1.64 m^3) canopy volume differing from rest of the sources.

Table 1 : Mean plant growth parameters in papaya as influenced by fertilizer doses and fertigation sources

Treatment	Plant height (m)	No. of leaves/plant	Stem girth (cm)	Canopy volume (m^3)
Main plot				
M_0	1.00	15.66	20.56	0.95
M_1	1.19	20.63	25.78	1.18
M_2	1.10	18.63	26.28	1.16
Subplot				
S_0	1.20	20.63	26.92	1.64
S_1	1.05	16.67	22.42	1.00
S_2	1.03	17.33	22.71	0.81
S_3	1.11	18.59	24.79	0.93
Interaction				
M_0S_0	1.10	21.00	24.75	1.44
M_0S_1	0.95	13.75	20.00	0.75
M_0S_2	0.90	12.00	17.00	0.65
M_0S_3	1.07	15.88	20.50	0.96
M_1S_0	1.32	21.00	26.75	1.78
M_1S_1	1.13	19.00	23.38	1.34
M_1S_2	1.15	21.50	27.50	0.87
M_1S_3	1.15	21.00	25.50	0.74
M_2S_0	1.18	19.88	29.25	1.71
M_2 S_1	1.06	17.25	23.88	0.92
M_2S_2	1.05	18.50	23.63	0.91
M_2S_3	1.12	18.88	28.38	1.10
S Em ± Main	0.02	0.65	0.37	0.07
Sub	0.03	0.66	0.80	0.21
Main x Sub^{-1}	0.05	1.19	1.25	0.32
C.D (P=0.05) Main	0.07	2.31	1.30	NS
Sub	0.08	1.93	2.33	0.60
Main x Sub^{-1}	NS	3.69	NS	NS

Physiological parameters in papaya

Although, the fertigation sources found to have non-significant impact on different physiological parameters recorded, the doses of fertilizers influenced the photosynthesis, respiration and stomatal conductance significantly (Table 2). Application of either 75% or 100% recommended fertilizers recorded significantly higher photosynthetic rate (16.34 µ mol m^{-2} s^{-1} and 16.24 mol m^{-2} s^{-1}, respectively), the former also recorded significantly higher stomatal conductance (0.14 mol H_2O m^{-2} s^{-1}) and transpiration rate (3.07 mol m^{-2} s^{-1}). Among the interactions, application of recommended fertilizers through fertigation (M_0S_0) recorded significantly higher photosynthetic rate (17.53 µ mol m^{-2} s^{-1}), which was followed by application of 75% RDF with organic sources of fertigation (M_2S_1), the latter also recording higher transpiration rate (3.14 mol m^{-2} s^{-1}). Application of 75% RDF through fertigation (M_2S_0) recorded significantly higher stomatal conductance (0.16 mol H_2O m^{-2} s^{-1}) also. Better physiological parameters in fertigated plants may be attributed to the higher nutritional status (N, P and K content), leaf N and K contents and physiological efficiency (Shirgure *et al.,* 2001), fertigated papaya plants recorded higher physiological efficiency (especially total chlorophyll content), photochemical efficiency, stomatal conductance and net photosynthesis, water use efficiency and relative water content compared with plants not subjected to fertigation.

Table 2 : Physiological parameters in papaya as influenced by fertilizer doses and fertigation sources

Treatment	Photosynthetic rate (μ mol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Transpiration rate (mol m^{-2} s^{-1})
Main plot			
M_0	16.24	0.09	1.94
M_1	12.61	0.07	2.32
M_2	16.34	0.14	3.07
Sub plot			
S_0	14.70	0.11	2.52
S_1	14.25	0.09	2.23
S_2	15.82	0.10	2.55
S_3	15.48	0.10	2.47
Interaction			
M_0S_0	17.53	0.11	2.54
M_0S_1	15.15	0.08	1.85
M_0S_2	16.26	0.09	1.58
M_0S_3	16.00	0.08	1.77
M_1S_0	9.69	0.05	1.91
M_1S_1	10.35	0.04	1.71
M_1S_2	16.10	0.09	3.01
M_1S_3	14.29	0.09	2.67
M_2S_0	16.88	0.16	3.11
M_2 S_1	17.26	0.14	3.14
M_2S_2	15.09	0.11	3.06
M_2S_3	16.15	0.13	2.97
S Em ± Main	0.20	0.01	0.15
Sub	0.41	0.01	0.15
Main x Sub^{-1}	0.64	0.01	0.27
C.D (P=0.05) Main	0.80	0.03	0.60
Sub	NS	NS	NS
Main x Sub^{-1}	1.98	0.04	0.89

Root growth

The impact of fertigation treatments on root growth parameters indicated that both the lateral and vertical root growth in papaya was significantly influenced by the doses and sources of fertigation although the root volume showed non-significant differences (Table 3). The vertical growth of the roots was significantly higher with application of 100 % RDF (84.1 cm) and especially with soil application of organic sources (97.5 cm) both of which differing significantly from other treatments. Although, the horizontal growth of roots was significantly influenced both by the fertilizer doses and the fertigation sources, their interaction was non- significant. In general, application of 75 % of RDF (163.8 cm) and among the sources, soil application of nutrients (174.5 cm) showed significantly higher lateral spread of roots. Root dry weight in general was significantly higher with 125 % RDF, and among the sources soil application of RDF shown significantly higher root dry weight (641.2 g $plant^{-1}$) differing significantly from rest of the fertigation sources.

Soil fertility

The pH of soil was influenced significantly both by the doses and sources of fertigation under papaya. Lowering the dose of fertilizers to 75 % RDF recorded pH 6.06 as compared to pH 5.96 in 100 % RDF. Among the sources, soil application of FYM and RDF recorded relatively better soil pH (6.11), while, among

Table 3 : Root growth in papaya as influenced by fertilizer doses and fertigation

Treatment	Root length (cm)	Root breadth (cm)	Root volume (cm^3 $plant^{-1}$)	Root fresh weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)
Main plot					
M_0	84.1	109.9	1222.5	1606.9	395.9
M_1	65.6	154.8	1530.6	2378.1	594.4
M_2	50.0	163.8	1332.5	2556.3	554.8
Subplot					
S_0	69.8	131.5	1260.8	1900.0	496.8
S_1	70.0	117.0	1215.8	1920.8	408.5
S_2	65.8	148.2	1490.8	2158.3	513.6
S_3	60.7	174.5	1480.0	2742.5	641.2
Interaction					
M_0S_0	74.5	105.5	1032.5	1187.5	323.0
M_0S_1	85.0	86.0	765.0	962.5	162.4
M_0S_2	97.5	109.5	1460.0	1900.0	477.2
M_0S_3	79.5	138.5	1632.5	2377.5	621.1
M_1S_0	85.0	114.0	1340.0	1887.5	677.6
M_1S_1	77.5	140.0	1612.5	2800.0	609.9
M_1S_2	52.5	180.0	1750.0	2275.0	501.2
M_1S_3	47.5	185.0	1420.0	2550.0	589.0
M_2S_0	50.0	175.0	1410.0	2625.0	489.9
M_2 S_1	47.5	125.0	1270.0	2000.0	453.2
M_2S_2	47.5	155.0	1262.5	2300.0	562.6
M_2S_3	55.0	200.0	1387.5	3300.0	713.7
S Em ± Main	0.2	2.7	145.9	37.0	20.4
Sub	2.0	7.5	281.5	155.7	52.7
Main x Sub^{-1}	3.0	11.6	446.7	236.4	81.6
C.D (P=0.05) Main	0.7	9.5	NS	130.5	72.1
Sub	5.8	21.9	NS	454.1	153.7
Main x Sub^{-1}	8.7	NS	NS	693.2	NS

the interactions, fertigation through organic sources with 75 % of RDF recorded a soil pH of 6.19. The lower pH of soil with recommended fertilizers may be attributed to the addition of acidic fertilizers and the same was relatively better when applied along with FYM.

The organic carbon content in soil was significantly influenced by doses and sources of fertigation. Application of 100 % RDF recorded significantly higher organic carbon (0.93 %) as compared to either 75 % (0.59 %) or 125 % (0.82 %). Among the sources, application through water soluble fertilizers recorded significantly higher organic carbon (0.92 %) as compared to other sources and the control. Among the interactions, 100% RDF through water soluble fertilizers recorded significantly higher organic carbon (1.16%) differing significantly from rest of the treatment combinations except the treatment application of 125% RDF through soil application of organics (1.05%). The higher organic carbon content with water soluble fertilizers may be attributed to the better availability of plant nutrients in turn favouring the accumulation of organic carbon in the soil.

The nitrogen content in soil was significantly influenced by doses and sources of fertigation. Application of 100 % RDF recorded significantly higher available nitrogen (150.7 kg ha^{-1}) as compared to either 75 % (96 kg ha^{-1}) or 125 % RDF

(133 kg ha^{-1}). Among the sources, application through water soluble fertilizers recorded significantly higher available nitrogen (148.2 kg ha^{-1}) as compared to other sources and the control. Among the interactions, 100 % RDF through water soluble fertilizers recorded significantly higher N (187.1 kg ha^{-1}) differing significantly from rest of the treatment combinations.

The available phosphorous content in soil was significantly influenced both by the doses and sources of fertigation. Application of 125 % RDF recorded significantly higher available phosphorous (40.92 kg ha^{-1}). Among the sources, soil application nutrients through organic sources recorded significantly higher available phosphorous (47.31 kg ha^{-1}) as compared to other sources and the control. Among the interactions, 75 % RDF through organic sources recorded higher available phosphorous content (58.46 kg ha^{-1}) differing significantly from rest of the treatment combinations except application of 125 % RDF through soil application of organic sources (55.91 kg ha^{-1}) and application of 100 % RDF through water soluble fertilizers (54.19 kg ha^{-1}).

The available potassium content in soil was significantly influenced by doses and sources of fertigation. Application of 125 % RDF recorded significantly higher soil available potassium (281.3 kg ha^{-1}). Among the sources, application through water soluble fertilizers recorded significantly higher available potassium (284.2 kg ha^{-1}) as compared to other sources and the control. Among the interactions, 100 % RDF through water soluble fertilizers recorded significantly higher potassium (353.8 kg ha^{-1}) differing

Table 4 : Soil fertility and major nutrients of soil in papaya as influenced by fertigation treatments

Treatment	pH	EC (dSm^{-1})	O.C. (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
Main plot						
M_0	5.93	0.20	0.93	150.7	34.12	256.3
M_1	5.96	0.16	0.82	133.0	40.92	281.3
M_2	6.06	0.17	0.59	96.0	39.20	243.1
Subplot						
S_0	5.96	0.22	0.92	148.2	40.59	284.2
S_1	5.99	0.15	0.76	122.3	32.16	251.3
S_2	5.88	0.21	0.76	123.1	47.31	252.1
S_3	6.11	0.13	0.70	112.6	32.26	253.4
Interaction						
M_0S_0	5.71	0.35	1.16	187.1	54.19	353.8
M_0S_1	6.03	0.16	0.95	153.1	26.20	261.3
M_0S_2	5.92	0.15	0.80	128.8	27.56	152.5
M_0S_3	6.09	0.15	0.83	133.7	28.53	257.5
M_1S_0	6.08	0.16	0.75	121.5	35.82	243.8
M_1S_1	5.76	0.13	0.68	109.4	38.46	258.8
M_1S_2	5.80	0.22	1.05	170.1	55.91	351.3
M_1S_3	6.19	0.13	0.81	131.2	33.50	271.3
M_2S_0	6.10	0.14	0.84	136.1	31.77	255.0
M_2 S_1	6.19	0.17	0.65	104.5	31.83	233.8
M_2S_2	5.92	0.26	0.44	70.5	58.46	252.5
M_2S_3	6.04	0.12	0.45	72.9	34.76	231.3
S Em ± Main	0.06	0.02	0.03	4.5	5.15	NS
Sub	NS	0.05	0.07	11.5	NS	NS
Main x Sub^{-1}	NS	0.08	0.11	17.7	NS	71.3
C.D (P=0.05) Main	0.02	0.01	0.01	1.3	1.46	12.1
Sub	0.08	0.02	0.02	3.9	4.51	13.1
Main x Sub^{-1}	0.12	0.03	0.04	6.0	6.92	23.1

significantly from rest of the treatment combinations except application of 125 % RDF through soil application of organic sources (351.3 kg ha^{-1}). These differences in NPK may be attributed to the movement of applied nutrients in the soil both horizontally and vertically as well as concentration of immobile elements (Sathya *et al*., 2008). The easy availability of water-soluble nutrients right at the root zone of the crop through fertigation in a balanced form through RDF might have favoured better availability of plant nutrients favouring their accumulation in the soil.

Fruit yield

The fruit yield in papaya was significantly influenced by fertilizer doses and fertigation sources (Table 5). Application of 75 % RDF through fertigation recorded significantly higher fruit yield (47.34 t ha^{-1}), which was followed by application of organic sources 125 % RDF (44.37 t ha^{-1}). The increase in yield of papaya was over 31 % with fertigation clearly indicating the relative advantage, which may be attributed to higher nutrient use efficiency resulting in more number of fruits, fruit weight, TSS and lower fruit cavity index.

Jeyakumar *et al*. (2010) reported that, application of 100 % recommended dose of N and K_2O through drip resulted in more number of fruits, fruit weight, TSS and low fruit cavity index with soil application of P_2O_5. Although significantly lower cavity index was observed when RDF was supplied with organics to the soil (3.12%), among the fertilizer dosages, relatively lower cavity index (10.51%) was observed with 125% RDF, while, among the sources of nutrients, soil application of only organic sources resulted in marginally lower cavity index (10.44%).

Table 5 : Fruit yield and quality in papaya with different fertilizer doses and fertigation sources

Treatment	No. of fruits plant^{-1}	Individual fruit weight (kg)	Fruit yield (kg plant^{-1})	Fruit yield (t ha^{-1})	TSS (^{o}B)	Cavity Index (%)
Main plot						
M_0	9.50	0.87	6.12	21.18	10.28	13.97
M_1	21.09	0.69	10.49	32.39	9.61	10.51
M_2	20.91	1.14	10.58	32.66	9.86	12.77
Subplot						
S_0	21.17	0.66	11.95	36.87	9.66	13.74
S_1	13.38	0.92	6.82	21.07	10.44	12.75
S_2	15.94	0.75	9.11	28.91	10.57	10.44
S_3	18.18	1.27	8.38	28.13	9.00	12.75
Interaction						
M_0S_0	19.25	0.70	12.73	39.27	10.10	19.06
M_0S_1	7.88	1.53	5.03	15.53	11.28	21.63
M_0S_2	3.25	0.71	1.73	7.70	11.30	3.12
M_0S_3	7.63	0.56	5.00	22.22	8.45	12.08
M_1S_0	23.50	0.54	7.78	24.00	9.30	11.60
M_1S_1	17.88	0.52	7.61	23.50	9.80	5.21
M_1S_2	21.75	0.89	14.38	44.37	9.38	11.23
M_1S_3	21.25	0.79	12.21	37.69	9.98	14.00
M_2S_0	20.75	0.73	15.34	47.34	9.58	10.55
M_2 S_1	14.38	0.72	7.83	24.17	10.25	11.42
M_2S_2	22.83	0.66	11.23	34.67	11.03	16.96
M_2S_3	25.67	2.47	7.93	24.48	8.58	12.18
S Em ± Main	1.33	NS	0.89	2.75	0.37	2.45
Sub	1.33	NS	1.17	3.62	0.47	2.19
Main x Sub^{-1}	2.39	NS	1.97	6.09	0.79	4.10
C.D (P=0.05) Main	4.69	0.248	3.14	9.72	NS	NS
Sub	3.87	0.276	3.41	10.57	NS	NS
Main x Sub^{-1}	7.44	0.483	5.98	18.53	NS	12.85

Table 6 : The economics of papaya cultivation under different fertilizer doses and sources of fertigation

Treatment	Fruit yield (t ha^{-1})	Gross returns (Rs. ha^{-1})	Total cost (Rs. ha^{-1})	Net returns (Rs. ha^{-1})	B:C ratio
Main plot					
M_0	21.18	3,17,734	2,46,228	71,506	1.28
M_1	32.39	4,85,820	2,58,475	2,27,345	1.87
M_2	32.67	4,89,975	2,34,119	2,55,856	2.08
Subplot					
S_0	36.87	5,53,050	2,54,147	2,98,903	2.21
S_1	21.07	3,15,990	2,26,582	89,408	1.40
S_2	28.91	4,33,675	2,52,532	1,81,143	1.70
S_3	28.13	4,21,990	2,51,833	1,70,157	1.67
Interaction					
M_0S_0	39.27	5,89,125	2,54,148	3,34,977	2.32
M_0S_1	15.53	2,32,980	2,26,582	6,398	1.03
M_0S_2	7.70	1,15,500	2,50,032	-1,34,532	0.46
M_0S_3	22.22	3,33,330	2,54,148	79,182	1.31
M_1S_0	24.00	3,59,955	2,68,238	91,717	1.34
M_1S_1	23.50	3,52,425	2,33,782	1,18,643	1.51
M_1S_2	44.37	6,65,505	2,70,595	3,94,910	2.46
M_1S_3	37.69	5,65,395	2,61,284	3,04,111	2.16
M_2S_0	47.34	7,10,070	2,40,055	4,70,015	2.96
M_2 S_1	24.17	3,62,565	2,19,382	1,43,183	1.65
M_2S_2	34.67	5,20,020	2,36,970	2,83,050	2.19
M_2S_3	24.48	3,67,245	2,40,068	1,27,177	1.53

The treatment combination M_2S_0 (75% RDF) recorded maximum fertilizer use efficiency (20.45 kg of yield /kg of nutrient applied) (Fig. 1). This may be due to the application of nutrients directly to the root zone through fertigation coupled with complete solubility of water soluble fertilizers increasing the efficiency of the applied nutrients. Similar results of 75% N and K when applied through drip recorded on par papaya yield with 100% RDF (Sadaraunnisa, 2010). It was attributed to the better yield components like number of fruits/ plant, fruit weight in the treatments where fertilizers were applied through drip compared to soil application of fertilizers. It was also concluded that since there was no significant difference between 100% and 75% N and K treatments through drip regarding yield and yield attributes, the later dosage is economical over the former.

The TSS in papaya fruits was not influenced significantly either by fertilizer doses and the sources of fertigation or their interaction (Table 5). However, relatively higher TSS was observed when RDF was supplied with organics either through soil (11.30 °Brix) or through fertigation (11.28 °Brix).

The cavity index in papaya was significantly influenced by the interaction of fertilizer doses and fertigation sources. Significantly, lower cavity index was observed when RDF was supplied with organics to the soil (3.12) and it was followed by application

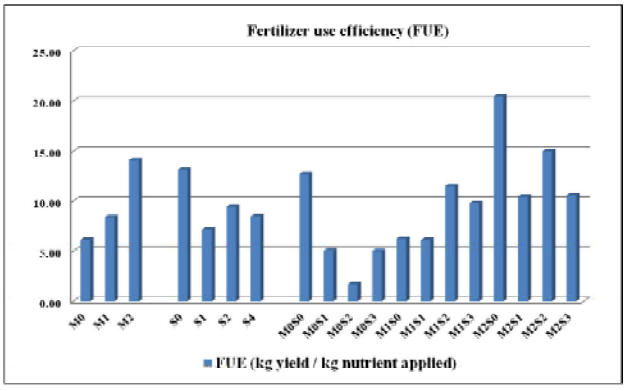

Fig. 1 : Fertilizer use efficiency in papaya as influenced by fertilizer doses and methods

of 125 % RDF through fertigation using organic sources (5.21). The lower cavity index recorded may be attributed to the production of more photosynthates due to more number of leaves and leaf area which might have resulted in better transfer to the sink, the developing fruit with thicker pulp and low cavity index. Jeyakumar *et al.* (2010) also observed that application of 100% recommended dose of N and K_2O through drip resulted in lower cavity index in papaya.

The economics

Fertigation of 75% RDF with inorganic fertilizers was found more economical with higher gross returns (Rs. 7.10 lakh ha^{-1}), net returns (Rs. 4.7 lakh ha^{-1}) and benefit cost ratio (2.96) (Table 6).

The higher net returns with the treatment (M_2S_0) may be attributed to the moderately higher papaya yield (47.34 t ha^{-1}). It was followed by soil application of 125 % RDF through organic sources with better gross returns (Rs. 6.65 lakh ha^{-1}), net returns (Rs.3.94 lakh ha^{-1}) and benefit cost ratio (2.46). In a similar study, Jeyakumar *et al.* (2010) also reported that the increase in number of fruits and fruit weight were attributed for higher fruit yield per tree and the resultant total fruit yield per hectare with high B:C ratio in plants treated with 100 % recommended dose of N & K_2O per plant through drip (50 g N and 50 g K_2O), in addition to soil application of 50 g P_2O_5.

CONCLUSION

The results of field experiment on fertigation in papaya indicated that application of 75% RDF through drip using water soluble fertilizers is beneficial to get higher fruit yield (47.34 t ha^{-1}) with higher nutrient use efficiency and was found economical with higher net returns (Rs.4.7 lakh ha^{-1}) and benefit cost ratio (2.96).

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Original Research Paper

Influence of container, potting media and nutrients on production and post-production consumer acceptance of potted marigold (*Tagetes patula* L.)

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ABSTRACT

Production of potted plants is influenced by factors viz., type of container, potting medium, nutrient dose. A study was conducted to standardize these factors for potted French marigold var. Arka Pari. The treatments comprised of two type of containers (plastic and coir), three potting media [red soil + FYM + sand (1:1:1 v/v), Arka fermented cocopeat (AFC), AFC + vermicompost (1:1 v/v)] and four nutrition concentrations (160:30:180 ppm N:P: K, 128:24:144 ppm N:P: K, 96:18:108 ppm N:P:K and 3% Jeevamrutha) laid out in factorial completely randomized design replicated thrice. Plants grown in potting media combination of Arka fermented cocopeat (AFC) + vermicompost (1:1 v/v) along with weekly application of nutrient solution (128:24:144 ppm NPK) produced maximum number of flowers plant^{-1} (147.61) and registered highest uptake of nitrogen (2.87 g plant^{-1}), phosphorus (0.53 g plant^{-1}), potassium (3.24 g plant^{-1}), magnesium (0.85 g plant^{-1}) and sulphur (0.21 g plant^{-1}). Based on the attributes of the potted plants, this treatment combination also registered the highest score (81.2 on a scale of 100), willingness of the consumers to purchase (4.5 on a scale of 5), overall acceptability (2.7 on a scale of 3) and the benefit cost ratio of 1.18.

Keywords : Consumer preference, container type, nutrition, potted marigold, potting media

INTRODUCTION

Potted plants occupy a sizable share in the floriculture trade both in the global and domestic markets.The indoor plants market was valued at USD 17.93 billion in 2021 and is expected to reach USD 26.23 billion by 2029, at a CAGR of 4.87% during the forecast period of 2022-2029 (Anon., 2022). Besides being a decorative element, potted flowering plants have positive effects on the human psychology and when placed indoors, improves the air quality. Popular flowering potted plants include marigold, petunia, geranium, chrysanthemum, orchids, anthurium and so on. French marigold (*Tagetes patula* L.), is one of the, most versatile, low-maintenance and popular flowering plants that can be grown in beds and as containers.

Production of floriferous and well maintained attractive canopy is imperative in enhancing the aesthetics and consumer appeal of the potted plants. The container type, potting media and the nutrient dose have a considerable effect on the growth, flowering and quality of the potted plants. Among the containers, plastic, ceramic, terracotta, metallic and biodegradable containers like coir pots are used for commercial production. According to Anil and Roshan (2022), the plastic segment was the highest contributor to the flower pots and planters market size, with $328.1 million in 2020, and is estimated to reach $479.6 million by 2030, at a CAGR of 3.6%. Conventional potting media in India is comprised of soil, sand and farmyard manure, whereas in other countries, peat and amended peat substrates were widely used. Problems of compaction, presence of soil borne pathogens in the soil based media and restriction on harvesting of peat due to environmental concerns has increased the need for alternate substrates. Similarly nutrition and in particular, the concentration of each of the major nutrients and the source of nutrients play an important role in the growth and development of the plant. Consumer acceptance and willingness to purchase the product is a key factor in successful production and marketing of the potted plants. The production of potted ornamental plants must be based on consumer preferences (Megersa *et al*., 2018).

Considering all these aspects, the present study was conducted to standardize the three important elements *viz*., container type, potting media composition and the

nutrient dose in potted plant production of marigold and to gauge the consumer preference.

MATERIALS AND METHODS

The study on potted plant production of French marigold var. Arka Pari, under open field conditions, was conducted at the ICAR - Indian Institute of Horticultural Research, Bengaluru, during 2019 and 2020, to standardize the container type, composition of the potting media, the nutrient doses and to evaluate the consumer acceptance of the containerized plants. French marigold var. Arka Pari is a short statured plant with spreading habit, bearing orange flowers and flowering duration is 30 to 45 days.

The treatments comprised of twenty four combinations laid out in factorial CRD design with three replications and ten pots per replication. Three factors *viz*., factor A: type of pots (P_1: 6" plastic pot; P_2: 6" coir pot); factor B: potting media [S_1: red soil + FYM + sand (1:1:1 v/v), S_2: Arka fermented cocopeat (AFC), S_3: AFC + vermicompost (1:1 v/v)]; factor C: nutrition concentration (N_1- 160:30:180 ppm, N_2- 128:24:144 ppm, N_3 - 96:18:108 ppm $N:P_2O_5:K_2O$, N_4 - Jeevamrutha @ 3% were imposed. Secondary and micronutrients were applied uniformly for the treatments N_1, N_2 and N_3. Nutrient application was scheduled at weekly intervals @ 50 ml pot^{-1}. One month old seedlings @ one seedling pot^{-1} were transplanted in the centre of the pot. Need based watering was done at regular intervals, taking into consideration the water holding capacity of the media (governed by the texture and porosity of the media) and the prevailing weather conditions. To encourage canopy spread through induction of more lateral branches, first pinch was done one month after transplanting and it was followed by the second pinching of the lateral branches. Prophylactic sprays of plant protection chemicals was done to check infestation of pest and diseases. Standard procedures were adopted to analyse the physical and chemical properties of the potting media. AFC recorded bulk density of 0.16 Mg m^{-3}; porosity 67.8%; pH 6.75; electrical conductivity 0.5 dSm^{-1}; total carbon 36.1%; total N 0.98%; total P 0.07%; total K 2.20% and Na 0.35%. The average concentration of macronutrients was estimated at 0.58% N, 0.26% P_2O_5 and 0.60% K_2O in FYM. Physical and chemical characteristics of the soil were recorded as bulk density (1.28 Mg m^{-3}); porosity (51.3%); pH (6.97), electrical conductivity (0.26 dSm^{-1}); organic carbon (7.8 g kg^{-1}); available N (0.13 g kg^{-1}); 18 mg kg^{-1} Olsen's P, ammonium acetate (CH_3COONH_4) extractable nutrients are as follow: 0.90 g Ca kg^{-1}, 0.174 g Mg kg^{-1} and 0.15 g K kg^{-1} and DTPA extractable micronutrients as follow: 10.3 mg kg^{-1} Fe, 5.70 mg kg^{-1}Mn, 2.24 mg kg^{-1} Cu and 1.35 mg kg^{-1} Zn. Analysis of N, P, K content and uptake by plant were done. Nitrogen (N) contents in the plant samples were analysed after mineralization with sulphuric acid by Kjeldahl method (Jackson, 1973). Phosphorus, potassium, calcium, magnesium, iron, manganese, zinc and copper were estimated after digesting with a triacid mixture of nitric acid, perchloric acid and sulphuric acid (9:4:1 v/v HNO_3: $HClO_4$: H_2SO_4) as described by Jackson (1973).

Observations were recorded on the vegetative growth, floral parameters and nutrient uptake during the cropping period, pooled and analysed using the OPSTAT statistical package (Sheoran *et al*., 1998). Post-production analysis of the potted plants was done with a sample size of 35 respondents, based on attributes such as cultural perfection (dense foliage, typical colour of cultivar, attractive), form (symmetrical appearance), plant size (height, spread and fullness), flower number (open flowers and buds), flower colour (true to the cultivar, clear, attractive, and free from blemishes) and distinctiveness (desirable characters) by assigning scores for these out of 30,15,15,20,10 and 10, respectively (Beck *et al*.,1985). According to Zeithaml (1988) the consumers' willingness to purchase is affected by objective price, perceived quality, perceived value, and product attributes. Willingness of the consumer to purchase the product was also ascertained by using the scale of 1-definitely would not; 2-probably would not; 3-might or might not; 4-probably would; 5-definitely would. The potted plants were also rated on an overall visual yardstick on a scale of 1 to 3 *viz*., 1-unacceptable; 2-acceptable and 3-visually excellent. The economics of potted plant production for varying container types, potting media and nutrients was calculated and the benefit: cost ratio was worked out.

RESULTS AND DISCUSSION

The canopy and flowering of the potted plants of marigold was influenced by the container type, potting media, nutrients and the interaction effect of these factors.

Container type potting media and nutrients : Pot type has significant influence on the number of leaves at flowering, internodal length, number of flowers plant^{-1} and root spread (Table 1). Plants grown in plastic pots (P_1) recorded the highest number of flowers plant^{-1} (124.54), root spread (18.17 cm), the lowest number of leaves plant^{-1} (54.75) and internodal length (1.94 cm), whereas coir pots (P_2) recorded the highest number of leaves at flowering (57.06), internodal length (2.06 cm), the lowest number of flowers plant^{-1} (112.17) and root spread (15.76 cm). In plastic pots, lesser permeability of the container walls, leading to better water and nutrient retention in the media, might have influenced the rhizosphere environment, contributed to better uptake of water and nutrients and thereby to better growth and development of the plant as compared to coir pots. This is in line with the findings of Evan and Hensley (2004) in *Vinca rosea.*

The number of flowers plant^{-1} was significantly influenced by the potting media composition (Table 1). The treatment S_3-Arka fermented cocopeat + vermicompost (1:1 v/v) produced the highest number of flowers plant^{-1}(123.68), whereas, AFC alone (S_2) recorded the lowest number of flowers plant^{-1} (113.65). This might be due to the fact that the AFC + vermicompost medium does not tend to compact, stores and allows uptake of nutrients, as opposed to the conventional soil based media. Further, the presence of vermicompost, a rich organic source of nutrition, would have contributed to better plant growth and thereby production of highest number of flowers. This corroborates the findings of Rawat *et al*. (2020) in Geranium.

Application of inorganic source of nutrients of varying concentrations and an organic source (Jeevamrutha) recorded significant differences for the number of leaves at flowering, flower diameter and number of flowers plant^{-1} (Table 1). The treatment N_3 - 96:18:108 ppm N:P_2O_5:K_2O, recorded the maximum number of leaves at flowering (59.39) and was at par with N_4 - Jeevamrutha @ 3% (57.02), whereas N_1- 160:30:180 ppm N: P_2O_5: K_2O recorded the minimum number of leaves (52.44). N_1- 160:30:180 ppm N: P_2O_5: K_2O recorded the highest flower diameter (4.48 cm) and number of flowers plant^{-1} (125.87), whereas the minimum flower diameter (4.32 cm) was recorded by application of N_2- 128:24:144 ppm

Table 1 : Influence of type of pot, potting media and nutrients on growth and flowering in marigold var. Arka Pari

Treatment	Plant spread at flowering (cm)	Number of leaves at flowering	Internodal length (cm)	Flower diameter (cm)	Number of flowers plant^{-1}	Root spread (cm)
P_1	33.20	54.75	1.94	4.37	124.54	18.17
P_2	33.45	57.06	2.06	4.41	112.17	15.76
SEm±	0.34	0.63	0.04	0.03	1.61	0.49
CD (P= 0.05)	NS	1.80	0.11	NS	4.58	1.39
S_1	33.24	54.72	1.98	4.37	117.73	16.10
S_2	33.66	55.88	2.03	4.38	113.65	18.36
S_3	33.07	57.11	2.00	4.41	123.68	16.43
SEm±	0.42	0.77	0.05	0,03	1.97	0.60
CD (P= 0.05)	NS	NS	NS	NS	5.62	1.71
N_1	33.41	52.44	2.00	4.48	125.87	16.01
N_2	33.65	54.76	1.92	4.32	117.18	17.81
N_3	33.95	59.39	2.03	4.41	112.78	17.48
N_4	32.28	57.02	2.05	4.33	117.60	16.55
SEm±	0.48	0.89	0.05	0.04	2.28	0.69
CD (P= 0.05)	NS	2.54	NS	0.1	6.48	NS

P_1: 6" plastic pot, P_2: 6" coir pot; S_1: red soil + FYM + sand (1:1:1 v/v), S_2: Arka fermented cocopeat (AFC), S_3: AFC + vermicompost (1:1 v/v)]; N_1- 160:30:180 ppm N:P_2O_5:K_2O, N_2- 128:24:144 ppm N:P_2O_5:K_2O, N_3 - 96:18:108 ppm N:P_2O_5:K_2O, N_4 - 3% Jeevamrutha

N: P_2O_5: K_2O and minimum number of flowers plant^{-1} (112.78) by N_3 - 96:18:108 ppm N: P_2O_5: K_2O. Higher concentrations of the major nutrients resulted in production of maximum number of flowers with larger flower size, which might be attributed to the availability of sufficient amount of nutrients to the plants, as also observed Kang and Van (2004) in *Salvia splendens*. However, it was observed the number of leaves did not increase with the increase in nutrient concentration and was also at par with the organic source of nutrients.

Interaction effect : Significant difference was observed with respect to the number of flowers plant^{-1}and root spread on account of the interaction of three factors *viz*., pot type, potting media composition and nutrients (Table 3), whereas, parameters like plant spread, number of leaves at flowering, internodal length (Table 2) and flower diameter (Table 3) did not vary significantly with these treatment combinations. Maximum number of flowers plant^{-1} (147.61) was produced by the plants grown in plastic pots, on a potting media combination of Arka fermented cocopeat + vermicompost (1:1 v/v) along with nutrient solution of concentration 128:24:144 ppm N: P_2O_5: K_2O ($P_1S_3N_2$) and it was on par with $P_1S_1N_4$ (142.06), $P_1S_2N_1$ (137.28) and $P_2S_1N_1$ (137.83), whereas $P_1S_3N_2$ (94.16) produced the minimum number of flowers plant^{-1}. Plastic pots containing the potting media combination of Arka fermented cocopeat and vermicompost with inorganic nutrient solution of concentration 128:24:144 ppm N: P_2O_5: K_2O produced most floriferous plants, which might be attributed to the walls of the plastic container and the nutrient rich porous potting media, holding adequate nutrients and moisture besides the key factor being the optimum concentration of the major nutrients applied at weekly intervals for the growth and production of the plant. According to Marinari *et al*. (2000) adding vermicompost to container media modifies the soil structure, increases availability of macro and micro-nutrients, stimulates microbial activity, augments production of plant growth-promoting substances by microorganisms through interactions with earthworms. Similar observation was made by Sahni *et al*. (2008) in strawberry. Root spread was significantly highest (23.70 cm) in plants grown in plastic pots on a potting media combination of red soil + FYM + sand (1:1:1 v/v) and nutrient solution of concentration 160:30:180 ppm N: P_2O_5: K_2O ($P_1S_1N_1$), which was on par with

Table 2 : Interaction effect of type of pot, potting media and nutrients on vegetative parameters at flowering stage

Treatment	Plant spread at flowering (cm)						Number of leaves at flowering						Internodal length (cm)					
	P_1			P_2			P_1			P_2			P_1			P_2		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
N_1	31.21	33.28	30.97	38.24	34.62	32.12	50.28	53.06	57.83	48.50	51.33	53.67	1.96	1.88	2.17	2.24	1.93	1.80
N_2	32.19	32.47	35.89	33.98	33.45	33.91	51.83	52.50	51.83	52.34	61.67	58.41	1.81	1.88	1.92	1.97	1.97	1.99
N_3	33.62	33.38	33.46	34.58	34.43	34.23	59.11	52.67	56.95	65.22	59.39	63.00	2.03	1.97	2.00	2.21	2.11	1.85
N_4	32.63	35.99	33.28	29.47	31.65	30.69	58.44	55.94	56.55	52.00	60.50	58.67	2.00	2.01	1.96	1.98	2.24	2.11
	SEm±						SEm±			CD (P= 0.05)			SEm±			CD (P= 0.05)		
P	0.34						0.63			1.80			0.04			0.11		
S	0.42						0.77			NS			0.05			NS		
P X S	0.59						1.09			NS			0.07			NS		
N	0.48						0.89			2.54			0.05			NS		
P X N	0.68						1.26			3.60			0.08			NS		
S X N	0.83						1.55			4.40			0.09			NS		
P X S X N	1.18						2.19			NS			0.13			NS		

P_1: 6" plastic pot; P_2: 6" coir pot; S_1: red soil + FYM + sand (1:1:1 v/v), S_2: Arka Fermented Cocopeat (AFC), S_3: AFC + vermicompost (1:1 v/v)]; N_1- 160:30:180 ppm N:P_2O_5:K_2O, N_2- 128:24:144 ppm N:P_2O_5:K_2O, N_3 - 96:18:108 ppm N:P_2O_5:K_2O, N_4–3% Jeevamrutha

Table 3 : Interaction effect of type of pot, potting media and nutrients on floral parameters and root spread

Treatment	Flower diameter (cm)						Number of flowers per plant						Root spread (cm)					
	P_1			P_2			P_1			P_2			P_1			P_2		
	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
N_1	4.55	4.49	4.42	4.38	4.44	4.62	127.11	137.28	130.67	137.83	116.06	106.26	23.70	18.17	19.27	11.40	11.31	12.23
N_2	4.21	4.44	4.32	4.30	4.36	4.28	114.89	110.33	147.61	108.11	104.44	117.67	21.90	11.53	18.60	17.83	19.20	17.80
N_3	4.33	4.25	4.35	4.70	4.26	4.57	107.67	111.72	118.89	110.00	103.78	124.61	21.57	18.37	13.53	17.00	17.33	17.10
N_4	4.30	4.34	4.40	4.20	4.47	4.28	142.06	121.45	124.78	94.16	104.17	118.95	21.40	17.67	13.67	12.07	17.90	16.58
	SEm±			CD (P= 0.05)			SEm±			CD (P= 0.05)			SEm±			CD (P= 0.05)		
P	0.03			NS			1.61			4.58			0.49			1.39		
S	0.03			NS			1.97			5.62			0.60			1.71		
P X S	0.05			NS			2.79			NS			0.85			2.41		
N	0.04			0.10			2.28			6.48			0.69			NS		
P X N	0.05			NS			3.22			9.17			0.98			2.79		
S X N	0.06			0.18			3.94			11.23			1.20			NS		
P X S X N	0.09			NS			5.58			15.88			1.69			4.82		

P_1: 6" plastic pot; P_2: 6" coir pot; S_1: red soil + FYM + sand (1:1:1 v/v), S_2: Arka Fermented Cocopeat (AFC), S_3: AFC + vermicompost (1:1 v/v)]; N_1 - 160:30:180 ppm N:P_2O_5:K_2O, N_2 - 128:24:144 ppm N:P_2O_5:K_2O, N_3 - 96:18:108 ppm N:P_2O_5:K_2O, N_4–3% Jeevamrutha

$P_1S_1N_2$ (21.90 cm), $P_1S_1N_3$ (21.57 cm), $P_1S_1N_4$ (21.40 cm), $P_1S_3N_1$ (19.27 cm) and $P_2S_2N_2$ (19.20 cm), whereas $P_2S_2N_1$ (11.31 cm) recorded the minimum root spread. This contradicts the findings that cocopeat based substrates encourage better root growth and spread, which might be due to the interaction of all the three factors. The longevity of flowers from bud stage to the end of display stage was assessed (Fig. 1) and the maximum longevity was recorded in the treatment combination $P_2S_2N_2$ (21.4 days), which was on par with $P_1S_3N_2$ (20 days), whereas $P_2S_1N_3$ had the least longevity (15 days). This might be attributed to the optimum dose of nutrients supplied to the plants at weekly intervals and the water and nutrient holding capacity of the potting media and the root spread that must have led to enhanced uptake of the nutrients.

Nutrient uptake: Maximum uptake of nitrogen (2.87g plant^{-1}), phosphorous (0.53g plant^{-1}), potassium (3.24g plant^{-1}), magnesium (0.85g plant^{-1}) and sulphur (0.21g plant^{-1}) was recorded by the plants grown in plastic pots, on a potting media combination of Arka fermented cocopeat + vermicompost (1:1 v/v) along with nutrient solution of concentration 128:24:144 ppm N: P_2O_5: K_2O ($P_1S_3N_2$), whereas the minimum uptake of nitrogen (0.84 g plant^{-1}), phosphorous (0.21g plant^{-1}), potassium (1.02g plant^{-1}), calcium (0.74g plant^{-1}), magnesium (0.25g plant^{-1}) and sulphur (0.07 g plant^{-1}) was recorded by the plants grown in coir pots, on a potting media combination of Arka fermented cocopeat + vermicompost (1:1 v/v) along with nutrient solution of concentration 96:18:108 ppm N: P_2O_5: K_2O ($P_2S_3N_3$) as is evident from Fig. 2 and 3. Profuse flowering and longevity might be attributed to the uptake of optimum amount of nutrients in this treatment combination. This corroborates the findings of Krol (2011) in pot marigold. Micronutrient uptake by the potted plants (Fig. 4 and 5) was the highest (3.29,4.57 and 8.10 mg plant^{-1} of Cu, Zn and Mn, respectively) in the plants grown in plastic pots, on a potting media combination of red soil + FYM + sand (1:1:1 v/v) along with nutrient solution of concentration 128:24:144 ppm N: P_2O_5: K_2O ($P_1S_1N_2$), whereas, the Fe uptake was the highest (34.09mg plant^{-1}) in $P_2S_1N_1$.

Scoring based on attributes, willingness of the consumer to purchase and the overall acceptability: The potted plants were scored based on attributes such as cultural perfection, form, plant size, flower number, flower colour and distinctiveness on a scale of 100 (Fig. 6 and 7).

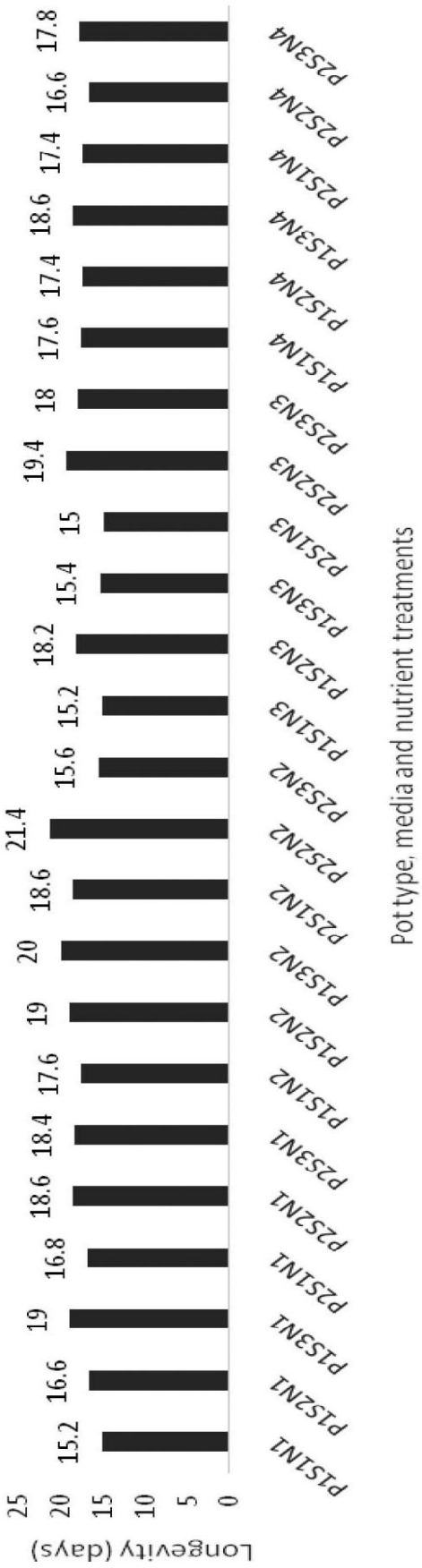

Fig. 1 : Effect of pot type, potting media and nutrients on longevity of individual flower on the plant

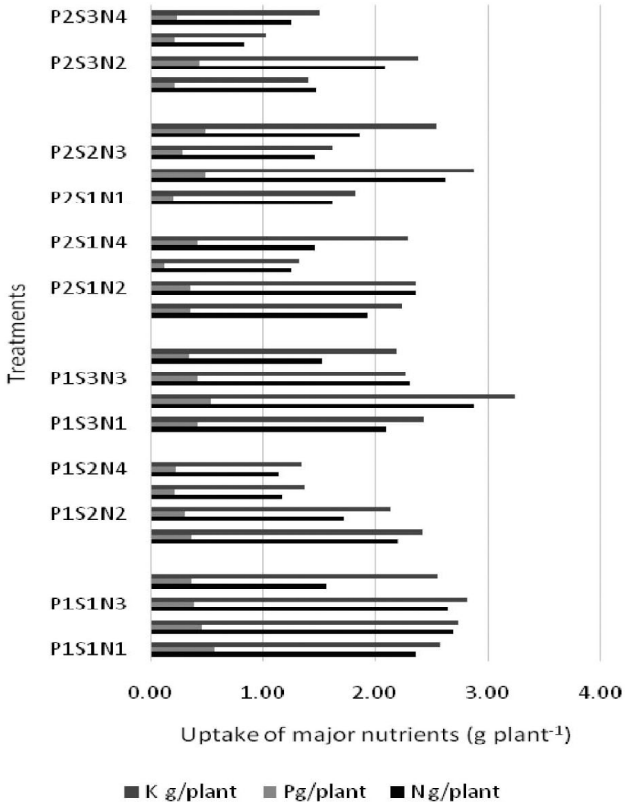

Fig. 2 : Effect of pot type, potting media and nutrients on uptake of major nutrients

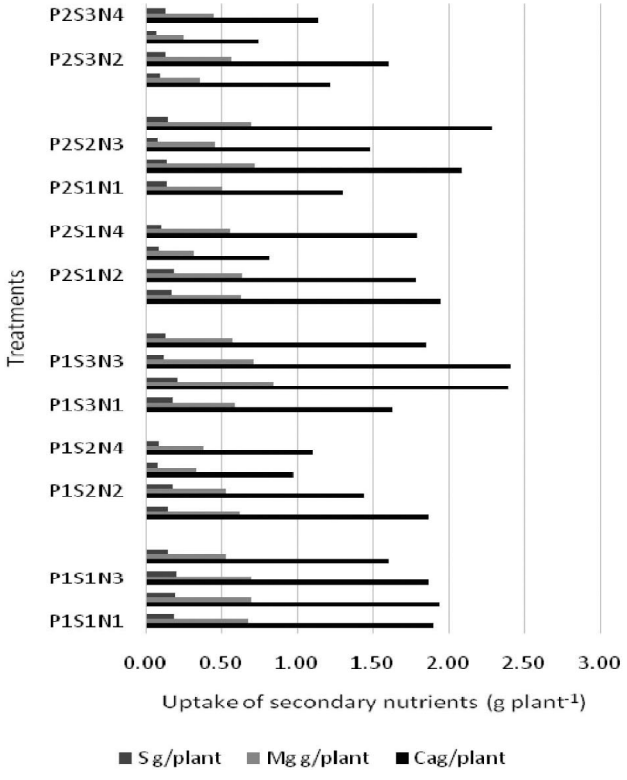

Fig. 3 : Effect of pot type, potting media and nutrients on uptake of secondary nutrients

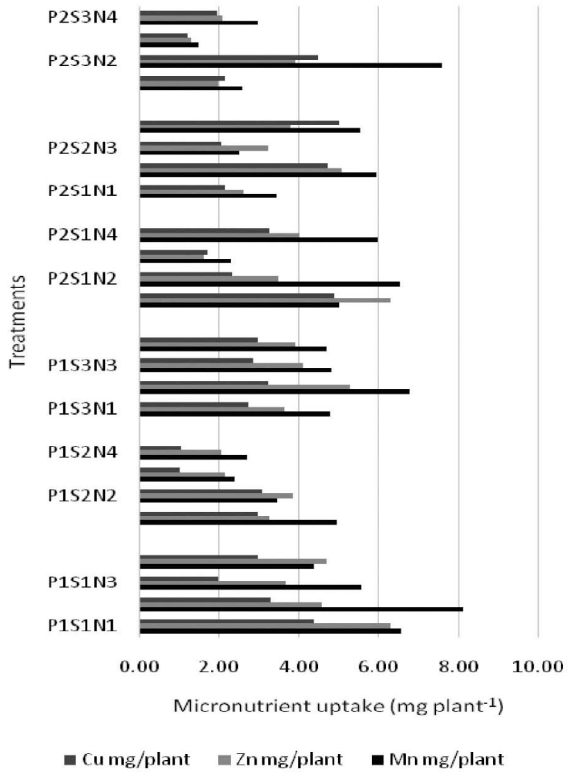

Fig. 4 : Effect of pot type , potting media and nutrients on uptake of Cu, Zn and Mn.

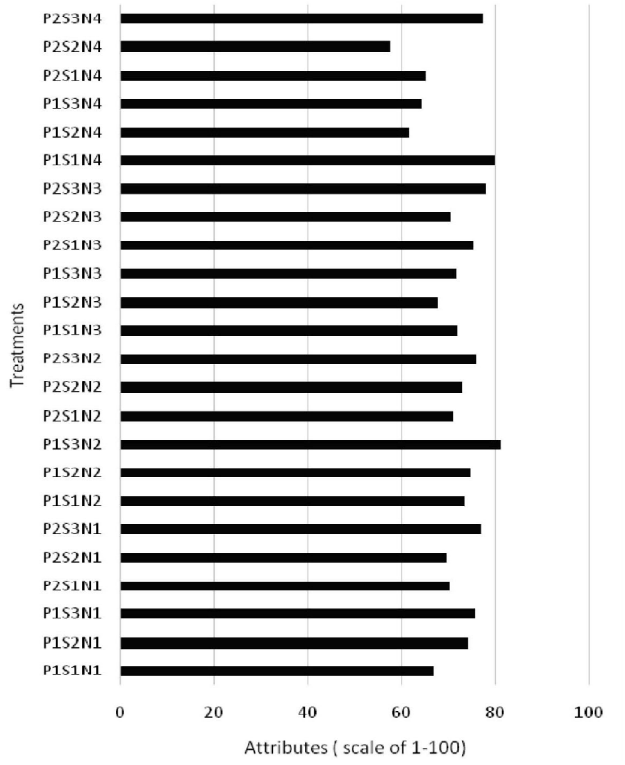

Fig. 6 : Effect of pot type, potting media on nutrient on attributes of consumer to purchase

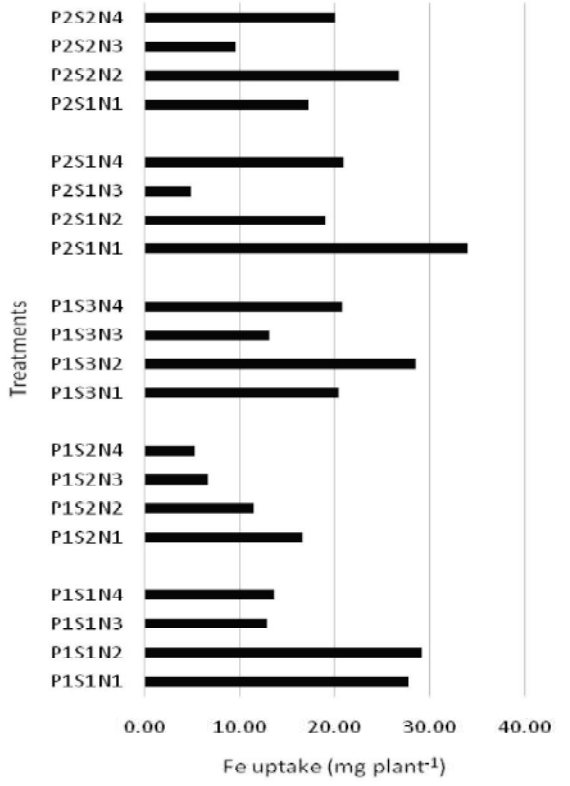

Fig. 5 : Effect of pot type, potting media and nutrients on uptake of Fe

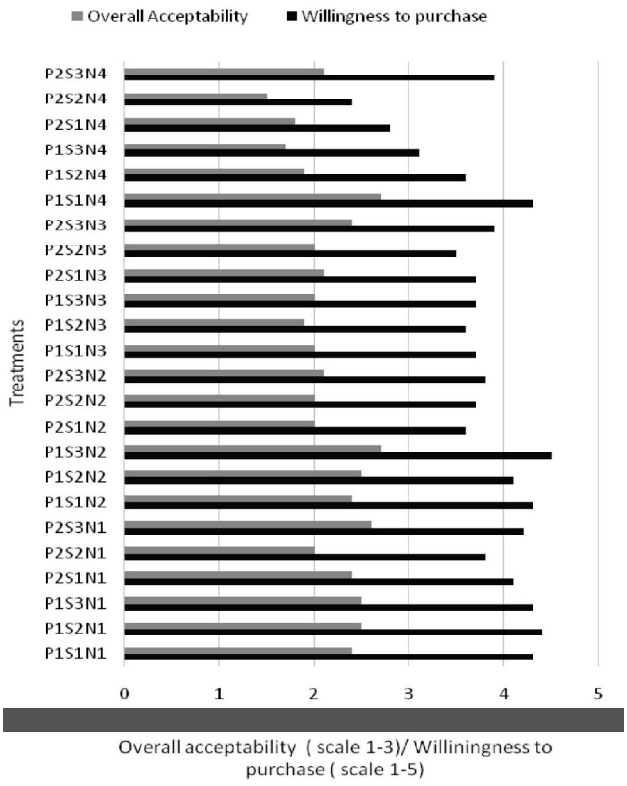

Fig. 7 : Effect of pot type, potting media and nutrients on scores based on the consumer to purchase

Plants grown in plastic pots on potting media combination of Arka fermented cocopeat + vermicompost (1:1 v/v) along with nutrient solution of concentration 128:24:144 ppm N: P_2O_5: K_2O ($P_1S_3N_2$) scored the highest (81.20), whereas, plants grown in coir pots, on a potting media combination of Arka fermented cocopeat along with 3% Jeevamrutha ($P_2S_2N_4$) registered the lowest score (57.70). Quality depends on the shape, size, colour of flowers and leaves, and number of flowers (Noordergraaf, 1994; Wang *et al*, 2005). Based on the consumers' willingness to purchase on a scale of 1-5, the same treatment combination $P_1S_3N_2$, registered the highest score of 4.5, and $P_2S_2N_4$ recorded the lowest score of 2.4. The overall acceptability based on visual appearance was assessed on a scale of 1-3. On visual assessment also $P_1S_3N_2$ and $P_1S_1N_4$ registered the highest score of 2.7 and $P_2S_2N_4$ recorded the lowest score of 1.5. According to Ferrante *et al*. (2015) improvement of visual quality of potted plants plays a key role in increasing the sale. Coir pots had poor mechanical properties and resulted in a faster degradation and tended to rupture during handling, transport and marketing. Besides, roots also protruded from the pot walls in some treatments with discolouration of the pots.

Economics: Plants grown in plastic pots on Arka fermented cocopeat alone along with nutrient solution of concentration 160:30:180 ppm N: P_2O_5: K_2O ($P_1S_2N_1$) recorded the highest benefit cost ratio of 2.03, however the number of flowers plant^{-1} and the consumer acceptance scores were lower for this treatment combination. The best performing treatment combination with respect to floriferousness, cultural attributes, willingness of the consumer to buy and overall visual acceptability ($P_1S_3N_2$) recorded a benefit cost ratio of 1.18 (Fig. 8). Coir pots increased the cost of production and have to be retailed at higher prices as compared to the plastic pots. Selling price of Rs.70 per coir potted plant resulted in B: C ratios ranging from 0.37 to 0.63.Willingness of the customer to purchase the coir pots depended on their purchasing power and concern for the environment by using eco-friendly products.

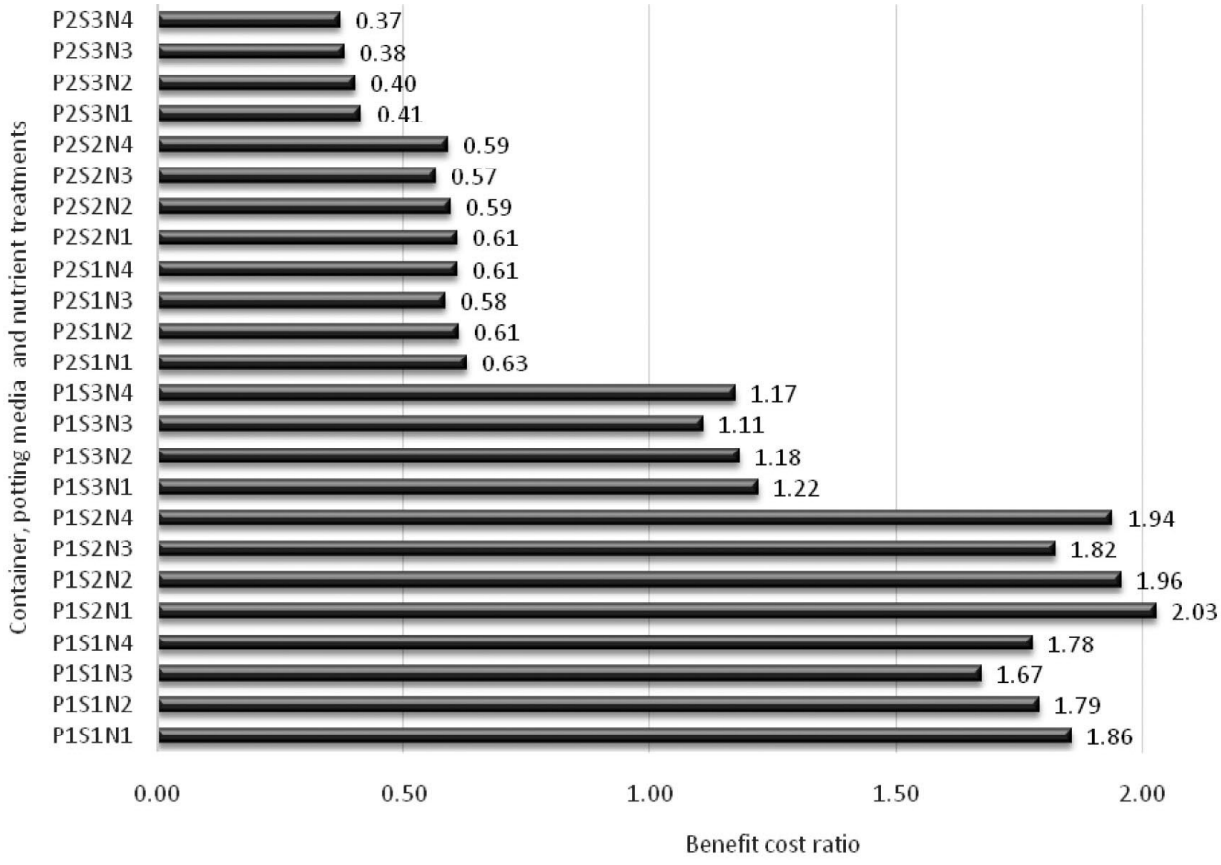

Fig. 8 : Effect of pot type, potting media and nutrients on the economics

CONCLUSION

From the present study, it can be concluded that potted plant production of marigold (*Tagetes patula* L.) can be taken up on a commercial basis by nurserymen in plastic pots on a potting media combination of Arka fermented cocopeat+ vermicompost (1:1 v/v) supplemented with weekly application of nutrient solution of 128:24:144 ppm N: P_2O_5: K_2O @ 50 ml pot^{-1}. This combination produced highly floriferous plants with attractive form and shape and had the highest consumer preference and overall visual acceptability.

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Original Research Paper

Impact of carbohydrate metabolism pathways on bearing habit of mango (*Mangifera indica* L.) genotypes

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ABSTRACT

Heterozygosity is the major constraint in perennial fruit crop like mango for regular bearing breeding. Majority of the popular mango varieties have irregular bearing habit. Many external and internal factors affect the bearing habit of perennial fruit crops. Among internal factors, the level of carbohydrate reserves and phytohormones plays a major role on bearing habit of fruit crops like apple, citrus, mango, litchi *etc*., Therefore, present research work aimed to study the carbohydrate metabolism pathways in regular and irregular mango genotypes of varying origin. A total of 30 primers were designed using *in silico* mining of four key genes coding for citrate synthase, alcohol dehydrogenase, sucrose phosphate synthase and trehalose phosphate synthase. These genes play important role in sugar and starch metabolism in mango. Of these specific primers, 14 showed polymorphism among the genotypes studied. Gene diversity (GD), average number of alleles per locus (An), polymorphism information content (PIC) and major allele frequency (Maf) observed were 0.45, 2.14, 0.35, 0.59, respectively. Simple sequence repeats markers grouped 63.15% studied mango genotypes of regular bearers together. Further, these markers could be utilized in a greater number of genotypes for regularity.

Keywords : Carbohydrate metabolism, irregular bearing, mango, molecular markers

INTRODUCTION

Mango (*Mangifera indica* L.), belongs to the family Anacardiaceae, has an important place among the fruits of the world and is popularly called as king of fruits in India because of its wide uses and nutritional qualification. It is the most widely cultivated tropical fruit species in India and its cultivation also spread to other tropical and subtropical parts of the world. It occupies the highest area of 2,317 thousand ha among fruit crops and contributes 20, 386 thousand metric tons fruit production (NHB, 2020-21) in India. Globally, Asia accounts for 75% of world mango production. Whereas, India holds first rank among world's mango producing countries with a share of 38 percent in total world's mango production (FAOSTAT, 2019). In India, most of the commercial cultivars behave as irregular bearers in north Indian conditions whereas produce regular crops under south Indian conditions (tropical climate). The irregular bearing behaviour of mango is the major obstacle in getting good yields during "off-year" cropping. Irregularity in mango crop bearing is may be due to different factors like C:N ratio, hormonal imbalance, *etc*. Carbohydrate metabolism plays a very important role in bearing behaviour of fruit crops (Fischer *et al*. 2012). Carbohydrates reserves depicted as the key energy producing chemicals which play important role in floral induction process in many crop species (Wahl *et al.,* 2013). Draining out of carbohydrate and nitrogen reserves during "On" year is known to lead to a lean crop in the "Off" year as they are important for fruit bud initiation i.e., high C/N ratio helps for fruit bud initiation (Sharma *et al*., 2019, 2020). It is well studied about the catalytic activity of the enzymes Sucrose Phosphate Synthase, Trehalose Phosphate Synthase, Citrate Synthase, Alcohol Dehydrogenase in carbohydrate metabolism of plants (Brownleader, 1997). The genes related to the enzymes of carbohydrate metabolism (Trehalose phosphate synthase, Sucrose phosphate synthase, Citrate synthase, Alcohol dehydrogenase) have been studied

by many researchers (Eldik *et al*., 1998, Coleman *et al*., 2010, Wahl *et al.,* 2013, Han *et al*., 2017, Benny *et al*., 2022) for their role in flowering related process of different plant species. Differential expression of the genes coding for sucrose synthase, sucrose phosphate synthase1, ATP synthase, polyphenol oxidase and auxin response factor are reported in the floral buds of mango cultivars Dashehari, Langra, Chausa and Amrapali (Bajpai *et al*., 2021). Carbohydrates levels in plants are generally analyzed by biochemical methods, recent advances in molecular biology and biotechnology fields helps us to find out the genes related to carbohydrate metabolism. In our present research we have designed carbohydrate metabolism specific primers for validation of regular and irregular bearing genotypes.

MATERIALS AND METHODS

The present experiment was carried out on 19 genotypes of mango (Mango Field Gene Bank, IARI) of varying origin and bearing habit *viz*., 9 hybrids (regular bearer) released from ICAR-Indian Agricultural Research Institute, New Delhi (Pusa Arunima, Pusa Surya, Pusa Peetamber, Pusa Lalima, Pusa Shresth, Pusa Manohari, Pusa Deepshikha, Amrapali and Mallika), six irregular bearer genotypes namely Dashehari, Kesar, Alphonso, Bombay Green, Langra, Chausa, two south Indian genotypes of regular bearer namely Totapuri and Neelum and two exotic genotypes viz; Tommy Atkins and Sensation. During the course of investigation, blocks were maintained as per the recommended cultural practices. New flushing and healthy leaves from single tree of each genotype were plucked, put into labelled polyethylene bags and placed in an icebox. Samples were wrapped in aluminium foil, tagged properly, frozen in liquid nitrogen for a few seconds, and stored at –80°C until DNA extraction. Genomic DNA was extracted by the cetyltrimethylammonium bromide (CTAB) method with some modifications (Doyle and Doyle 1987).The genomic DNA was further purified by successive RNase treatment followed by phenol: chloroform extraction. The pellet dissolved in TE buffer and stored at -20 °C temperature. The quality of the extracted DNA was assessed by agarose gel electrophoresis and quantified using Nanodrop 8000 spectrophotometer (Thermo Scientific, USA).

A total of 4 key gene sequences coding for Trehalose phosphate synthase, Sucrose phosphate synthase, Citrate synthase and Alcohol dehydrogenase of *Mangifera indica* L. were retrieved from National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov). These gene nucleotide sequences play important role in carbohydrate metabolism. A total of 30 primers were synthesized for wet lab validation. Carbohydrate metabolism genes coding for Trehalose phosphate synthase, Sucrose phosphate synthase, citrate synthase, Alcohol dehydrogenase generated 9, 10, 5 and 6 primers, respectively. Nucleotide accession number GU233771, GU233770 and GU233769 of *alcohol dehydrogenase* gene of M. *indica* L. var. Dashehari was used for simple sequence repeats (SSRs) mining. A total of 10 SSRs were identified and 6 primers were synthesized. For *citrate synthase* gene JN001196, XM_044609816, XM_044609329 nucleotide accession of mango varieties Jinhuang and Alphonso were used for SSRs mining. A total of 5 primers were generated from identified 5 SSRs sequences. *Trehalose phosphate synthase* gene (nucleotide accession number MH759789) sequence of mango variety Kensington Pride resulted into 13 SSRs and a total of 9 primers were generated. *Sucrose phosphate synthase* gene (nucleotide accession number AB724402, AB724401, AB724400 and AB724399) sequences of mango varieties namely N-13, Cat Trang, Glenn, Valencia Pride resulted into 25 SSRs sequences and a total of 10 primers were generated. Primer 3 software (www.frodo.wi/mit.edu/primer3) was used for primer designing. PCR was carried out in 10µl reaction mixture containing 0.5µl each primer (10 pico mole each of forward and reverse), 2 µl of 25ng/µl genomic DNA as template and 5µl of *Taq* polymerase buffer 2X master mix (G Bioscience, USA). The volume was made up to 10µl with sterile distilled water. Thermocycling was carried out in a PE-Thermo cycler (C1000 Touch Thermal cycler, Bio-Rad, USA). Initial denaturation carried out at 94 °C for 5 minutes followed by 35 cycles (denaturation at 94°C, annealing at 55 ° C and extension at 72°C for 1 minute). Final extension was carried out at 72°C for 10 minutes. PCR amplified products were resolved in 3% high resolution agarose gels (Sisco Research Laboratories Pvt. Ltd). Electrophoresis was carried out at 120 V for 3 to 4 hours. DNA profiles were visualized on UV trans-illuminator and photographed on gel documentation system (Alpha Innotech, USA). Power Marker 3.5 was used to calculate gene diversity, heterozygosity and polymorphic information content of the markers (Liu and Spencer, 2005).

RESULTS AND DISCUSSION

A total of 30 carbohydrate metabolism specific markers were designed (Online Resource 1). Carbohydrate metabolism genes coding for Trehalose phosphate synthase, Sucrose phosphate synthase, Citrate synthase, Alcohol dehydrogenase generated 9, 10, 5 and 6 primers, respectively (Online Resource 1).These markers were validated in 19 mango genotypes. Genomic DNA yield was found varied in all 19 studied mango genotypes and highest yield in Totapuri (1360.30 ng/µl) and lowest in Pusa Surya (405.80 ng/µl) with 752.91 ng/µl average yield. The average value of DNA quality on the basis of nanodrop reading (A260/280) was 1.68 and maximum value was found in Pusa Surya (1.79) and minimum value was found in Dashehari (1.65). However, A260/230 ratio was found maximum in Pusa Shresth (2.07) and minimum in Neelum (1.83) with 1.90 average values. A total of 14 markers were found polymorphic (Table 1). Agarose gel profile of mango genotypes using *alcohol dehydrogenase* gene-based primer NMAD1 shown in Fig. 1. The major allelic frequency (Maf) ranged from the 0.44 to 0.94 among the markers with a mean value of 0.59 per locus. The marker NMSPS4 had the highest allelic frequency (0.94), while NMTPS7 had the lowest value (0.44). Further, among all primers, maximum and minimum PIC value was found in NMTPS7 primers (0.49) and NMSPS4 (0.09), respectively. However, average PIC value was 0.35 per locus. The gene diversity of the primers was calculated which ranged from 0.09 to 0.58 with an average of 0.45 per locus. The NMTPS7 had the highest gene diversity (0.58), while the lowest value (0.09) was recorded in NMSPS4. The observed heterozygosity among primers was also estimated, which varied from 0.10 to 1.00 with an average value of 0.67 per locus.

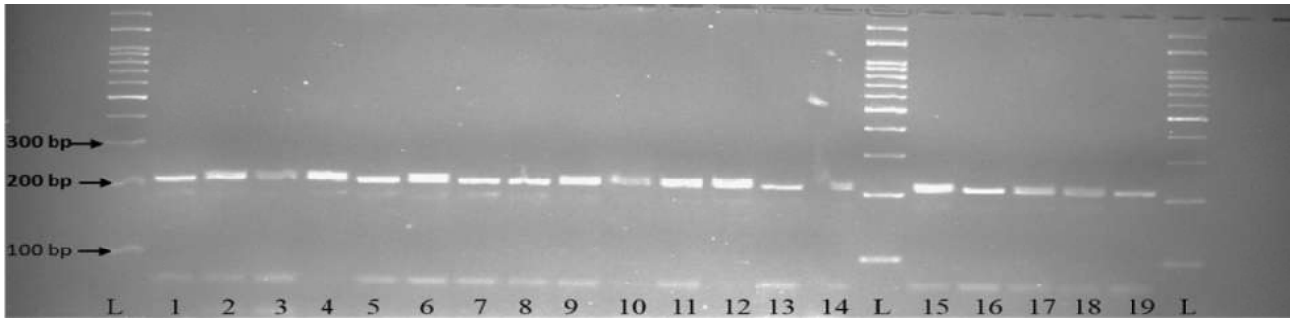

Fig. 1 : Agarose gel profile of mango genotypes using *alcohol dehydrogenase* gene-based primer NMAD1
L- 100 BP Ladder, 1. Pusa Arunima, 2. Pusa Surya, 3. Mallika, 4. Tommy Atkins, 5. Sensation, 6. Neelum, 7. Totapuri, 8. Pusa Shreshth, 9. Pusa Deepshikha, 10. Amrapali, 11. Pusa Manohari, 12. Pusa Peetamber, 13. Pusa Lalima, 14. Kesar, 15. Dashehari, 16. Bombay Green, 17. Langra, 18. Chausa, 19. Alphonso, L- 100 BP Ladder

Table 1 : Genetic variability indices of the 14 polymorphic carbohydrate metabolism specific primers among the set of 19 mango genotypes

Marker ID	Maf	An	GD	Ho	PIC
NMAD1	0.7105	2.0000	0.4114	0.5789	0.3267
NMAD2	0.5000	2.0000	0.5000	1.0000	0.3750
NMAD3	0.6579	2.0000	0.4501	0.6842	0.3488
NMAD4	0.5526	2.0000	0.4945	0.7895	0.3722
NMAD5	0.5000	2.0000	0.5000	0.8947	0.3750
NMAD6	0.6579	2.0000	0.4501	0.5789	0.3488
NMCS1	0.5526	2.0000	0.4945	0.5789	0.3722
NMCS2	0.4737	2.0000	0.5485	0.7368	0.4453
NMCS3	0.5000	3.0000	0.5000	0.5789	0.3750
NMSPS4	0.9474	2.0000	0.0997	0.1053	0.0948
NMSPS5	0.5526	2.0000	0.4945	0.8947	0.3722
NMSPS7	0.5789	2.0000	0.4875	0.7368	0.3687
NMTPS1	0.7105	2.0000	0.4114	0.5789	0.3267
NMTPS7	0.4474	3.0000	0.5886	0.6842	0.4997
Mean	0.5959	2.1429	0.4593	0.6729	0.3572

Foot note required? Maf, An, GD, Ho, PIC

A dendrogram generated based on molecular data grouped all the 19 genotypes of mango into one major cluster B and one out group A. Major cluster B, comprised most of the studied genotypes and further sub-divided into two clusters as B1 and B2. Cluster B2 further sub-divided into cluster B2.1 and B2.2. Only the Amrapali and Pusa Arunima genotypes were found in sub-cluster B2.1. Most of the mango genotypes (89.46%) come under sub-group B2.2 (Table 2). Genetic tree showed the relatedness among the studied mango genotypes (Fig.2). Operational taxonomic units (OTU) for all combinations given in Online Resource 2.

Table 2 : Distribution of mango genotypes into groups based on carbohydrate metabolism specific markers

Cluster	Alternate bearing genotypes (Bombay Green, Kesar, Dashehari, Alphonso, Langra, Chausa)	Regular bearing genotypes(Tommy Atkins, Pusa Arunima, Amrapali, Totapuri, Pusa Shreshth, Pusa Peetamber, Pusa Lalima, Pusa Deepshikha, Pusa Manohari, Neelum, Mallika, Pusa Surya, Sensation)	Total
A	1(5.2%)	0	5.2%
B.1	0	1 (5.2%)	5.2%
B.2	5(26.31%)	12 (63.15%)	89.46%

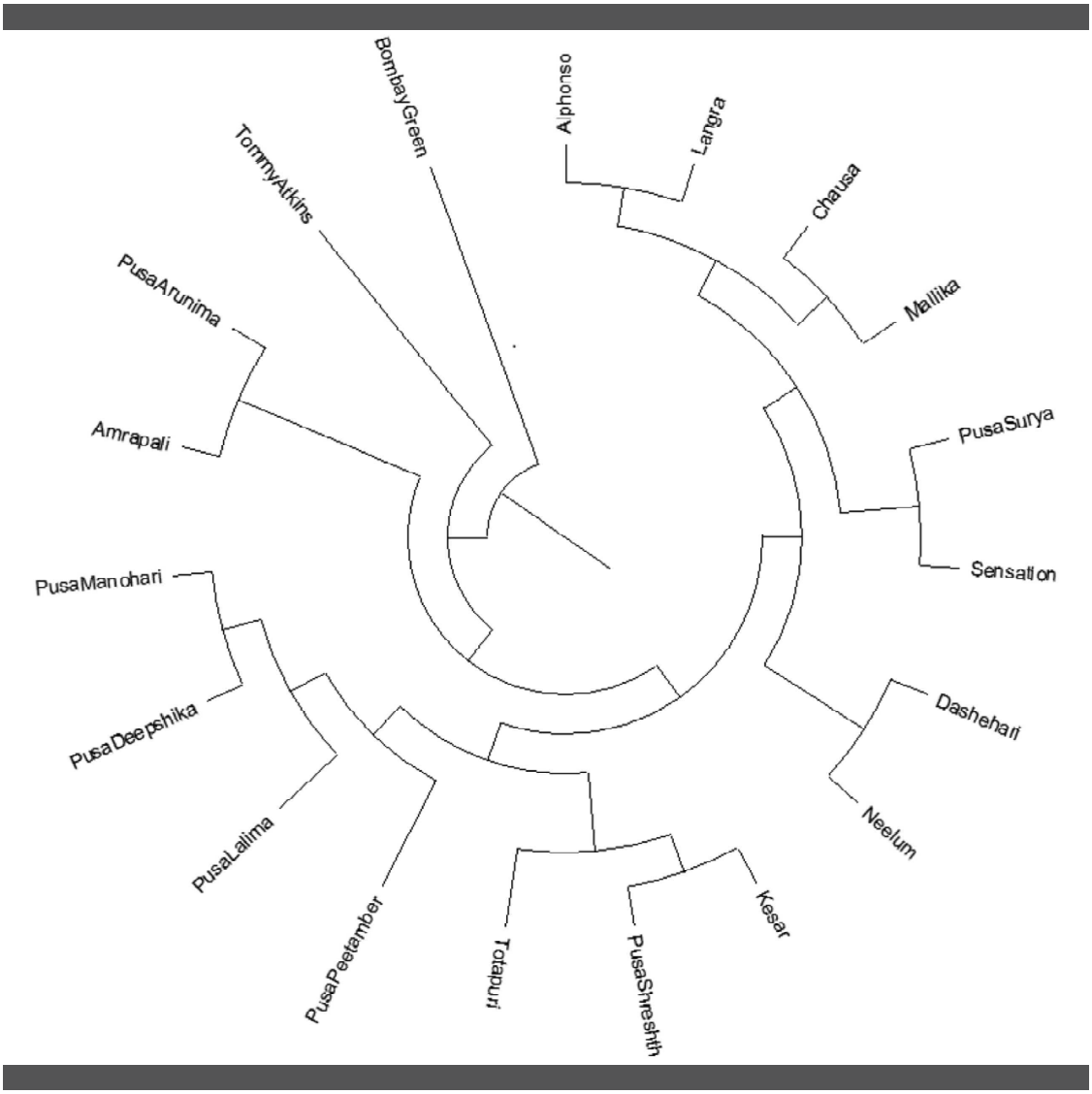

Fig. 2 : Genetic tree of 19 mango genotypes using carbohydrate metabolism specifc primers

The SSR markers were used with a view to characterize and analyze the 19 mango genotypes with respect to bearing habit (regular or alternate bearing) of mango tree. Out of 30 SSR markers used, 14 were found polymorphic. PIC values aid in forecasting the potential use of DNA markers for genotypes assessment in molecular breeding. Markers with high PIC values (NMTPS7) have greater potential in showing allelic variation according to Spandana (2012) findings in Sesamum crop. And our SSR markers exhibited lower level of gene diversity (0.45). Low level of genetic diversity indicates the frequent use of only few parents in breeding among selected cultivars (Kumar *et al*., 2013). Though the dendrogram in the present study did not indicate very clear pattern of clustering according to the bearing habit. The cluster B2 consists of 63.15 % regular bearing genotypes as one group which may indicate that these markers have some potential to use and to improve in future studies for differentiating mango cultivars based on their bearing nature.

CONCLUSION

For characterization and evolution of mango genotypes with respect to bearing habit, SSR markers can be used as they are globally accepted for their efficient and effective management and analysis of the genetic diversity of the germplasm. Though clustering is not clear in dendrogram, our markers grouped more than 60 % regular bearing cultivars in one cluster which need further improvement in future studies. Therefore, the research work further will be helpful in selection of suitable recombinants and hybrids having regular bearing habit in early nursery stage itself overcoming the problem of long gestation periods and other economic constraints.

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Original Research Paper

Effect of maturity stages on the quality indices of wood apple (*Feronia limonia*) and modeling of its kinetics by applying machine learning approaches

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ABSTRACT

In the present investigation, an inexpensive and non-destructive method was tested for the appropriate maturity classification of wood apple (*Feronia limonia*). The investigation was conducted to establish the pronounced effect of maturity stages on the growth kinetics, physico-chemical properties, and other quality indices of wood apple. A systematic trend was observed for all the properties namely sphericity, bulk density (g/cm^3), true density (g/cm^3), pH, total soluble solids TSS (°Brix), titratable acidity (%) and TSS/TA ratio, *etc*. of the fruit. In contrast, regular changes were also observed in the color properties at various maturity stages of the wood apple. The maturity kinetics was formulated by applying recurrent neural network (RNN) in compliance with K means cluster algorithm. RNN modeling was applied by considering color property (redness value) as input and six maturity indices as the output of the formulated structure. The RNN architecture, 1-6-6 showed the best results for forecasting the wood apple maturity based on color features. Further, based on the results of the K means cluster algorithm, the maturity stages were classified into three main categories, illustrated in the form of a simplified color chart. Hence, this investigation can be useful for proper control and identification of wood apple maturity during the processing.

Keywords : Bio-chemical properties, K-means cluster algorithm, maturity stages, wood apple

INTRODUCTION

Fruits play a vital role in balancing diet. It is a widely recommended diet in daily intake of meals (Slavin and Lloyd, 2012). There is a sharp demand and increased interest in fruits, as their consumption appears to reduce certain chronic diseases. In terms of fruit production, India stands second in the world after China. The total area was reported to be 25.43 million hectares and total production was approximately 311.71 million tonnes in the year 2017 - 2018, which is one of the leading positions in the world (Anon., 2018). Despite the fact that India is among the leading position in the world in horticultural crop production, it seems inadequate. The reason behind this may be unscientific ways of processing perishable horticultural products. Therefore, it is high time that all the fruits and vegetables be processed in such a way that all parts including waste of them can be brought to maximum utilization.

Wood apple (*Feronia limonia*), a non-commercial and commonly found fruit is still under-utilized and has great potential in terms of health benefits but it lacks in-depth scientific knowledge. It belongs to *Rutaceae* family and is native to India (Mani and Mitra, 2020). The seedlings of the fruit are seen growing naturally in scattered and isolated at various agro- climatic zones (Yadav *et al*., 2018b). It is generally round in shape and turns brown to yellow when mature. The soft yellow pulp has net-like protective fiber with numerous small white seeds scattered. The average length, width, and thickness are 8.92 cm, 8.22 cm, and 7.95 cm respectively (Sonawane *et al*., 2020). The fruit has a heavy hard protective shell that comprises of 55.67% of the total weight of the fruit (Devi and Kulkarni, 2018) and an edible portion of 42.9 to 60.60% (Sharma *et al*., 2014). It contains properties that are of medicinal importance (Bobade *et al*., 2020), nutritionally rich (Yadav *et al*., 2018a), and rich in iron, protein, and minerals (Rao *et al*., 2011), and many more to count. The titratable acid and pectin

content increases with maturity and thereafter decreases (Kumar and Deen, 2017). It can be processed into various food items, its pulp can also be dried effectively at 70°C and milled to powder for value addition (Goyary *et al*., 2021).

The palatability of fruit in terms of its quality is a closely linked factor that has an effect on the consumer perspective and can be correlated with fruit maturity and harvesting (Gupta *et al*., 2020). Ripening is the critical phase where fruits attain their desirable composition and makes fruit palatable. In the northeastern part of India, wood apples can be harvested from mid of November to the end of March. It has a longer period of duration of harvesting as compared to other fruits. The flesh has an appetizing flavor and tastes sour-sweet. The combination of excellent flavor, nutritive value, and medicinal characteristics possess great potential for processing into valuable products. There are various parameters and indices that are used to determine the harvesting of fruits. The degree of ripening of fruit can be determined by the colors charts that express color in terms of L*, a* and b* values in numerical forms along the axes (from white to black, green to red, and blue to yellow, respectively) that can be combined mathematically for calculating the color indexes (López and Gómez, 2004). A fruit ripening chart can be prepared for monitoring the ripening stages by using L*, a*, and b* and the RNN mechanism. Based on the color properties of the ripening stages, a color chart can be prepared using L*, a*, and b* and various other qualities by using RNN (Gupta *et al*., 2020). In present research work, an attempt was made to classify the maturity stages of wood apples using a clustering algorithm (CA) and mapping of color properties versus various physico-chemical properties by implementing a recurrent neural network (RNN). This study will ultimately provide a brief idea to the cultivators that the fruit has attained its maximum maturity level and its pulp has softened enough and is ready to be harvested.

MATERIALS AND METHODS

In this research work, 100 healthy fruits were harvested thrice in a season first at the initial fruit development phase, second at its peak period of maturity, and third at the final ripe. Each harvested lot was taken for determining its various physico-chemical properties, and color properties. After that, the properties were mapped for maturity kinetics and its integrated simulation with physico-chemical properties.

Determination of physico-chemical properties

Weight, arithmetic mean diameter, and sphericity ratio

The fruit sample was randomly collected from different trees around Assam University Silchar campus. One hundred fruits were taken as a sample size for analysis. The weight (mass) of each fruit was measured with electronic weight balance (Scale-tec brand, capacity 1 kg and 0.1 mg precision). The arithmetic mean diameter (D_a) was measured using a vernier caliper (Kristeel Precision brand, 0 to 150 mm range and least count 0.01 mm) along the X, Y, and Z axes and calculated using the Equation 1. (Mohsenin, 1970; Bayram, 2005) and the sphericity ratio (φ) was calculated using Equation. 2 (Mansouri *et al*., 2017).

$$D_a = \frac{X \times Y \times Z}{3} \quad (1)$$

$$\emptyset = \frac{(X \times Y \times Z)^{\frac{1}{3}}}{X} \quad (2)$$

Bulk density (ρ_b)

The bulk density of wood apple was determined as per the standard procedure. A rectangular box container of 0.36 m × 0.28 m× 0.95 m volume was used. Firstly, the container was weighed empty to determine its mass, and then secondly it was filled with wood apple and weighed once again. The mass of the empty container was deducted and then the actual mass of the wood apple was divided by the volume of the container (Khalloufi *et al*., 2010). The bulk density was calculated by using Equation. 3.

$$\rho_b = \frac{\text{Weight of sample in container}}{\text{Volume of container}} \quad (3)$$

True density (ρ_t)

It is the density excluding the pores of the mass. The true density of wood apple was determined by the displacement method (Tscheuschner, 1987) and calculated by the Equation. 4.

$$\rho t = \frac{\text{Mass of the sample}}{\text{Volume of toluene displaced}} \quad (4)$$

pH

The pH of the wood apple pulp was measured with a pocket pH meter (PAL-pH). Prior to the test pH meter was calibrated against standard buffer solutions of known hydrogen ion activity. The pulp of wood apple was put at the sensor unit and the result was displayed on the display unit with two decimals. The method was followed according to Karastogianni *et al*. (2016).

TSS (°Brix)

Total soluble solids were measured using a pocket refractometer (LABART ERMA). The pulp sample of wood apple was put in the sample holder and the °Brix was recorded (AOAC, 2005; Jamil *et al*., 2010).

Titratable acidity

The titratable acid was determined by titration method. The pulp of the wood apple was titrated with 0.1 N NaOH using phenolphthalein indicator (AOAC, 2005). The result was expressed in terms of percentage of malic acid equivalent (gram of malic acid equivalent per 100-gram fruit pulp weight) (Ranganna, 1986; Zhang *et al*., 2005).

TSS/TA ratio

The ratio of total soluble solids to titratable acidity (TSS/TA) was calculated by dividing TSS by TA (Su *et al*., 2013).

Color properties determination of wood apple

The color properties of the wood apple were determined by using Image J software. For the measurement of color properties, the image was captured by using an HD camera from six different angles of the fruit. L*, a*, b* values were considered for estimation of the color properties. The methodology for the image processing of wood apples is illustrated in Fig.1. The method of image analysis was followed according to Mohammadi *et al*., (2015) with minor modifications such as increasing the megapixel of the camera lens and widening the capture angle.

Classification of maturity stages by using K-means cluster algorithm

For finding the clusters, N points were randomly selected from the "dataset" (database) for initializing the K-means algorithm. The appropriate operation for allocating and renovating the clusters was, to establish up to their convergence or to the point where they

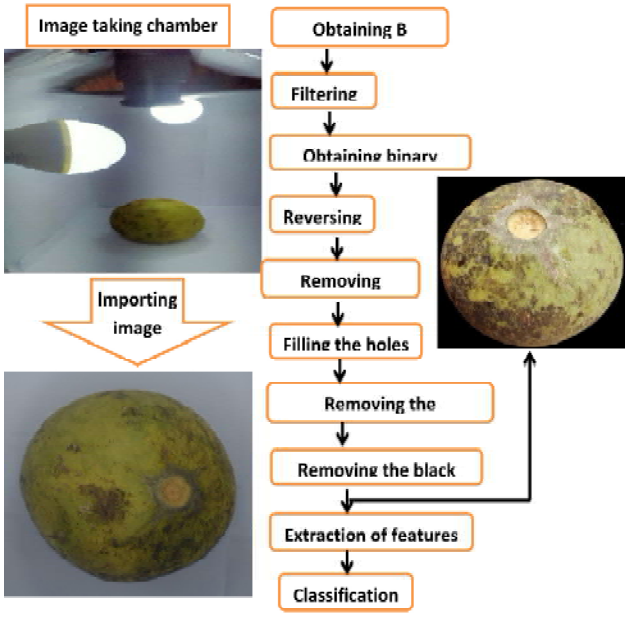

Fig. 1 : Classification of wood apple using color categorizing algorithm

reach a maximum limit of recurrence number. Alliterations were grouped within the same cluster to the nearest the centroid (assignment) point in each of the dataset points (Pacheco and Lopez, 2019).

Mapping of maturity kinetics and its integrated simulation with physico-chemical properties :

Recurrent neural network for the mapping of various properties

In the present study, a recurrent multilayer structure containing three layers input, hidden, and output of one each was used. The construction of RNN was done according to Cheroutre-Vialette and Lebert, (2002) with modification by improving the temperature in the input layer. There is no scientific or theoretical, set of principles/ rules for the decision of the unknown framework. The framework may be determined empirically and the best results showing arrangement be selected. The ideal neuron number of the unknown layer was recursively determined by developing a number of RNNs that differ with the size of the unknown layer (the tested neurons were three to nine). The statistical parameters namely mean square of error (MSE) and coefficient of determination were utilized as the measures for the choice of best RNN architecture. It was carried out by training and testing the data. From the unknown layer seven neurons were considered to be best framework. The testing was selected to be an activation function for

each neuron. The RNN was trained recursively by using repetitive representative pairs of vector exemplar input/output. The weights of the neural connections were primarily selected at random and are adjusted by a non-linear optimization technique. In order to minimize the cost function equal to the mean square of the output error, the quasi-Newtonian formula (Shanno, 1970) was used. To adjust the weights the learning base was used, to allow over-learning during weight optimization the testing base was used and for the confirmation of the output, the validation base was used. Of the total experiments, a minimum of 60% of experiments comprises of the learning and testing bases and 40% in the validation base. MATLAB software and the Optimization Toolbox (Mathworks) were used for developing the recurrent network software. This software includes all the pre-requisite parameters such as pre-initialization of the information (data), the instruction of the recurrent network and the perception of the result.

RESULTS AND DISCUSSION

Effect of maturity stages on the physical properties of wood apple

The physical properties of wood apple were measured with standard procedures. All the literature that referred to the physical properties of wood apple was found to be not similar. A lot of variations were observed in shape and size, diameter, bulk, and true density. The data obtained by Sonawane *et al*. (2020) was different from the data obtained by Murakonda *et al*. (2021). The data of the physical properties like weight, diameter, sphericity, true and bulk density are shown in Table 1. At the early stage of fruit development, the weight was found to be 252.786 $\pm$ 17.431g. The arithmetic mean diameter was found to be 7.944$\pm$ 0.199 cm. The bulk and true densities were observed to be 0.447$\pm$ 0.018 and 1.029$\pm$ 0.158 g/cm^3 respectively. The wood apple grew up gradually and comparatively at a slow rate, unlike any other climacteric fruit. At the fully matured stage, the fruit became plumpy and changes occurred in all the physical properties. During that stage, weight increased up to 473.506$\pm$ 18.828 g, whereas arithmetic mean diameter was observed to be 9.505$\pm$ 0.212 cm. The changes were observed for the sphericity, bulk, and true densities of the wood apple also. During the ripening stage, no such changes were observed in case of physical properties excluding weight. Increased weight may be due to the development of high moisture content (77.02 g per 100 g of pulp according to Devi and Kulkarni, (2018)). During ripening stage, the weight was found to be 520.118$\pm$ 15.578 g, whereas arithmetic mean diameter was 9.621$\pm$ 0.223 cm. Sphericity, bulk and true densities were found to be 20.334$\pm$ 0.508,

Table 1 : Change of physical properties in wood apple during different stages of maturity

Stages	Variables	Weight (g)	Arithmetic mean diameter (cm)	Sphericity	Bulk density (g/cm^3)	True density (g/cm^3)
Early Stage	Mean	252.786	7.994	15.781	0.447	1.029
	Min.	192.460	7.426	14.155	0.414	0.876
	Max.	288.240	8.613	17.731	0.481	1.422
	Var.	303.871	0.039	0.393	0.0003	0.025
	SD	17.431	0.199	0.627	0.018	0.158
Fully Matured Stage	Mean	473.506	9.505	19.974	0.880	1.932
	Min.	441.530	8.990	18.678	0.759	0.987
	Max.	546.970	10.110	21.681	0.988	2.506
	Var.	354.499	0.045	0.290	0.006	0.164
	SD	18.828	0.212	0.539	0.080	0.405
Ripe stage	Mean	520.118	9.621	20.334	0.943	2.230
	Min.	493.060	9.313	19.501	0.853	0.984
	Max.	568.260	10.503	22.307	1.121	2.984
	Var.	242.676	0.050	0.258	0.007	0.392
	SD	15.578	0.223	0.508	0.083	0.626

0.943± 0.083 and 2.230± 0.626 g/cm^3 respectively. The variation in the physical property data of the wood apple might be due to collection of the fruit samples from different climacteric zones, soil condition and variety.

Effect of maturity stages on the biochemical properties of wood apple

The changes in the biochemical properties of wood apples during different maturity stages are represented in Table 2. The wood apple pulp was slightly acidic in nature at the initial stage. As the fruit gradually matured, an increasing trend was observed in the pH values. At the early stage, it was found to be 3.55± 0.02, whereas during the matured stage it increased up to 4± 0.02. During the final ripened stage, the pH was found to be 5.24± 0.04 which was almost similar to any other such fruit. This result implies that there is more concentration of hydrogen ions at the initial stage and more hydroxyl ions at the ripened stage. Further, variation in the TSS in terms of °Brix values was also observed along with the changes in wood apple maturity. At the early stage of wood apple maturity, the TSS was found to be 15.8± 0.14° Brix. During the maturation period it was 16.90± 0.26° Brix followed by the ripening stage, the value was elevated to 18.22± 0.29° Brix. This sudden elevation in the °Brix value indicates that sugar content increased considerably during the ripening. Total acid concentration which generally measures the titratable acidity of any fruit was found at a high percentage during the initial stage and declination was observed during further stages. The ratio of TSS and TA also provides the characteristic flavor and texture of the fruit. Sometimes this ratio is also considered as the indicator for the harvesting period of the fruit. In case of wood apple, TSS/TA ratio followed a rising trend with the increase of maturity stages. At initial stage the ratio was found to be 3.80± 0.54 and further increased from 9.66± 0.26 to 14.07± 0.29 during matured to ripened stages. Hence, the bio-chemical properties of the wood apple changed with the advancement of maturity stages and ripening. From these properties it could be also observed that pH had positive impact on °Brix and negative impact on the titratable acidity, indicating formation of ethylene and ultimate ripening of the fruit. Hence, the ethylene helped wood apple to develop its sweetness and musky flavor with the advancement of maturity stages.

Color based mapping of maturity kinetics

Collection of color properties at various stages

The colour properties of the wood apple pulp and shell with respect to different maturity stages were evaluated based on the 'L*','a*','b*' values. Table 3 represents the color properties of the wood apple. With the advancement of maturity, increasing trend was observed in terms of all the color values. The L* value was found to be 59± 4.72, 61± 2.12 and 72± 1.92 at early, matured and ripened stages respectively, whereas a* followed the trend -15± 0.02, 0.52± 0.02 and 50.92 during these stages. Similarly, the b* value was found to be 39± 3.92, 41± 2.09 and 50± 1.09 at these three stages respectively. The changes in the colour values could be observed based on the pigment of the fruit. Fig. 2 demonstrates the classification of wood apple maturity based on changing colour properties. At early stage there might be more

Table 2 : Changes of bio-chemical properties of different stages of maturity in wood apple

Properties	Early stage	Matured stage	Ripened stage
pH	3.55+0.02	4.00+0.02	5.24+0.04
TSS (°Brix)	15.8+0.14	16.90+0.26	18.22+0.29
Titratable acidity (%)	4.16 +0.28	1.75 +0.01	1.29 +0.01
TSS/TA ratio	3.80+0.54	9.66+0.26	14.07+0.29

Table 3 : Color properties at various stages of wood apple

Colour properties	Early stage	Matured stage	Ripened stage
L* value	59+4.724	61+2.12	72+1.92
a* value	-15+0.002	0.52+0.02	5+0.92
b* value	39+3.92	41+2.09	50+1.09

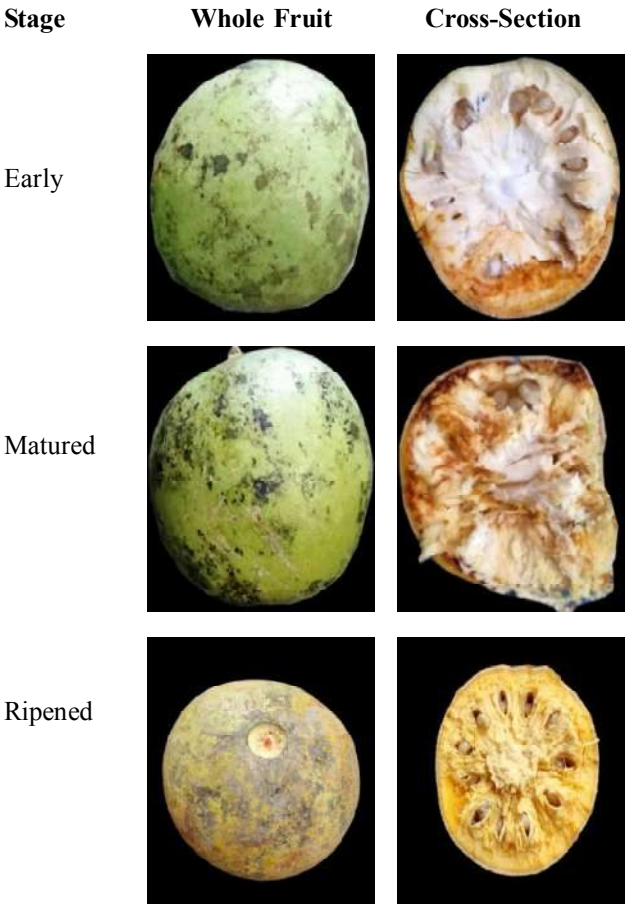

Fig. 2 : Pictorial representation for different stages of wood apple

deposition of chlorophyll pigments that reduced gradually with the advancement of maturity stages and resulted in the formation of carotenoids. During the matured stage, the carotenoids dominated chlorophyll pigments, bringing about the change of fruit colour to red-yellow.

Classification maturity kinetics by k-means cluster algorithm

For the classification of maturity stages of wood apple k- means cluster algorithm was used. Data of six physio-chemical properties namely bulk density (g/cm^3), true density (g/cm^3), pH, TSS (°Brix), titratable acidity (%) and TSS/TA ratio were considered along with the variation of redness value (a* value). From Fig. 3, classification of maturity stages based on k-means cluster algorithm can be observed. Three major stages *viz*., early stage, matured stage and fully ripened stage can be classified for recognizing ripening of wood apple. By considering these three stages, variation in other properties was discussed.

Application of RNN for the mapping of maturity kinetics

Fruit maturity properties determination based on color features is a challenging aspect to keep up the quality

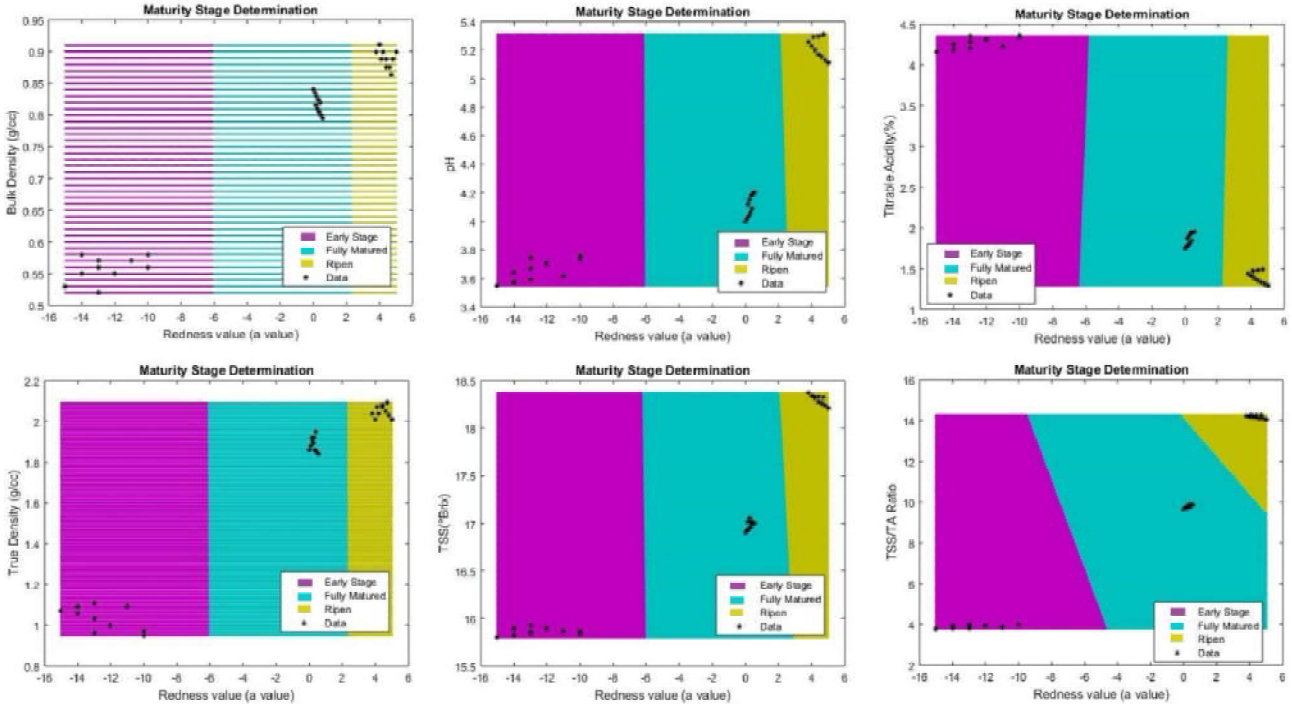

Fig. 3 : Classification of maturity stages by using k-means cluster algorithm

of the product during processing. To accomplish this particular task, a mathematical based relationship is made between the color features and maturity properties which are always highly reliable. Maturity kinetics of various fruits and vegetables based on colour parameters are reported by various researchers. ANN modeling can successfully draw a best relationship between colour properties and quality attributes of plucking time of fruit (Gupta *et al*., 2020). The evaluation of changes of colour of fruit throughout the maturity can be differentiated by color scales with the help of multi-dimensional regression based on Support Vector Regression (SVR) (Avila *et al*., 2015). In this study, RNN modeling was applied for the mapping of colour parameters and various maturity indices of wood apple.

Fig. 2 illustrates the sections for different stages of wood apple color properties. Fig. 4 shows different combination of RNN architectures with hidden layer neurons varying from 2 and 7. Selection of best RNN architecture on the basis MSE is illustrated in Fig. 5. The weight and bias values for the best RNN architecture is represented in Table 4. This can be used for further simulations of color-based quality indices and sensitivity analysis.

Color chart for wood apple

A color chart was developed for figuring out the distinctive maturity stages of wood apple. Fig. 6 illustrates the color chart of the wood apple. This chart will be beneficial to perceive different maturity stages of the fruit. pBy spotting the proper maturity levels, probable properties or quality of the fruit also can be predicted. This can be in the long run beneficial to govern required satisfactory of the product during processing. Hence, incorporation of the color chart as

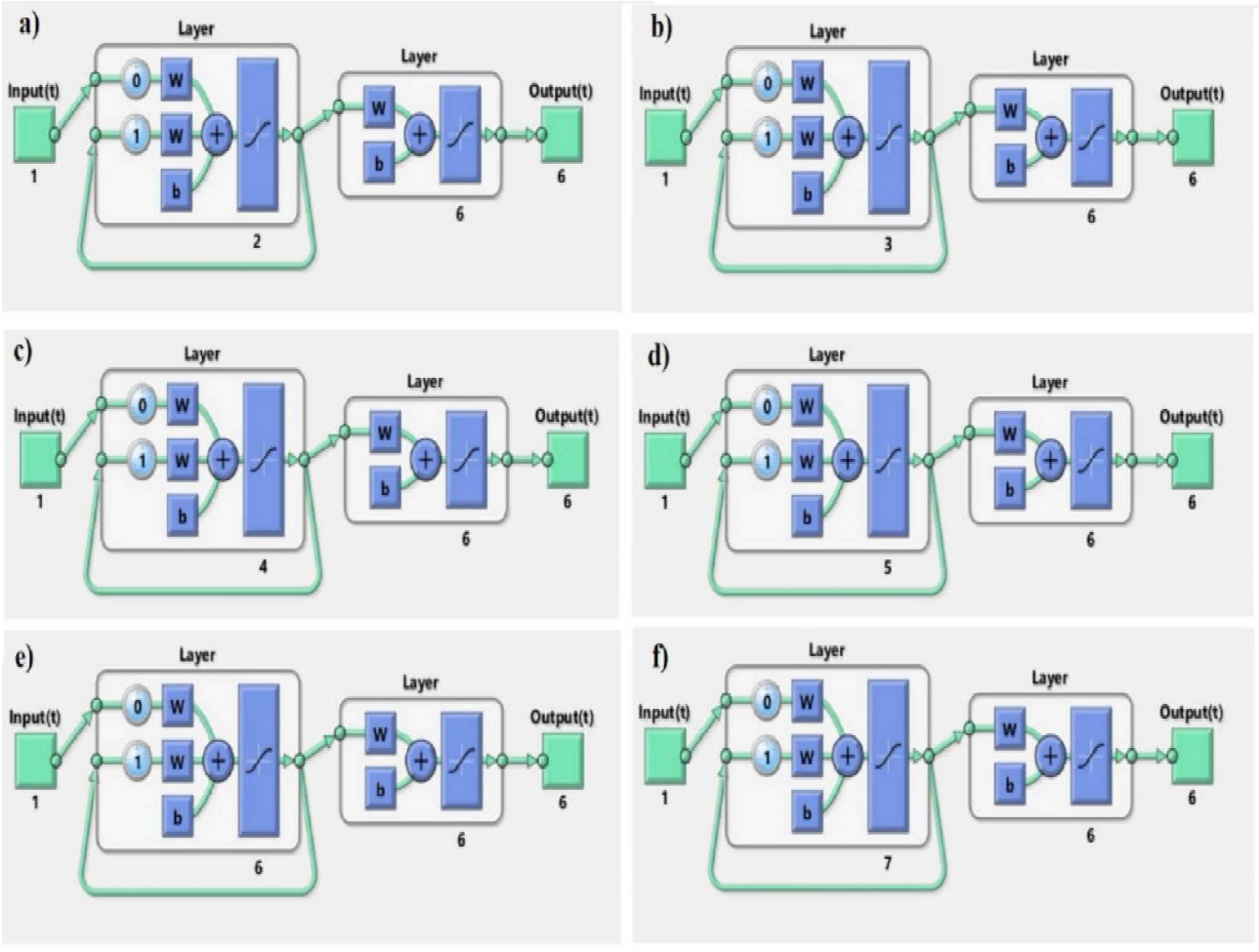

Fig. 4 : RNN modeling for the mapping of maturity kinetics total calculation time during run-time phase. The best network was selected based on the highest R^2 (0.99) and lowest MSE values.

Table 4 : Weight and bias values of best RNN architecture

IW{1,1}	-0.0378	LW{1,1}	-0.970	0.673	0.973	0.881	0.009	-0.389
	-1.453		0.383	-0.547	-0.873	-0.622	-0.336	0.709
	-1.367		-0.492	-0.208	-0.770	-0.883	0.847	0.411
	-1.343		-0.723	-0.191	-0.237	0.955	0.553	0.939
	0.757		0.241	0.190	0.867	0.794	-0.829	-0.601
	-0.914		0.587	0.643	-0.379	0.329	-1.066	0.278
LW{2,1}	-0.672	-1.041	-0.624	-0.977	0.302	1.208	B(1)	-1.847
	-0.60309	-1.078	-1.079	-0.963	-0.463	0.032		0.892
	0.93018	-0.14786	-1.318	-0.993	0.833	-0.428		0.919
	0.67438	-0.868	-0.969	-1.012	0.255	-0.502		0.455
	-0.1785	0.932	-0.184	1.5948	0.50812	-0.436		1.006
	-1.3929	-0.710	-1.414	-0.981	0.81681	0.498		-1.883
B(2)	1.582							
	0.838							
	-0.534							
	-0.089;							
	-1.457							
	-1.291							

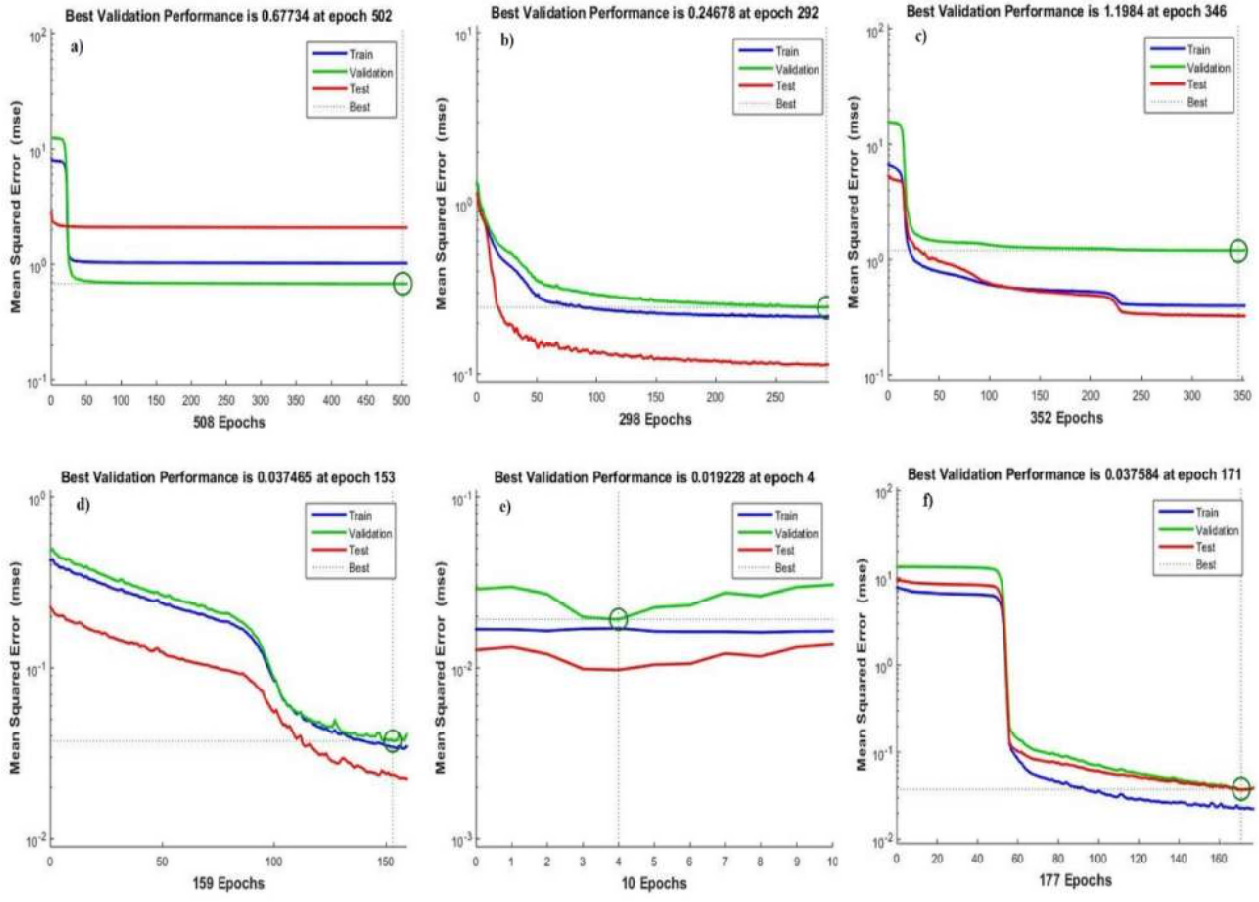

Fig. 5 : Selection of best architecture for RNN model

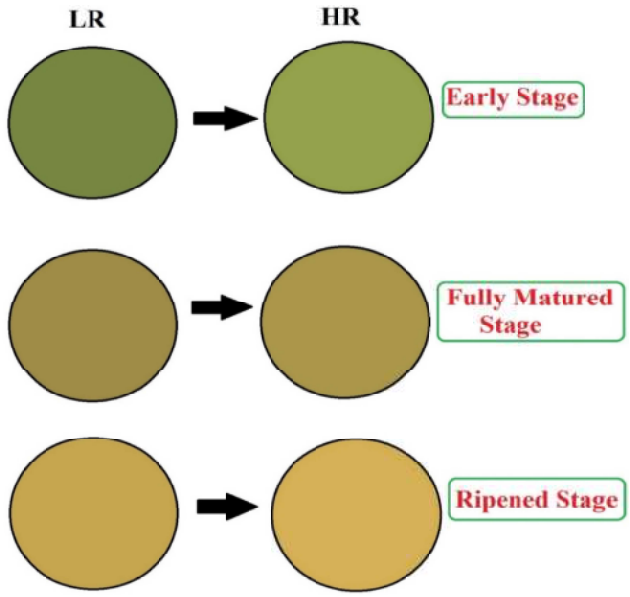

Fig. 6 : Color chart for identifying maturity stages of wood apple

reference handout, will exceptionally help farmers as an outcome of this unique investigation.

CONCLUSION

In this study, the effect of maturity stages on colour, physical and bio-chemical properties of the wood apple were investigated. An attempt was made to differentiate the changes in the properties with respect to three different stages of the wood apple *viz*. early, matured and ripe. A systematic trend was observed for all the properties namely sphericity, bulk density (g/cm^3), true density (g/cm^3), pH, TSS (°Brix), titratable acidity (%) and TSS/TA ratio, *etc*. of the fruit. In contrast, regular changes were also observed in the color properties at various maturity of the wood apple. The maturity kinetics was formulated by applying recurrent neural network (RNN) in compliance with K means cluster algorithm. RNN modeling was applied by considering color property (redness value) as input and six maturity indices as the output of the formulated structure. The RNN architecture, 1-6-6 showed the best results for forecasting the wood apple maturity based on color features. Further, based on the results of the K means cluster algorithm, the maturity stages were classified into three main categories. A color chart was also developed for figuring out distinctive maturity stages of wood apple. This chart can be utilized as a reference handout by the farmers for the maturity detection of the wood apple.

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Original Research Paper

Manipulating female flower intensity in 'Yu Her Pau' Litchi by delayed winter pruning

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ABSTRACT

'Yu Her Pau' litchi (*Litchi chinensis*) has excellent fruit quality. However, its production on Taiwan is limited by low productivity despite being regarded as a high-quality fruit. It is known that litchi's leaves play a critical role in floral induction under low temperature. Thus, we hypothesized that the flower intensity in spring could be manipulated by altering the leaf quality in winter, thereby increasing crop load. In this pilot study, 'Yu Her Pau' trees were pruned in mid-December [early pruning (EP)], one of the common cultural practices carried out by growers in the region, as control or mid-January [late pruning (LP)]. This resulted in 50% and 100% canopy foliage for EP and LP trees, respectively, between mid-December and mid-January. At the peak blooming time in March, LP trees produced significantly more female flowers than EP trees (95.8 and 56.1/panicle, respectively) with no negative effects on initial fruit set number, fruitlet abscission, or fruit quality at harvest. Our results suggest additional mature leaves present on trees in mid-December onward may benefit litchi flower formation without affecting fruit retention. Thus, preserving leaves with delayed pruning might potentially mitigate the negative impacts of warmer winters due to climate change on litchi flowering.

Keywords : Crop load, flowering, fruitlet retention, *Litchi chinensis*, low-temperature induction

INTRODUCTION

'Yu Her Pau' is an early-maturing litchi (*Litchi chinensis*) cultivar with outstanding fruit quality, but low crop load is a perpetual issue for its production on Taiwan (Chen *et al*., 2013; Chang *et al*., 2022). To obtain better fruit development and retention, some litchi growers would prune lateral branches at the end of vegetative flushing to maximize light interception for enhancing photosynthesis on the remaining fruit-bearing branches. Nevertheless, the benefit of this practice on yields has been anecdotal without empirical evidence. Increasing spring female flowers, which form fruitlets, could be another approach to enhance productivity but has never been explored for 'Yu Her Pau' litchi.

Litchi flower formation is a result of signaling cascades initiated by leaf perceiving winter low temperatures (< 20 °C) (Menzel and Simpson, 1995), which upregulate a litchi *flowering locus t* (*FT*), *LcFT1*, in leaves (Ding *et al*., 2015; Lu *et al*., 2022). Similar responses involving leaf *FT* transcription under floral-inductive low-temperature conditions were reported in citrus (*Citrus* sp.) (Nishikawa *et al*., 2007), mango (*Mangifera indica*) (Nakagawa *et al*., 2012), and avocado (*Persea americana*) (Ziv *et al*., 2014), indicating that the leave's role may be conserved among evergreen woody perennials. Interestingly, in low-temperature-treated citrus, reducing leaf numbers resulted in a progressive decrease in flower buds (Nishikawa *et al*., 2013). This leads to the assumption that litchi's flowering could be manipulated by altering the quantity of leaves to increase productivity. In this pilot study, our objective was to test this hypothesis through evaluating effects of leaf quantity during winter low-temperature exposure on spring female flowering in field-grown 'Yu Her Pau' litchi. Despite the positive correlation between leaf number during fruit development and final crop load in litchi (Chang and Lin, 2008), whether mature leaf appearance as early as floral-inductive period also helps subsequent fruit set and retention is unclear and thus was also investigated in this research as a subsidiary objective.

MATERIALS AND METHODS

This trial was conducted with ten, 31-year old 'Yu Her Pau' trees at Chiayi Agricultural Experiment Branch, Chiayi City, Taiwan (Lat. 23°29' N, Long. 120°28' E, Alt. 70 m). Except pruning times, all trees were subjected to the same management practices. Experiment was in a randomized complete block design; each of the five blocks contained two treatments, early pruning (EP) as control and late pruning (LP).

For EP, five trees were pruned on 17 Dec 2016 by removing most lateral branches, resulting in about 50% of mature leaves removed from the tree canopy. This treatment, including the extent of branch excision and pruning time, was carried out according to one of growers' common practices in the regions, hence serving as the control in this study. For late pruning (LP), the other five 'Yu Her Pau' trees were thinned on 16 Jan 2017 using the same criteria as for EP. Therefore, from mid-December through mid-January, EP trees had 50% less canopy foliage than LP trees. Spring inflorescence pruning, a conventional practice for litchi production in Taiwan (Chang *et al*., 2022), was done to all trees at the same level on 9 Mar 2017, with the onset of male blooming.

Ten panicles were randomly selected per tree for quantifying flower intensity and fruitlet retention. Newly emerged female flowers were counted every 2 to 3 days from 20 Mar through 7 Apr 2017, followed by the weekly quantification of fruitlets for 11 weeks after full female bloom (AFFB). Weekly fruitlet retention was calculated by dividing the number of fruitlets remaining on the panicles by the number of fruitlets obtained at week 1AFFB. At harvest on 7 June 2017, five randomly selected fruits per tree were evaluated for pericarp, aril, seed and whole fruit weight, and total soluble solids content. The treatment effects of LP in comparison with EP (control) on all parameters measured were determined using one-way analysis of variance with SAS Enterprise Guide (version 7.1; SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Both EP and LP 'Yu Her Pau' trees started to produce female flowers from 24 Mar 2017 and had the peak bloom time on 29 Mar (Fig. 1), during which LP trees produced significantly more female flowers than EP trees (Fig. 1). Total female flowers produced in spring were also significantly greater in LP trees than EP trees (208.8 and 153.0/panicle, respectively; $P = 0.022$) (Table 1). These results demonstrated that delayed pruning increased flower intensity without affecting phenology. Notably, from mid-December to mid-January, LP trees had twice as much canopy foliage as that of EP trees, suggesting more mature leaves during this period plays a pivotal role in promoting flowering promotion. Since flowering phenology was unaltered by pruning times, the relationship between the number of mature leaves in winter and spring female flower intensity in litchi is likely quantitative, consistent with the results in citrus (Nishikawa *et al*., 2013). Given the positive

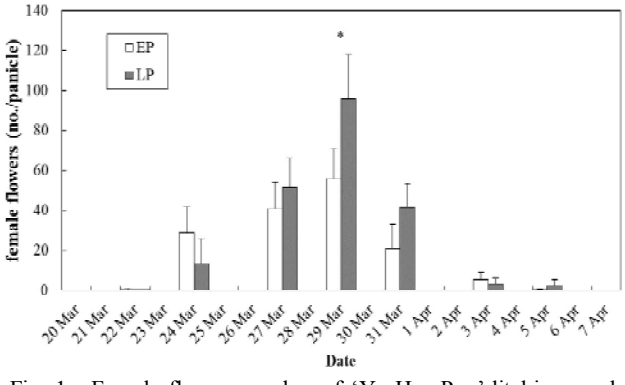

Fig. 1 : Female flower number of 'Yu Her Pau' litchi pruned in mid-December [early pruning (EP)] and mid-January [late pruning (LP)]. *significant differences between two treatments on 29 Mar at P< 0.05.

Table 1 : Flower and Fruit characteristics in 'Yu Her Pau' litchi pruned in mid-December [early pruning (EP)] and mid-January [late pruning (LP)]

Treatment	Female flower no. / panicle	Fruit no. / panicle immediately before harvest	Fw (g)	Pw (g)	Aw (g)	Sw (g)	TSS (^{0}Brix)
EP	153.0	2.8	29.29	5.75	22.15	1.38	19.65
LP	208.8	1.9	30.16	5.96	22.63	1.57	19.69
ANOVA	*	NS	NS	NS	NS	NS	NS

FW: fruit weight, PW: pericarp weight, AW: aril weight, SW: seed weight, TSS: total soluble solids (TSS)

correlation between winter leaf carbohydrate levels and spring flower numbers reported in citrus (Garcia-Luis *et al*.,1995), the additional litchi leaves present in December due to LP might constitutes a greater carbohydrate pool to support more flower buds. Alternatively, greater leaf number and area in LP trees may result in a higher *FT* accumulation (Kinmonth-Schultz *et al*., 2019), which corresponded to flower intensity in response to floral-inductive conditions (Nishikawa *et al*., 2007; Tang *et al*., 2021; Lu *et al*., 2022). Relevantly, litchi grown in the North Hemisphere had maximum *LcFT1* expression between mid-December and mid-January (Ding *et al*., 2015), when low temperatures (< 20 ◦C) guaranteed floral induction (Menzel and Simpson, 1995). The mean monthly temperature during this trial was 19.8 ◦C in December 2016, and 18.3 ◦C in January at the orchard that met the low temperature requirement. Together, keeping more leaves under flowering-promoting low temperatures (mid-December through January) may positively affect floral signaling involving *LcFT1*, thereby enhancing flowering.

Litchi inflorescences are heterocladic pleiothyrsoids; each female flower is surrounded by multiple subsequently produced male flowers within a dichasium (Robbertse *et al*., 1995). For 'Yu Her Pau' litchi, the resource competition between new fruitlets (from female flowers) and male flowers is one predominant cause of low fruit set (Chen *et al.,* 2013). Thus, it is possible that, with increased female flowers (Fig. 1), fruit set would be reduced in LP trees due to the concomitant increment in male flowers (Jiang *et al*., 2012; Lee and Chang 2019). In contrast, our results demonstrated that by week 1 AFFB, fruitlet numbers in LP and EP trees (160.3 and 150.0/panicle, respectively) were not different (Fig. 2), suggesting the initial fruit set was not reduced by delayed pruning. As more fruitlets abscised from LP (87.0%) than EP trees (78.7%), fruitlet number remained undistinguishable at week 2 AFFB (Fig. 2). Final fruit number per panicle of both treatments stayed similar through harvest (Table 1), with no difference in pericarp, aril, seed, and total fruit weight or total soluble solids content (Table 1). The results of this study indicate that the increase in floral intensity as a result of delayed pruning did not have a significant negative impact on fruitlet retention or fruit quality in 'Yu Her Pau' litchi.

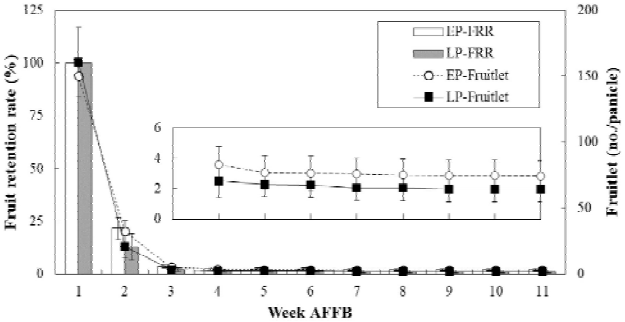

Fig. 2 : Fruitlet number and fruit retention rate (FRR) of 'Yu Her Pau' litchi pruned in mid-December [early pruning (EP)] and mid-January [late pruning (LP)]. (Week AFFB-Week after full female bloom).

Mature leaves during the early to mid-stages of fruit development are the main photo assimilate source for fruitlets nearby (Chang and Lin, 2008). Hence, similar crop load and fruit quality traits of EP and LP trees could be attributed to similar leaf quantity and canopy light interception, achieved by the same extent of pruning (albeit done at different times), past mid-January. This inference further suggests that the presence of mature leaves during the floral-inductive period (mid-December to mid-January), relative to fruit development period, might play an inconsequential part in fruit retention and maturation thereafter.

CONCLUSION

While literature has provided evidence for the role of leaves, regarding carbohydrate reserves and *FT* transcription, in litchi flower formation, this study was the first to put such knowledge into practice i.e., to effectively manipulate female flowering by increasing leaf exposure to floral-inductive low temperatures with delayed pruning. Our results presented a tool to mitigate low flower intensity in litchi in the event of warmer winters due to climate change. Although our study demonstrated no negative effects of increased flowering on initial fruit set, delayed pruning did not result in an increase in final crop load in 'Yu Her Pau' litchi. This reflects the fact that flower formation is just one component with regard to yields. Therefore, other practices that improve fruitlet retention, like inflorescence pruning and cincturing (Chang *et al.,* 2022), could be used in conjunction with delayed pruning to enhance overall litchi productivity.

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Original Research Paper

Stionic effects on leaf mineral nutrient contents in Pummelo (*Citrus maxima* Merr.) grafted on different rootstocks

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ABSTRACT

A study was conducted to determine the mineral nutrients concentration in the index leaf of pummelo accessions. Index leaf samples from 25 pummelo accessions grafted on pummelo and 12 pummelo clones grafted on Rangpur lime rootstocks were collected for assessing leaf mineral nutrient status. The results revealed that pummelo plants grafted on pummelo, the concentration of leaf N (1.43-2.49 %), P (0.17-0.22 %), K (0.75-4.45 %), Ca (2.37-6.29 %), Mg (0.60-1.04 %), S (0.06-0.22 %), Fe (124-245.45 mg kg^{-1}), Mn (9.85-50.05 mg kg^{-1}), Zn (17-69 mg kg^{-1}) and Cu (8.8-25.15 mg kg^{-1}) showed significant variation with different accessions. Out of 25 pummelo accessions, twenty-four accessions were deficient in N and S, fourteen were deficient in K, four were deficient in Mn and five were deficient in Zn and all accessions were sufficient in P, Ca, Mg, Fe and Cu. The observed trends in the leaf nutrient concentration of pummelo accessions clearly indicated the significance of the genotypic variation when chemical analysis is used for diagnosing the leaf nutrient status of pummelo trees. Similarly, leaf N, P, K, Ca, Mn, Cu and Zn varied significantly among twelve pummelo clones grafted on Rangpur lime. Among the clones grafted on Rangpur lime, 18-3 and 18-5 found to have higher and lower leaf nutrient content in most of the mineral nutrients, respectively. The leaf nutrient content of pummelo varies among genotypes, but there is no genotype that stands out in all macro and micronutrients evaluated. The N, P, K, Ca, S, Fe, Mn and Cu leaf contents in pummelo were always higher in plants grafted on Rangpur lime. However, the foliar Mg and Zn contents were continually higher in plants grafted on 'pummelo' compared to Rangpur lime which eventually reduces leaf yellowing/chlorosis in pummelo. Pummelo rootstocks were found to respond well in terms of Mg and Zn nutrient uptake and tolerance to *Phytophthora* as compared to Rangpur lime. Therefore, it is concluded that pummelo can be an ideal rootstock for commercial pummelo cultivation.

Keywords : Accessions, grafting, leaf mineral nutrient, pummelo, Rangpur lime, rootstock

INTRODUCTION

Pummelo [*Citrus maxima* Merr., (*C. grandis* Osbeck; *C. decumana* L.)], family Rutaceae, was known in the western world mainly as the principal ancestor of the grapefruit. The areas in southern Thailand and northern Malaysia are most likely the centre of origin of pummelos (Wen *et al.*, 2010). In India, it is grown in home gardens in all states of India and maximum diversity is reported from North-East (NE) Region Bihar and Bengal (Roy *et al.*, 2014). Pummelo is now gaining popularity in India due to its high nutritional value and antioxidant property. It has played an important role as a parent of many citrus fruits, such as lemon, oranges and grapefruit (Youseif *et al.*, 2014). Pummelo fruit has several health benefits because of its super-rich Vitamin C and Vitamin B content. It also contains Vitamin A, bioflavonoids, healthy fats, protein, fiber, antioxidants and enzymes. It contains high amount of beta carotene and folic acid and is very beneficial for pregnant women.

Nutrients are required for supporting the metabolism within the tree-ecosystem and also to support quality fruit production (Thamrin *et al.*, 2014). Both maximum fruit quality and yield of pummelo will occur only in the presence of optimum nutrient balance and intensity. Maintaining orchards at optimal leaf nutrient concentrations is one of the key issues for maximizing yield. Low fruit quality and yield is often associated with poor soil fertility and poor nutrient management (Zhuang, 1995). Leaf analysis is a method of determining plant nutrient requirement based on assumption that within certain limits, there

is positive relationship between nutrient availability, leaf nutrient content, yield and quality of fruits (Srivastava and Singh, 2004a; 2004b). Stebbins and Wilder (2003) reported that leaf nutrient concentrations can be used as a guide to determine nutrient status of plant that are directly linked/related to the pattern of growth and development.

Impact of stock on scion and scion on stock is known as stionic effect. Rootstock choice is one of the most important aspects in orchard management because scion cultivars respond differently to growth, fruit quality, disease resistance and nutrients accumulation when grown on diverse rootstocks. Plant nutrient concentrations in scion cultivar may differ even though they are grown in the same conditions. Rootstocks directly affect the ability of plants to uptake the water and nutrients from the soil. Similarly, different scions exhibit variable quantities of nutrients from different rootstocks. The long-term performance of stionic combinations and their significant effects on leaf nutrient levels in different fruit crops have been studied for different climatic conditions across the world (Dubey and Sharma, 2016). However, no such studies were carried out in pummelo. Hence, selection of an appropriate graft/stionic combination with better leaf nutrient absorption is very critical to produce pummelo commercially. Therefore, the main purpose of the present research was to determine the status of various macro and micronutrients in the leaf of pummelo genotypes grafted on Pummelo and Rangpur lime for choosing the right graft combination with enhanced nutrient absorption. It is also possible to reduce the application of nutrients in pummelo by employing perfect stionic combination that have high nutrient absorption capacity.

MATERIALS AND METHODS

To determine the nutrient concentration of leaf as influenced by genotypes and rootstocks, index leaf samples (4th and 5th leaf from tip of new shoots/flush with age of 4 to 6 months) from 25 pummelo genotypes (>15 years old) grafted on pummelo and 12 pummelo clones (4 years old) grafted on Rangpur lime rootstocks were collected in the month of June 2019 from the field gene bank maintained at ICAR-IIHR, Bengaluru, which is situated in south-east tract of Karnataka state at 12^{0}58 North latitude and 77^{0}34 East longitude and at an altitude of 900 m above mean sea level. The study area comes under semi-arid, sub-tropical climate with hot summer and cold winter with an average rainfall of 866 mm. Most of the rainfall is received from the south-west monsoon during July to August. Twenty leaf samples, five leaves from each direction of east, west, north and south were taken individually from five trees per genotypes from non bearing fruit terminals. The samples were washed first under tap water followed by 0.1 N HCl, distilled water and finally with double distilled water. The cleaned leaf samples were then dried by spreading on clean blotting papers and final drying was done in an oven at 68^{o}C (Chapman and Pratt, 1961) by separately packing in labeled paper bags. The dried leaf samples were sequentially ground by electrical grinder for further analysis.

The nitrogen (N) content in the leaf samples was analysed by Kjeldahl method (AOAC, 1970). Phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) were estimated by diacid mixture (9:4 HNO_3: $HClO_4$) as given by Jackson (1973). Phosphorus content in leaf samples was determined by vanadomolybdo phosphoric acid yellow colour method (Jackson, 1973). The intensity of yellow colour was read at 430 nm in the spectrophotometer. Potassium content was estimated using flame photometer (Jackson, 1973). Calcium and magnesium content was determined by Atomic Absorption Spectrophotometer (AAS) (Sarma *et al.,* 1987). Micronutrient content *viz.* Fe, Mn, Cu and Zn was determined using Atomic Absorption Spectrophotometer (AAS) (Sarma *et al.,* 1987). The data were statistically scrutinized using analysis of variance of SAS 9.3 statistical package.

RESULTS AND DISCUSSION

Leaf macronutrients

Index leaf samples of 23 pummelo genotypes grafted on pummelo rootstock and 12 pummelo clones grafted on Rangpur lime rootstock were analyzed for N, P, K, Ca, Mg, S, Fe, Mn, Zn and Cu contents and presented in Table 1, 2, 3 and 4 and Fig.1, 2. The data on leaf macronutrients content in pummelo grafted on pummelo and Rangpur lime is presented in Table 1 & 2 and Fig. 1. The concentration of different nutrients in leaf exhibited a wide variation among the genotypes irrespective of the rootstocks. However, the N concentration of leaves was not differed significantly among pummelo genotypes grafted on own rootstocks. Genotype 'Kunigal selection' had the highest leaf N

Table 1 : Leaf macronutrient content in pummelo grafted on pummelo

Genotype	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Devenahalli Selection-1	1.53	0.19	0.95^{C}	4.70^{BCDEF}	0.74	0.13
Midnapur Selection 1	1.57	0.21	1.90^{BC}	4.10^{DEFG}	0.80	0.22
Midnapur Selection 1	1.64	0.21	1.30^{BC}	3.83^{EFGHI}	0.86	0.09
Tirupati-1	1.86	0.18	1.45^{BC}	4.17^{DEFG}	0.77	0.06
Hyderabad Selection	1.97	0.20	2.00^{BC}	5.51^{ABCD}	0.85	0.15
Kallar Selection	2.11	0.20	0.85^{C}	3.46^{FGHI}	0.82	0.15
Raichur Selection	1.58	0.22	1.15^{C}	3.77^{EFGHI}	0.72	0.11
Khanapur Selection	1.95	0.21	2.10^{BC}	4.11^{DEFG}	0.76	0.12
IKP-1	2.03	0.17	0.75^{C}	4.37^{CDEFG}	0.82	0.17
IKP-2	1.92	0.20	1.15^{C}	2.40^{HI}	0.60	0.07
Tirupati-2	1.43	0.19	0.90^{C}	4.26^{CDEFG}	0.81	0.11
Tirupati -2A	1.89	0.19	0.75^{C}	5.76^{ABC}	1.04	0.12
Kalenahalli-1	1.53	0.19	1.55^{BC}	3.97^{DEFG}	0.75	0.15
Devenahalli Selection-2	1.54	0.19	1.95^{BC}	5.95^{AB}	0.88	0.08
Midnapur Selection-2A	1.90	0.19	2.75^{B}	6.29^{A}	0.93	0.14
Kunigal Selection	2.59	0.20	4.45^{A}	3.94^{EFGH}	0.83	0.11
Devenahalli Selection-3	1.64	0.17	1.00^{C}	4.02^{DEFG}	0.83	0.14
Accession-18	1.54	0.20	1.75^{BC}	3.84^{EFGHI}	0.69	0.13
Accession-19	1.62	0.22	1.25^{BC}	3.41^{FGHI}	0.75	0.17
Devenahalli Selection -4	1.93	0.18	1.25^{BC}	2.37^{I}	0.71	0.12
Devenahalli Selection-5	1.61	0.18	2.25^{BC}	4.45^{BCDEFG}	0.92	0.14
Devenahalli Selection-6	1.69	0.19	1.05^{C}	3.02^{GHI}	0.70	0.14
Devenahalli Selection-7	1.79	0.20	1.10^{C}	5.27^{ABCDE}	1.01	0.12
Gollehalli	2.07	0.20	1.65^{BC}	4.01^{DEFG}	0.94	0.13
Kalenahalli-1A	1.62	0.19	0.80^{C}	2.99^{GHI}	0.73	0.15
General Mean	1.78	0.19	1.52	4.16	0.81	0.13
p-Value	0.7806	0.3407	0.0176	0.0014	0.3237	0.9886
SE(d)	0.413	0.018	0.734	0.753	0.133	0.075
LSD at 5%	NS	NS	1.5139	1.5549	NS	NS

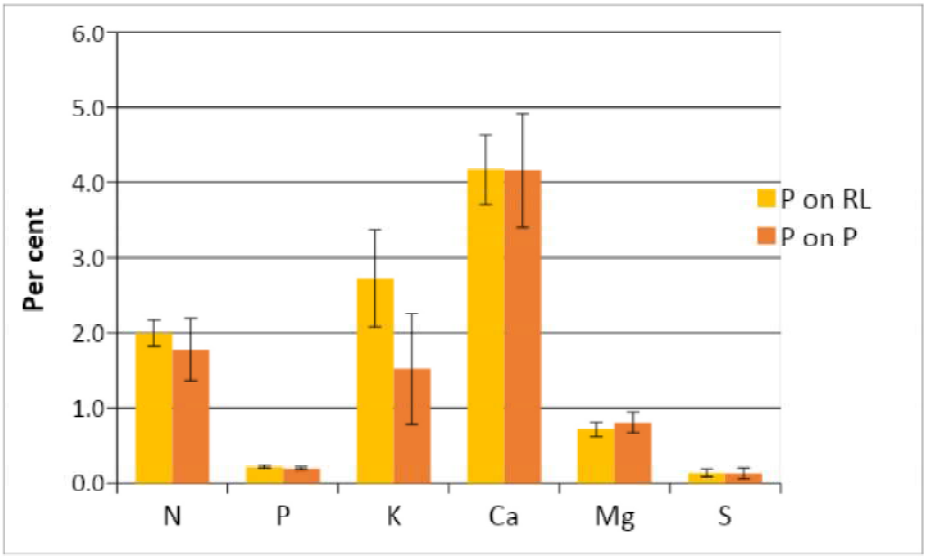

Fig. 1 : Leaf macronutrient content in pummelo grafted on pummelo (P) and Rangpur lime (RL)

Table 2 : Leaf macronutrient content in pummelo grafted on Rangpur lime

Genotype	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Clone 19-1	1.59^{D}	0.23^{BCD}	3.30^{AB}	3.03^{EF}	0.72	0.13
Clone 18-5	1.57^{D}	0.17^{G}	2.30^{BCD}	2.72^{F}	0.67	0.13
Clone 24-4	2.39^{A}	0.18^{FG}	3.07^{ABC}	4.80^{ABC}	0.81	0.14
Clone 8-4	1.83^{CD}	0.22^{BCDE}	1.10^{D}	5.04^{A}	0.77	0.14
Clone 25-5	2.03^{BC}	0.19^{EFG}	1.73^{CD}	3.85^{CDE}	0.73	0.10
Clone 18-4	1.79^{CD}	0.24^{BC}	2.73^{ABC}	3.96^{CDE}	0.75	0.13
Clone 21-4	2.33^{AB}	0.21^{CDEF}	2.40^{ABCD}	4.95^{AB}	0.71	0.15
Clone 18-1	1.66^{D}	0.24^{B}	3.23^{AB}	4.00^{BCD}	0.67	0.12
Clone 10-5	2.25^{AB}	0.20^{DEFG}	3.17^{AB}	3.57^{DEF}	0.65	0.10
Clone 18-3	2.52^{A}	0.31^{A}	3.67^{A}	4.95^{AB}	0.69	0.20
Clone 25-2	1.73^{CD}	0.22^{BCDE}	2.53^{ABC}	4.74^{ABC}	0.75	0.13
Clone 20-4	2.28^{AB}	0.21^{BCDEF}	3.47^{AB}	4.40^{ABCD}	0.72	0.13
General Mean	2.00	0.22	2.73	4.17	0.72	0.13
p-Value	<.0001	<.0001	0.0224	0.0003	0.9196	0.9228
SE(d)	0.171	0.016	0.649	0.461	0.096	0.054
LSD at 5%	0.3542	0.0328	1.3465	0.9568	NS	NS

(2.59%) and 'Tirupati Selection' had the lowest leaf N (1.43%) when grafted with pummelo. Zhuang *et al*. (1991) reported that leaf N content ranging from 2.5 to 3.1% indicated sufficiency in Guanximiyou' pummelo leaves. The concentration of leaf N in most of the pummelo accessions was below the critical value of 2.50% except in genotype 'Kunigal Selection. Similar trend was observed in leaf N content of pummelo accessions grafted on Rangpur lime also (Table 2). The highest mean leaf N content (2.00%) was observed in the pummelo grafted on Rangpur lime rootstock compared to pummelo grafted on pummelo (1.78%). The range of N levels in leaf of pummelo accessions grafted on pummelo and Rangpur lime was compared well with the reported values of 1.7 to 2.81 per cent in citrus by Srivastava and Singh (2002; 2003; 2005; 2006; 2008).

The pummelo accessions grafted on pummelo did not influence the P concentration of leaves significantly. The values ranged from 0.17 to 0.22% with a mean value of 0.19%. The range of P levels in leaf matched well with the values 0.14-0.18 % reported by Zhuang *et al*. (1991) in pummelo and 0.09-0.17 % reported by Srivastava and Singh (2002; 2003; 2005; 2006; 2008) in citrus. The concentration of leaf P was the highest in the genotype 'Raichur selection' (0.22%) and was the least in 'Devenahalli Selection-3' (0.17%) accession. Of the 23 accessions, the values of P in the foliage were found to be sufficient in five accessions and excess in twenty accessions. According to Zhuang *et al*. (1991), leaf P content ranging from 0.14 to 0.18% indicated sufficiency, whereas, P content below 0.14% indicated P deficiency in pummelo. Similar to leaf N, the highest mean leaf P content (0.22%) was also observed in the pummelo grafted on Rangpur lime rootstock compared to pummelo grafted on pummelo (0.19%).

The leaf K concentration of pummelo accessions grafted on pummelo ranged from 0.75 to 4.45% with a mean value of 1.52%. Leaf K was significantly higher in the Kunigal Selection (4.45%) and genotypes Tirupati-2 (0.75%) recorded the lowest leaf K content. However, the concentration of leaf K was below the critical value of 1.40% in 14 pummelo accessions. The range of K levels in leaf were almost similar to that reported by Srivastava and Singh (2002; 2003; 2005; 2008) (1.02-2.59%) in citrus. According to Zhuang *et al*. (1991), the leaf K content ranging from 1.4 to 2.2% indicated sufficiency. Like leaf N and P, the highest mean leaf K content was observed in the pummelo grafted on Rangpur lime (2.73%) rootstock compared to pummelo on pummelo (1.52%). The concentration of Ca and Mg in leaf of pummelo genotypes exhibited wide variation. Highest Ca concentration of 6.29% was observed in accession 'Midnapur Selection-2A' and the lowest Ca concentration of 2.37% was in genotype 'Devenahalli Selection-4'. The Ca concentration of leaf was at par in 'Devenahalli

Selection-2, 'Tirupati-2' and 'Hyderabad Selection' genotypes. The range of Ca level in leaf (2.37-6.29%) was higher than the range reported by Zhuang *et al*. (1991) (2.0-3.8%) and Srivastava and Singh (2002; 2003; 2005; 2008) in citrus (1.80-3.28%). The concentration of leaf Ca in all the genotypes was higher than the critical level (2.0%). The concentration of Mg in leaves of pummelo genotypes varied from as low as 0.6% in genotype 'Tirupati-2' to as high as 1.04% in genotype 'A-10' which was above the critical levels (0.32%). The range of Mg level in leaf was compared well with standards of Zhuang *et al*. (1991) (0.32-0.47%) and Srivastava and Singh (2002; 2003; 2005; 2008) (0.43-0.92%). The mean leaf Ca (4.16%) and S (0.13%) in pummelo genotypes grafted on pummelo were found almost comparable with the pummelo genotypes grafted on Rangpur lime (4.17% and 0.13%). However, the pummelo genotypes grafted on pummelo had better mean leaf Mg content (0.81%) than the plants grafted on Rangpur lime (0.72%). With respect to leaf sulphur content, no significant difference was observed in different pummelo genotypes grafted on pummelo nevertheless found to be matching with leaf S content of pummelo clones grafted on Rangpur lime. The range of S levels in leaf (0.06-0.22%) was noticeably lower than those reported by Zhuang *et al*. (1991) (0.2-0.39%).

Leaf micronutrients

The data on leaf micronutrients in pummelo grafted on pummelo and Rangpur lime is presented in Table 3 & 4 and Fig. 2. Considerable differences were observed, in the micronutrient concentration of leaf in pummelo genotypes. A relatively wide range of leaf Fe was found among the pummelo genotypes. The concentration of leaf Fe was found to be statistically significant in pummelo genotypes grafted on pummelo and the genotype 'IKP-1' recorded the highest leaf Fe (245.45 mg kg^{-1}). The lowest leaf Fe content (124 mg kg^{-1}) was recorded in genotype 'Devenahalli Selection-4'. The range of Fe levels in pummelo leaf (124-245.45 mg kg^{-1}) of the present study was compared well with standards of Srivastava and Singh (2002) reported in Khasi mandarin (84.6-249.0 mg kg^{-1}). Pummelo genotypes differed significantly with respect to leaf Mn concentration. Higher concentration of leaf Mn was recorded in genotype 'Tirupati-2' (50.05 mg kg^{-1}), and 'IKP-1' (36.40 mg kg^{-1}). The range of Mn levels in leaf (9.85-50.05 ppm) was appreciably lower than those reported by Zhuang *et al*. (1991) (15-140 mg kg^{-1}). The concentration of leaf Zn ranged from 17 to 69 mg kg^{-1} with a mean value of 33.3 mg kg^{-1}. The accession 'IKP-1' recorded the highest leaf Zn concentration of 69 mg kg^{-1} whereas; accession 'A-20' had the lowest leaf Zn concentration of 17 mg kg^{-1}. The values of Zn levels in leaf of most of accessions were relatively higher than those reported by Zhuang *et al*. (1991) (24-44 mg kg^{-1}). The concentration of Zn in the leaves were in the deficient range (<24 mg kg^{-1}) in few pummelo genotypes (A-18, Devenahalli selection-4, Devenahalli Selection-5, Devenahalli Selection-6 and Kalenahalli) grafted on pummelo. Copper concentration of the pummelo leaf ranged from 8.80 to 25.15 mg kg^{-1} with a mean of 15.36 mg kg^{-1}. Higher concentration of leaf Cu was recorded in accessions 'Gollehalli Selection, 'Devenahalli Selection-2' and 'Kalenahalli. The concentration of leaf Cu was statistically significant among the pummelo accessions. The ranges of Cu levels in leaves were much higher than the values of 8-17 mg kg^{-1} reported by Zhuang *et al*. (1991). The values of Cu in the foliage of all the accessions under study were above the critical value of 8.0 mg kg^{-1}. Pummelo genotypes grafted on Rangpur lime recorded the highest Fe, Mn, and Cu except Zn. Low leaf Zn might be one of the causes for wide spread appearance of severe yellowing/chlorosis in pummelo trees grafted on Rangpur lime. The highest leaf Zn was recorded in the pummelo accessions grafted on pummelo might be reason for reduced level of leaf yellowing/chlorosis. The Zn is required in plants for synthesis of auxins which act as plant growth promoter in various phenophases of the plant.

The data recorded on leaf macro and micronutrient status of pummelo grafted on different rootstocks revealed that the nutrient content of the leaf samples was significantly influenced by the rootstocks and scions as well. Differences in leaf nutrient content among the stionic combinations could be due to the variances among the rootstocks in the morphology, density of the roots in the soil profile, rooting pattern, root volume, and variations in nutrient absorption capacity of the roots (Zhuang *et al*., 1991; Srivastava and Singh 2002). The rootstock having higher root volume can be more efficient in absorbing nutrients from the soil. Variation in leaf nutrient content could also be caused by scions of different genotypes, and

Table 3 : Leaf micronutrient content in pummelo grafted on pummelo

Genotype	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
Devenahalli Selection-1	180.45^{BC}	19.30^{GHIJ}	31.60^{FG}	13.85^{FGHI}
Midnapur Selection 1	163.60^{BCDE}	19.35^{GHIJ}	27.45^{GH}	15.70^{EF}
Midnapur Selection 1	133.05^{DE}	18.60^{GHIJ}	32.40^{F}	15.05^{EFGH}
Tirupati-1	176.05^{BCD}	21.45^{FGHI}	39.65^{D}	15.45^{EFG}
Hyderabad Selection	176.35^{BCD}	24.90^{DEF}	34.70^{EF}	11.75^{HIJ}
Kallar Selection	157.50^{BCDE}	17.45^{HIJKL}	29.95^{FG}	11.50^{IJ}
Raichur Selection	192.00^{B}	17.80^{GHIJK}	46.90^{C}	16.00^{EF}
Khanapur Selection	170.65^{BCD}	21.90^{FGHI}	33.40^{EF}	12.05^{GHIJ}
IKP-1	245.45^{A}	36.40^{C}	69.00^{A}	13.70^{FGHI}
IKP-2	186.45^{BC}	16.95^{IJKL}	27.40^{GH}	11.70^{HIJ}
Tirupati-2	195.40^{B}	50.05^{A}	47.35^{BC}	14.95^{EFGHI}
Tirupati -2A	172.70^{BCD}	44.30^{B}	42.90^{CD}	20.35^{BCD}
Kalenahalli-1	174.90^{BCD}	22.90^{EFG}	38.15^{DE}	16.90^{DEF}
Devenahalli Selection-2	182.40^{BC}	29.45^{D}	52.20^{B}	22.50^{AB}
Midnapur Selection-2A	157.60^{BCDE}	19.30^{GHIJ}	30.35^{FG}	18.25^{CDE}
Kunigal Selection	146.40^{CDE}	12.45^{LM}	26.85^{GHI}	11.90^{HIJ}
Devenahalli Selection-3	146.55^{CDE}	15.15^{JKL}	24.00^{HIJ}	8.80^{J}
Accession-18	134.95^{DE}	14.45^{JKLM}	23.50^{HIJ}	9.35^{J}
Accession-19	162.05^{BCDE}	15.75^{JKL}	24.10^{HIJ}	12.10^{GHIJ}
Devenahalli Selection -4	124.00^{E}	9.85^{M}	17.00^{K}	10.10^{J}
Devenahalli Selection-5	146.80^{CDE}	17.20^{HIJKL}	23.30^{HIJ}	21.25^{BC}
Devenahalli Selection-6	145.30^{CDE}	12.80^{KLM}	20.10^{JK}	18.00^{CDE}
Devenahalli Selection-7	181.90^{BC}	22.30^{EFGH}	33.85^{EF}	16.25^{EF}
Gollehalli	188.10^{BC}	27.25^{DE}	34.35^{EF}	25.15^{A}
Kalenahalli-1A	145.55^{CDE}	22.00^{FGHI}	22.05^{IJ}	21.50^{BC}
General Mean	167.45	21.97	33.30	15.36
p-Value	0.0080	<.0001	<.0001	<.0001
SE(d)	21.819	2.513	2.391	1.717
LSD at 5%	45.033	5.1872	4.9356	3.5442

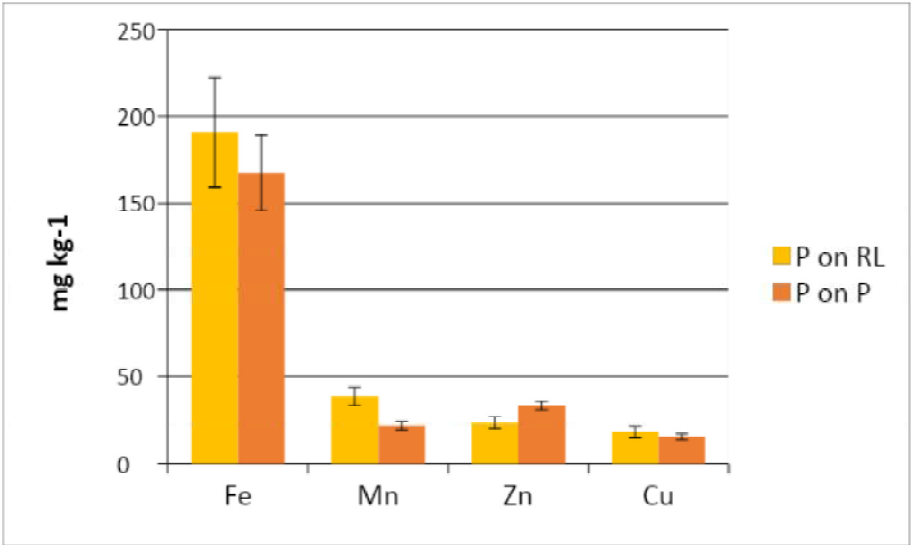

Fig. 2 : Leaf micronutrient content in pummelo grafted on pummelo (P) and Rangpur lime (RL)

Table 4 : Leaf micronutrient content in pummelo grafted on Rangpur lime

Genotype	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Zn (mg kg^{-1})	Cu (mg kg^{-1})
Clone 19-1	147.20	38.37^{CDE}	21.67^{BC}	13.57^{D}
Clone 18-5	156.40	33.80^{CDEF}	15.73^{C}	14.30^{CD}
Clone 24-4	223.27	28.50^{EF}	26.60^{AB}	13.73^{D}
Clone 8-4	221.37	42.70^{BC}	26.80^{AB}	25.60^{A}
Clone 25-5	201.23	50.27^{AB}	21.27^{BC}	17.87^{BCD}
Clone 18-4	170.73	42.70^{BC}	24.03^{AB}	22.43^{AB}
Clone 21-4	175.87	27.27^{F}	23.97^{AB}	20.83^{ABC}
Clone 18-1	167.43	39.97^{BCD}	22.33^{BC}	16.03^{BCD}
Clone 10-5	188.97	54.80^{A}	21.90^{BC}	14.33^{CD}
Clone 18-3	183.73	36.00^{CDEF}	23.10^{B}	21.90^{AB}
Clone 25-2	230.97	30.50^{DEF}	25.90^{AB}	19.00^{ABCD}
Clone 20-4	223.97	37.93^{CDEF}	30.77^{A}	18.63^{BCD}
General Mean	190.93	38.57	23.67	18.19
p-Value	0.1478	0.0007	0.0396	0.0202
SE(d)	31.527	5.273	3.401	3.346
LSD at 5%	NS	10.935	7.0538	6.939

differences in the incorporation from the roots to shoots and then leaves (Srivastava and Singh 2003; 2005 and 2008).

CONCLUSION

Present investigation, clearly indicated the leaf nutrient content of pummelo varies among genotypes, but there is no genotype that stands out in all macro (N, P, K, Ca, Mg and S) and micronutrients (Fe, Mn, Zn and Cu) analyzed. However, Hyderabad Selection, Raichur Selection, IKP-1, Tirupathi-2, and Kalenahalli Selction-1 could be considered as superior pummelo accessions offering a great scope for genetic improvement programmes and maximizing productivity with less inputs. Average leaf nitrogen, potassium, iron, and manganese contents in pummelo were higher in plants grafted on Rangpur lime. Phosphorus, calcium, sulphur, and copper contents in pummelo-pummelo stionic combination were found almost comparable with the pummelo-Rangpur lime stionic combination. However, the foliar magnesium and zinc contents were found higher in pummelo - pummelo stionic combination which eventually reduces leaf yellowing/chlorosis in pummelo. Pummelo rootstocks were found to respond well in terms of P, Ca, Mg, S, Zn and Cu nutrient uptake and tolerance to Phytophthora as compared to Rangpur lime. Therefore, it can be concluded that pummelo can be an ideal rootstock for commercial pummelo cultivation with better nutrient absorption capacity, reduced chlorosis, and phytophthora incidence. Wider variations in leaf nutrients contents in pummelo accessions indicated the differential response of pummelo germplasm under similar soil-climatic conditions which emphasize due consideration while formulating leaf nutrient standards of pummelo for diagnostic and future nutrient management strategy as well.

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Original Research Paper

Physio-biochemical responses of polyembryonic mango (*Mangifera indica* L.) genotypes to varying levels of salinity stress

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ABSTRACT

Mango genotypes that are salinity tolerant can possibly be used as clonal rootstock for sustained production of salinity sensitive commercial mango cultivars in salt affected soils. Present study was carried out to elucidate the effect of salinity stress induced by salts of NaCl+$CaCl_2$ (1:1) at concentrations of 0, 25, 50 and 100 mM on fifteen polyembryonic mango genotypes. The physio-biochemical parameters such as relative water content, chlorophyll content, epicuticular wax content, water potential (Ψ), carbohydrate content, lipid peroxidation, proline accumulation and antioxidant enzymes were determined at each level of salinity in all genotypes. On the basis of these physio-biochemical changes, the study illustrated that the polyembryonic genotypes, Turpentine, Deorakhio, Olour, Bappakkai, Vattam, Nekkare, Kurukkan, Kensington, Muvandan, EC-95862, Manipur, Sabre, Vellaikolamban, Kitchener and Mylepelian were in the decreasing order in response to salinity stress tolerance.

Keywords : Antioxidant enzymes, lipid peroxidation, proline, RWC, salinity tolerance

INTRODUCTION

Mango (*Mangifera indica* L.) is grown in tropical and sub-tropical parts of the world and in India it occupies around 2.217 million hectares with 18.506 million MT production (Abd-Allatif *et al*., 2015). In this era of climate change, various abiotic stresses such as drought, salinity, high/low temperature are becoming serious issues for crop production acting as a principal cause for crop failure, yield reduction and decline in productivity. Among these stresses, salinity is a matter of great concern which covers nearly 6.74 million hectares of agricultural land in India and it might touch 16.2 million hectares by 2050 (Anonymous, 2015). Area under salt affected soils is increasing steadily due to several factors like insufficient precipitation, deforestation, amount of salt concentration in river basins, poor drainage, increasing rate of evaporation *etc*. Mango being a salt sensitive crop, shows scorching of leaf tips and margins, leaf curling and in severe cases growth reduction, low chlorophyll content, increased abscission of leaves and death of trees, particularly at early stages of growth under salinity stress (Srivastav *et al*., 2007). Though several strategies like leaching, good drainage, application of high-quality irrigation water, tillage and amendments of coarse organic matter *etc*. can be applicable to maintain the soil and plant health under saline condition, these are expensive and temporary. Breeding for resistant genotypes could be laborious and complex due to the polygenic nature of salt resistance. If good-quality water or adequate drainage facilities are not available in salt affected soils, the only option is to introduce the salt tolerant crops to make use of such soils.

Of late, use of salt tolerant rootstocks has gained wide attention in management of salinity and sustained production of different fruit crops like mango, citrus and grape. Though rootstocks have widely been studied for manipulating growth and flowering in mango, there is limited information on its use for alleviating adverse effects of salinity. The rootstocks induce salinity tolerance by restricting the movement and/or avoiding absorption and accumulation of toxic ions from the saline soils by undergoing various physio-biochemical changes. However, evaluation of mango rootstocks

employing physio-biochemical attributes under imposed salinity and assessing the relative tolerance has drawn little attention. In the present investigation, physio-biochemical response of 15 polyembryonic mango genotypes to varying levels of salinity stress was studied with an objective to identify rootstocks exhibiting better salinity tolerance and their further use in grafting for commercial mango cultivation in salt affected soils.

MATERIALS AND METHODS

Plant material and growing conditions

The present study was conducted at ICAR-Indian Institute of Horticultural Research (IIHR), Bengaluru during 2015 to 2017, to study the response of different polyembryonic mango genotypes to salinity stress levels. Five months old seedlings of fifteen genotypes *viz*., EC-95862, Bappakkai, Vellaikolamban, Nekkare, Turpentine, Muvandan, Kurukkan, Kensington, Olour, Manipur, Deorakhio, Vattam, Mylepelian, Sabre and Kitchener, raised in polythene bags filled with 1.5 kg mixture of soil, sand and FYM (1:1:1 v/v), were subjected to salinity stress levels by irrigating with 0 mM, 25 mM, 50 mM and 100 mM solutions of NaCl+$CaCl_2$ (1:1, w/w) at regular interval of four days. When visual symptoms of salinity stress as marginal leaf burning started to appear on leaves after 40 days under 100 mM salinity stress, leaf samples at each level of salinity stress were collected from all genotypes for estimation of physio-biochemical parameters. For estimation of antioxidant activities, the fresh leaf samples were ground in liquid nitrogen and preserved at -80°C. The different physio-biochemical parameters were estimated using procedures as described below.

Relative water content (RWC)

RWC was estimated by following the procedure given by Barrs and Wheather (1962). Uniform leaf discs of one centimeter diameter were cut using cork borer and fresh weight of twenty discs was recorded. These discs were placed in petri plates containing 15-20 mL distilled water. After 4-5 hours, turgid weight of the discs was recorded and kept in hot air oven at 60±5°C temperature until constant dry weight was attained. RWC was expressed in *per cent* by using formula RWC = [(Fresh weight - Dry weight) / (Turgid weight - Dry weight)] x 100.

Carbohydrate content

The carbohydrate content of the leaves was estimated using the anthrone reagent method (Hedge and Hofreiter, 1962). Fresh leaf sample of 100 mg was taken into test tube and hydrolysed with 5 mL of 2.5 N HCl and kept in a boiling water bath for three hours and then cooled to room temperature. Solution was neutralized by adding solid Na_2CO_3 until the effervescence ceased. Total volume was then made up to 50 mL with distilled water and supernatant was collected. To 0.5 mL supernatant, 4 mL of anthrone reagent was added and the solution was heated for 8 minutes in a boiling water bath and cooled rapidly. The resultant green to dark green colour was read at 630 nm using UV-Visible spectrophotometer (T80+ UV/VIS, PG Instruments Ltd. UK). The final values were calculated using following formula and expressed in mg/ g fresh weight of sample

$$\text{Carbohydrate content (mg/g)} = \frac{(OD_{630nm} \times \text{Std. value})}{\text{Aliquot took (ml)}} \times \frac{\text{Total vol. of the extract (ml)}}{\text{Wt. of the sample (g)}} \times \frac{100}{1000}$$

Membrane stability index (MSI)

The MSI was determined through *per cent* electrolyte leakage as discussed by Laxman (2014). Ten leaf discs having 10 mm diameter were taken using cork borer and transferred to test tube containing 30 mL distilled water and incubated for 30 minutes at room temperature and initial electrical conductivity (EC_1) was recorded using conductivity meter (model SYSTRONICS; India). The tubes were incubated at 50°C for half an hour and EC_2 was recorded. The test tubes were placed in hot water bath at 100°C for one hour and EC_3 was noted. The MSI was calculated using the following formula MSI= [1-((EC_2-EC_1)/ EC_3)] ×100.

Malondialdehyde content

Leaf tissue weighing 0.25 g was homogenized with 5 mL of distilled water using pestle and mortar. The sample was taken into test tube and 5 mL of thiobarbituric acid (TBA) and 5 mL of trichloroacetic acid (TCA) were added. The contents were heated for 30 minutes in a boiling water bath and later centrifuged at 10000 rpm for 10 minutes. The absorbance of supernatant was read at 532 and 600 nm in UV-Visible spectrophotometer (T80+ UV/VIS, PG Instruments Ltd.). The malondialdehyde (MDA) content was calculated using its extinction coefficient 155 mM^{-1} cm^{-1} (Heath and Packer, 1968) and formula:

MDA content (nmol g^{-1} FW) = [(A532-A600) ×V×1000/$\mathfrak{E}$] ×W, where $\mathfrak{E}$ is the specific extinction coefficient (155 mM cm^{-1}), V is the volume of sample and W is the fresh weight of leaf.

Chlorophyll content

Leaf sample weighing 100 mg was placed into test tube and 10 mL of dimethyl sulphoxide (DMSO) was added. The tubes were then incubated in an oven at temperature 65°C for 3½ hours and the contents filtered. The chlorophyll content was determined using spectrophotometer at wavelengths of 663 and 645 nm, against pure DMSO as blank. The amount of chlorophyll a, chlorophyll b and total chlorophyll in terms of mg g^{-1} fresh weight basis was estimated using the method described by Hiscox and Isrealstam (1979). The values were calculated using formula:

$$\text{Chl a} = \frac{[12.7(A663) - 2.69(A645)] \times V}{W} \times 1000$$

$$\text{Chl b} = \frac{[22.9(A645) - 4.68(A663)] \times V}{W} \times 1000$$

Total chlorophyll = Chl a+Chl b

Where, A = absorbance, V = volume of DMSO solution, W = weight of sample

Epicuticular wax content (ECW)

ECW was estimated using the method described by Ebercon *et al*. (1977) with some modifications. Fully expanded mature leaves were cleaned using cotton to remove dust particles. Three leaf segments (3 cm^2) were immersed in test tube containing 10 mL chloroform and shaken with electronic shaker for 30 seconds. Then chloroform was transferred to another test tube and allowed to evaporate completely. A five mL of acidic $K_2Cr_2O_7$ was added and the solution was heated in boiling water bath for 30 minutes. After cooling, final volume was adjusted to 12 mL with distilled water and optical density was read at 590 nm using UV-VIS spectrophotometer (T80+ UV/VIS, PG Instrument Ltd., UK).

Leaf water potential (ψ)

The Ψ of fully mature and expanded leaf of each genotype was determined instantly after collecting between 11.30 am and 12.30 pm, using pressure bomb apparatus (ARIMAD, v3000 MRC) and expressed in -MPa.

Proline content

Proline content was estimated using rapid colorimetric method (Bates *et al*., 1973). Leaf sample of 0.25 g was extracted by homogenizing in 5 mL of 3% aqueous sulphosalicylic acid followed by centrifugation at 10000 rpm for 10 min at 4^0C Two mL supernatant was collected in test tube, and 2 mL of glacial acetic acid and 2 mL acid ninhydrin were added. The test tubes with sample were heated in the boiling water bath for 1 hour, and then placed in ice bath to terminate the reaction. After addition of 4 mL toluene to the reaction mixture and stirring it for 20-30 seconds using electronic shaker, the toluene layer was separated and warmed at room temperature. The intensity of dark pink color produced was measured at 520 nm with toluene as blank using a UV-visible spectrophotometer (T80+ UV/VIS, PG Instruments Ltd. UK) and expressed in $mg100^{-1}g$ FW.

Antioxidant enzymes activity

Superoxide dismutase (SOD) activity was estimated using the method described by Du and Bramlage (1994). Leaf tissue weighing 0.25 g was homogenized with 5 mL of 0.05 M potassium phosphate buffer (pH 7.8) containing 0.2% PVP, 0.1 mM EDTA and 3 mM $MgCl_2$. Activity was assessed using a reaction mixture containing buffer, 39 mM methionine, 450 µM nitro blue-tetrazolium (NBT), 12 µM riboflavin, 10 mM EDTA and enzyme extract 0.03 mL. The inhibition of photo-oxidation of NBT under fluorescent light was measured at 560 nm and expressed in units of enzyme mg^{-1} of protein. The catalase (CAT) activity was determined using the method described by Masia (1998). Enzyme was extracted from 0.25 g leaf sample using 5 mL of 0.067 M sodium phosphate buffer (pH 7.0) containing 0.2% PVP, 0.1 mM EDTA and 3 mM $MgCl_2$. Activity was measured by the reduction in absorbance at 240 nm in a mixture containing buffer, 0.3 mL H_2O_2 solution and enzyme extract for 5 min at 1 min interval and expressed in units of enzyme $min^{-1}mg^{-1}$of protein. The POX activity was determined as described by Chander (1990). Enzyme was extracted using 5 mL of 0.05 M citrate buffer (pH 6.4) containing 0.2% PVP, 0.1 mM EDTA and 3 mM $MgCl_2$ and the activity was estimated by measuring the H_2O_2 (0.2 mL) dependent oxidation of o-phenylenediamine (0.2 mL) at 450 nm for 5 min at 1 min interval and expressed in unitsμg^{-1} protein. The polyphenol oxidase (PPO) was determined following Selvaraj and Kumar (1995)

using pyrogallol as substrate. Frozen tissue of 0.25 g weight was thoroughly ground with 5 mL of 50 µM citrate buffer (pH 6.8) with 0.2% PVP 0.1 mM and EDTA. The extract was centrifuged at 10000 rpm for 10 minutes and the supernatant was collected. After initiating the reaction by adding pyrogallol, the increase in absorbance was measured at 450 nm for 5 minutes at 1 minute interval and values expressed as OD change $min^{-1}\mu g^{-1}$ of protein.

Experimental design and statistical analysis

The experiment was laid out in factorial completely randomized design with six plants each genotype under each treatment. The data were analysed by using statistical software SAS 9.3 versionand subjected to the analysis of variance (ANOVA). Significant differences among the genotypes induced by salinity stress were compared using Fisher's test at $P \leq 0.05$. The resulted values mentined in text are per cent increased and decreased over control.

RESULTS AND DISCUSSION

Relative water content (RWC) and carbohydrate content under salinity stress

The RWC of the mango leaves in the plants subjected to different levels of salinity varied from lower (25 mM) to higher (100 mM) salt concentration and indicated decreasing percentage within the range of (1.97 to 28.31%) over control. Turpentine maintained good amount of RWC (13.91%), while, Mylepelian showed maximum reduction (28.31%) at highest salt concentration (100 mM) over control (Table 1). Data indicated increased carbohydrate content with increased levels of salinity. Carbohydrate content increased from 26.90% to 71.63% with 25 mM and 50 mM salt solutions compared to control. Higher carbohydrate content was observed in genotypes Turpentine (82.87%) followed by Deorakhio (82.39%) and Olour (80.68%) compared to Mylepelian (35.90%), Kitchener (38.15%) and Vellaikolamban (47.26%) over control at 100 mM salt. Quantifying the effects of salinity on RWC is required to know the plant water status under salinity stress condition. Reduction of RWC in leaves of mango genotypes imposed with 25 mM, 50 mM and 100mM salinity levels may be because of osmotic stress and cellular dehydration induced by salt, as pointed out by Lata *et al*. (2011) in mango. Increasing level of salinity stress tended to increase the carbohydrate content in leaves of all the mango genotypes. Among the genotypes, maximum increase in carbohydrate content was found in Turpentine while Mylepelian depicted the

Table 1 : Changes in RWC (%) and carbohydrate content (mg g^{-1} FW) in polyembryonic mango genotypes under varying levels of salinity

Genotype	RWC (%)				Carbohydrate content (mg g^{-1} FW)			
	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM
EC-95862	84.77^{ef}	80.37^{de}	75.94^{efg}	69.55^{defg}	32.33	45.43^{bcd}	47.67^{cdef}	52.33^{cd}
Vattam	88.60^{ab}	85.39^{ab}	81.20^{abc}	75.04^{ab}	35.33	52.60^{abc}	57.70^{abc}	63.00^{ab}
Vellaikolamban	87.66^{bcd}	81.99^{cde}	77.31^{def}	69.20^{efg}	32.23	43.30^{bcd}	44.17^{ef}	47.47^{de}
Nekkare	84.80^{ef}	81.50^{cde}	77.49^{def}	71.61^{cde}	33.33	49.17^{abc}	53.90^{abcde}	58.33^{bc}
Mylepelian	86.82^{cd}	79.86^{e}	73.10^{g}	62.24^{h}	30.73	39.00^{d}	39.83^{f}	41.77^{e}
Turpentine	87.36^{bcd}	85.63^{a}	81.62^{a}	75.21^{a}	37.37	58.57^{a}	64.13^{a}	68.33^{a}
Sabre	84.44^{f}	79.22^{e}	74.82^{fg}	68.11^{fg}	31.37	43.03^{cd}	44.43^{def}	48.73^{de}
Manipur	88.50^{ab}	83.43^{abcd}	78.97^{abcde}	71.52^{cde}	30.67	43.07^{cd}	44.43^{def}	49.20^{de}
Kitchener	88.28^{bc}	82.18^{bcde}	76.42^{ef}	66.66^{g}	34.17	44.73^{bcd}	45.47^{def}	47.20^{de}
Kensington	89.90^{a}	85.99^{a}	81.80^{a}	74.86^{ab}	31.97	45.10^{bcd}	47.67^{cdef}	53.97^{cd}
Olour	84.89^{ef}	82.30^{bcde}	78.22^{cde}	72.17^{bcd}	33.13	50.23^{abc}	55.77^{abcd}	59.87^{bc}
Kurukkan	84.71^{ef}	81.11^{cde}	77.31^{def}	70.99^{cdef}	31.03	45.77^{bcd}	49.97^{bcdef}	54.30^{cd}
Bappakkai	88.56^{ab}	85.74^{a}	81.39^{ab}	75.17^{a}	35.63	53.33^{ab}	59.87^{ab}	64.07^{ab}
Muvandan	87.28^{bcd}	83.33^{abcd}	78.52^{bcde}	71.78^{cde}	31.53	44.47^{bcd}	46.67^{cdef}	52.97^{cd}
Deorakhio	86.20^{de}	83.75^{abc}	79.68^{abcd}	73.37^{abc}	37.30	57.67^{a}	63.13^{a}	68.03^{a}
S.E.m(±)	0.74	1.56	1.48	1.42	3.26	3.43	3.87	2.59
LSD ($P \leq 0.05$)	1.52	3.21	3.05	2.92	NS	10.04	11.41	7.63

least increase over control. Increased carbohydrate content under salinity might be indicative of stress adaptation as it not only functions as osmoprotectant but also helps in osmotic adjustment, carbon storage and radical scavenging which could be altered under salinity stress. Singh *et al*. (2000) also reported increase in CHO (glucose, fructose, sucrose and fructans) under salinity stress in grape cvs. Perlette, Pusa Seedless and Beuty Seedless.

Amount of malondialdehyde (MDA) and Membrane stability index (MSI) under salinity stress

At lower salt concentration (25 mM), least amount of MDA content was recorded in Turpentine (10.20%), whereas, maximum was recorded in Mylepelian (29.20%) (Table 2). At 50 mM and 100 mM salt stress also, similar trends were observed. MSI reduced with increase in salt concentration (Table 2). In Mylepelian at 25 mM and 50 mM level of salinity stress, MSI was reduced to15% and 25%, respectively, and in Turpentine only 1% and 3%, respectively compared to 0 mM salt stress. The genotypes Turpentine, Deorakhio and Olour shown better membrane stability (16.09%, 19.51% and 19.72% reduction, respectively) while Mylepelian, Kitchener and Vellaikolamban had more reduction (36.75%, 35.88% and 33.80%, respectively) at 100 mM salt concentration when compared with 0 mM concentration. MDA content serves as an indicator for amount of cell damage during osmotic stress induced by salinity. Lower accumulation of MDA content in salt tolerant genotypes may be due to the potential of genotypes to protect cell damage during salinity stress. Oxidative stress, induced by saline condition, leads to the formation of lipid peroxidation in the form of MDA has been used as essential marker or indication for identifying the amount of cell damage (Tayebimeigooni, 2012). Study conducted by Dayal *et al*. (2014) also depicted the increase in accumulation of content in NaCl stressed Amrapali, Kurrukan and Olour mango plants. Higher membrane stability and lower level of MDA in genotypes Turpentine, Deorakhio and Olour might be due to the increased activities of antioxidant enzymes (POX, CAT, SOD and PPO) which serve as a protective mechanism against oxidative stress. The maintenance of membrane stability under salinity stress might be associated to the less MDA accumulation and thereby less membrane injury which can be considered as an effective parameter for finding the tolerance and sensitive nature of plant under salt stress. The results on MSI are in conformity with the findings of Gora *et al*. (2017) where MSI in monoembryonic and polyembryonic cultivars of mango drastically

Table 2 : Changes in MDA (nmoles g^{-1} FW) and MSI (%) in polyembryonic mango genotypes under varying levels of salinity

Genotype	MDA (nmoles g^{-1} FW)				MSI (%)			
	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM
EC-95862	25.03^{abcd}	30.24^{bcde}	33.44^{def}	40.52^{c}	89.25	79.29^{bcd}	75.46^{de}	65.38^{d}
Vattam	23.27^{cd}	27.05^{efg}	30.11^{hi}	35.57^{fg}	89.95	86.24^{ab}	80.42^{bc}	71.07^{b}
Vellaikolamban	27.29^{ab}	33.55^{ab}	37.63^{ab}	47.01^{b}	89.87	77.56^{cd}	71.86^{fg}	59.49^{fg}
Nekkare	24.26^{bcd}	28.24^{defg}	31.51^{fgh}	37.12^{ef}	90.23	84.68^{abc}	78.22^{cd}	69.99^{bc}
Mylepelian	27.76^{a}	35.87^{a}	38.75^{a}	51.57^{a}	87.53	73.76^{d}	65.58^{h}	55.36^{g}
Turpentine	23.18^{cd}	25.55^{g}	28.47^{i}	33.89^{g}	90.62	88.85^{a}	87.05^{a}	76.03^{a}
Sabre	26.15^{abc}	32.13^{bc}	35.73^{bcd}	44.62^{b}	89.75	79.10^{bcd}	72.76^{ef}	61.04^{ef}
Manipur	27.38^{a}	33.55^{ab}	37.12^{abc}	45.68^{b}	89.79	79.54^{bcd}	73.09^{ef}	65.19^{de}
Kitchener	25.16^{abcd}	31.87^{bcd}	35.10^{cde}	44.60^{b}	89.67	77.29^{cd}	68.38^{gh}	57.49^{fg}
Kensington	23.44^{cd}	27.87^{efg}	30.71^{ghi}	36.90^{ef}	89.98	80.95^{abcd}	77.19^{cd}	66.75^{cd}
Olour	23.48^{cd}	26.28^{fg}	29.72^{hi}	35.48^{fg}	90.82	88.30^{a}	85.95^{a}	72.91^{ab}
Kurukkan	24.17^{cd}	28.69^{cdefg}	31.42^{fgh}	37.59^{def}	90.25	82.23^{abcd}	78.02^{cd}	68.96^{bcd}
Bappakkai	26.13^{abc}	29.48^{cdef}	33.38^{def}	39.83^{cd}	89.97	86.67^{ab}	83.60^{ab}	71.46^{b}
Muvandan	24.88^{abcd}	29.85^{bcdef}	32.99^{efg}	39.18^{cde}	89.80	80.52^{abcd}	76.08^{de}	65.81^{cd}
Deorakhio	22.90^{d}	25.31^{g}	28.43^{i}	34.15^{g}	90.77	88.65^{a}	86.50^{a}	73.05^{ab}
S.E.m(±)	1.52	1.81	1.21	1.31	0.968	4.23	1.75	2.05
LSD ($P \leq 0.05$)	3.11	3.71	2.48	2.69	NS	8.67	3.59	4.21

decreased with increasing NaCl stress. The maintenance of high MSI in Turpentine, Deorakhio and Olour pointed out their ability to maintain good amount of cell turgidity by improving their RWC and water potential compared to Mylepelian, Vellaikolamban and Kitchener, which depicted more reduction in MSI with increasing levels of salinity.

Leaf chlorophyll status and proline accumulation in response to salinity stress

There was variation in chlorophyll content among all the genotypes under salt stress (Table 3). The reduction in chlorophyll 'a' (2.18% to 31.80%), chlorophyll 'b' (11.01% to 34.85%) and total chlorophyll (4.59% to 32.52%) content among all genotypes which imposed with 25 mM and 50 mM salt concentrations. The genotypes Turpentine (15.32%), Deorakhio (20.87%) and Olour (22.50%) responded with less reduction in chlorophyll 'a' content, while, Mylepelian (51.42%), Kitchener (51.23%) and Vellaikolamban (47.03%) showed more reduction. Similar trend was recorded with relation to chlorophyll 'b' and total chlorophyll content (Table 3) in the same genotypes at higher level of salinity stress over control. It was noticed that the proline content increased with increase in salt concentration from 25 mM to 100 mM and it varied significantly among the genotypes under each level of salinity (Fig. 1). Higher proline accumulation in leaves of genotypes Turpentine (66.40%) and Deorakhio (64.39%) was recorded at 100 mM salt concentration, whereas, genotypes Mylepelian (48.65%) and Kitchener (52.22%) were found to limits the accumulation of proline at the same salinity stress. Decrease in chlorophyll content with increased salinity stress could be due to adverse effect of salinity on chlorophyll metabolism leading to its decline. Another cause for reduction in chlorophyll content could be variation in chlorophyll synthesis linked to the induction of 'chlorophyllase' a chlorophyll degrading enzyme in a plant species under saline conditions (Gunes *et al*., 2007). Proline is considered as a compatible osmolyte involved in carbon and nitrogen storage that helps in stress adaptation. In the present study, the extent of proline accumulation was influenced in mango genotypes by salinity stress. The maximum increased accumulation of proline over control was in cv. Turpentine and this could be an indication for its ability to combat the adverse effect of salinity stress through osmotic adjustment, reactive oxygen species (ROS) scavenging or by enhancing the anti-oxidant activity (Sharma and Dietz, 2006; Patel *et al*., 2011).

Epicuticular wax (ECW) deposition and leaf Ψ under salinity stress

ECW depositions on leaf surface varied across the genotypes at different levels of salt concentration. The ECW content increased as salt stress increased. Higher amount of ECW deposition was recorded in Turpentine at 25 mM and 50 mM, whereas, less content in Mylepelian. At higher salt concentration (100 mM), maximum increase of ECW was observed in Turpentine (91.28%) followed by Deorakhio (89.92%) and Olour (85.33%), whereas, minimum (50.47% to 62.35%) was recorded in Mylepelian, Kitchener and Vellaikolamban (Fig. 2). The Ψ in leaves generally declined with increasing salinity stress (Fig. 3). Among the fifteen genotypes

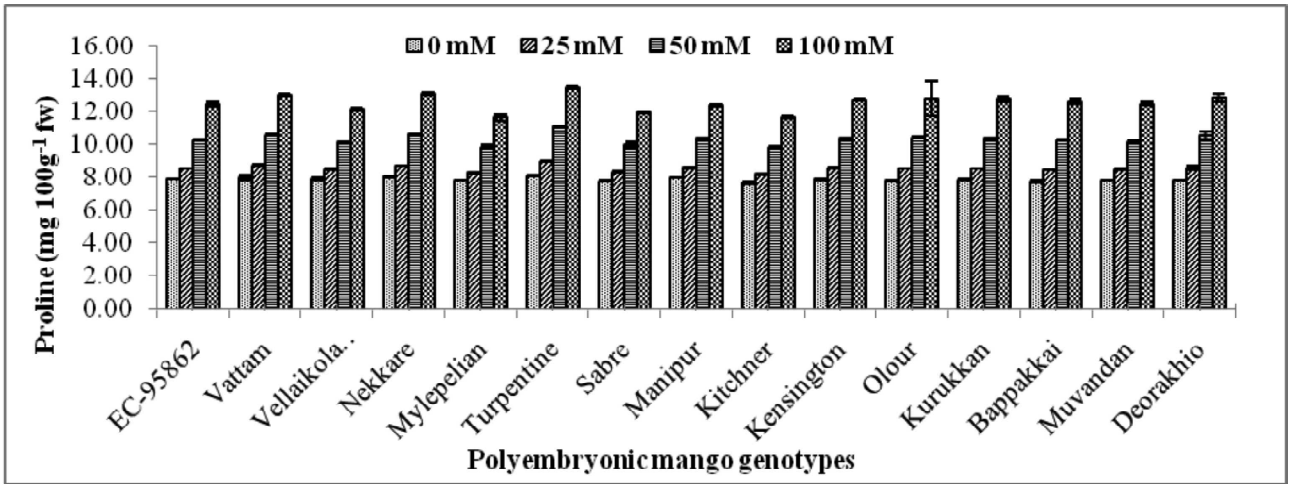

Fig. 1 : Changes in proline content (mg 100g $^{-1}$FW) in polyembryonic mango genotypes under varying levels of salinity

Table 3 : Changes in chlorophyll 'a', chlorophyll 'b' and total chlorophyll (mgg^{-1} FW) in polyembryonic mango genotypes under varying levels of salinity

Genotype	Chlorophyll 'a'				Chlorophyll 'b'				Total chlorophyll			
	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM	0 mM	25 mM	50 mM	100 mM
EC-95862	1.71	1.55^{c}	1.40^{bcde}	1.10^{cdef}	0.88	0.67^{abc}	0.64	0.50	2.59	2.22^{cde}	2.04^{cdef}	1.60^{cdefg}
Vattam	1.67	1.58^{c}	1.46^{bcde}	1.25^{bcdef}	0.69	0.56^{c}	0.54	0.47	2.36	2.14^{def}	2.01^{cdef}	1.72^{cde}
Vellaikolamban	1.54	1.34^{d}	1.14^{e}	0.82^{f}	0.89	0.63^{abc}	0.61	0.49	2.43	1.97^{f}	1.75^{f}	1.31^{fg}
Nekkare	1.94	1.81^{b}	1.68^{abc}	1.46^{abc}	0.77	0.61^{bc}	0.60	0.51	2.71	2.42^{bc}	2.28^{bc}	1.97^{abc}
Mylepelian	2.00	1.65^{bc}	1.37^{cde}	0.97^{def}	0.62	0.41^{d}	0.40	0.31	2.62	2.06^{ef}	1.77^{f}	1.28^{g}
Turpentine	2.10	2.05^{a}	1.97^{a}	1.77^{a}	0.79	0.70^{ab}	0.69	0.59	2.88	2.75^{a}	2.66^{a}	2.37^{a}
Sabre	1.77	1.56^{c}	1.34^{de}	0.99^{cdef}	0.92	0.66^{abc}	0.63	0.52	2.69	2.22^{cde}	1.97^{def}	1.51^{defg}
Manipur	1.70	1.51^{cd}	1.32^{de}	1.02^{cdef}	0.86	0.64^{abc}	0.60	0.49	2.56	2.15^{def}	1.92^{ef}	1.51^{defg}
Kitchener	1.84	1.56^{c}	1.30^{de}	0.90^{ef}	0.90	0.63^{abc}	0.60	0.47	2.74	2.19^{cdef}	1.90^{ef}	1.37^{efg}
Kensington	2.02	1.84^{b}	1.72^{ab}	1.38^{abcd}	0.97	0.75^{a}	0.72	0.59	2.99	2.59^{ab}	2.44^{ab}	1.97^{abc}
Olour	1.75	1.68^{bc}	1.61^{bcd}	1.36^{abcde}	0.78	0.66^{abc}	0.66	0.57	2.54	2.35^{cd}	2.27^{bcd}	1.93^{bcd}
Kurukkan	2.26	2.07^{a}	1.95^{a}	1.65^{ab}	0.88	0.70^{ab}	0.67	0.56	3.14	2.76^{a}	2.62^{a}	2.20^{ab}
Bappakkai	1.74	1.65^{bc}	1.56^{bcd}	1.33^{abcde}	0.77	0.63^{abc}	0.63	0.54	2.51	2.28^{cde}	2.19^{bcde}	1.87^{bcd}
Muvandan	1.72	1.56^{c}	1.42^{bcde}	1.13^{cdef}	0.94	0.72^{ab}	0.69	0.57	2.66	2.28^{cde}	2.11^{cde}	1.70^{cdef}
Deorakhio	1.54	1.48^{cd}	1.43^{bcde}	1.22^{bcdef}	0.79	0.68^{abc}	0.68	0.59	2.33	2.17^{def}	2.11^{cde}	1.81^{bcd}
S.E.m(±)	0.408	0.100	0.159	0.227	0.224	0.067	0.11	0.141	0.337	0.115	0.152	0.203
LSD ($P \leq 0.05$)	NS	0.205	0.326	0.465	NS	0.136	NS	NS	NS	0.234	0.310	0.415

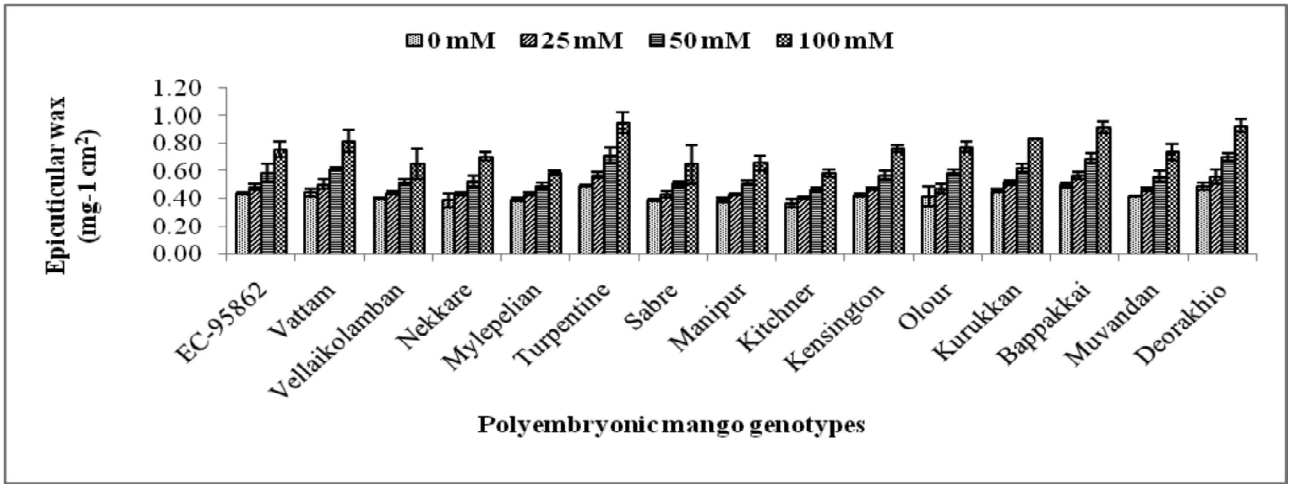

Fig. 2 : Changes in epicuticular wax content ($mg^{-1}cm^2$) of polyembryonic mango genotypes under varying levels of salinity

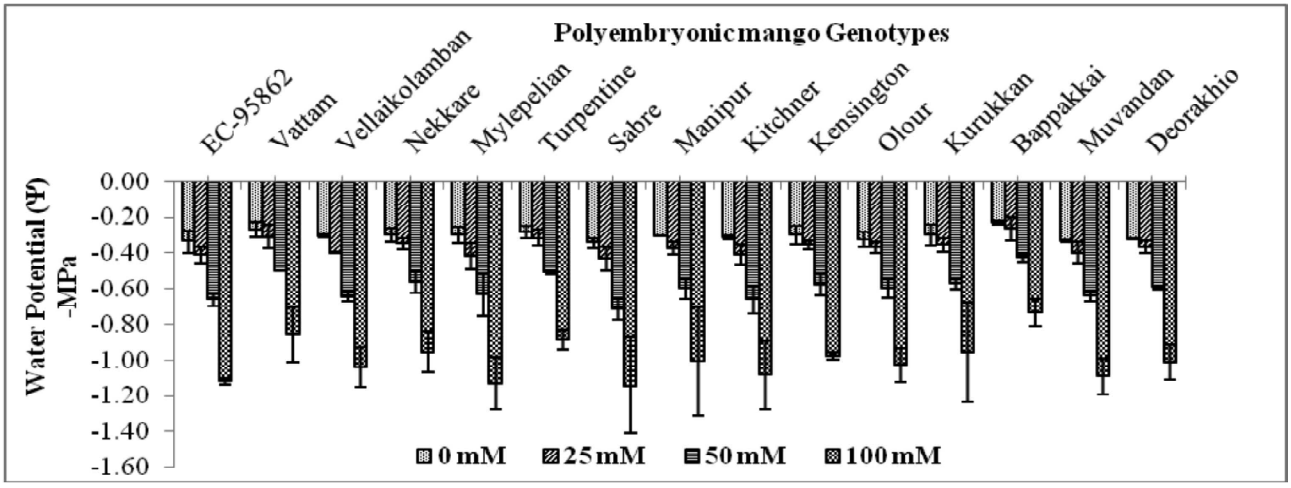

Fig. 3 : Changes in Ψ (-MPa) in leaves of polyembryonic mango genotypes under varying levels of salinity

it was varying from (-0.267 to -0.433 MPa) in 25 mM and (-0.427 to 0.717 MPa) in 50 mM salinity stress. The genotypes Turpentine, Deorakhio and Olour showed less reduction in Ψ (212.94% to 215.31%) while Mylepelian, Kitchener and Vellaikolamban exhibited more reduction (276.67% to 239.13%) in Ψ at higher level of salt concentration (100 mM) over control. The epicuticular wax serves as protective layer and helps in regulating the gas exchange, leaf temperature and light reflectance properties under abiotic stress conditions which imparts tolerance (Mansour, 2007). ECW deposition during the stress condition is important for balancing the transpiration and overheating of leaves which affect the photosynthesis. Though non-significant differences among the genotypes with respect to ECW, the rate of deposition on the cuticular surface was found to increase with increase in salinity stress. This adaptive mechanism can relate to the tolerance of genotypes *viz.*, Turpentine, Deorakhio and Olour, which exhibited the maximum increased ECW content over control in comparison to other rootstocks (Mylepelian, Kitchener and Vellaikolamban). Leaf Ψ became more negative as level of salinity increased. High osmotic pressure induced by salinity stress adversely affects the ability of plant cells to uptake water and nutrients. The study of Fozouni *et al*. (2012) has recently confirmed that leaf Ψ reduced under salinity stress in grape cv. Dastarchin and red Sultana. Maintenance of high water potential under salinity stress is evidence for salt tolerance nature of Turpentine, Deorakhio and Olour. This ability might be generated through multi-factors like greater maintenance of RWC and MSI, higher accumulation of proline *etc*.

Antioxidant enzymes activity

Peroxidase (POX) activity increased as the level of salt oncentration increased compared to control plants (Fig. 4). It was in the ranged 6.00% to 14.04% under 25 mM and 19.77% to 37.82% in 50 mM treated plants compare to control. When treated with 100 mM salt concentration, the genotypes Turpentine, Deorakhio and Olour had higher activity of POX (89.23%, 85.02% and 83.85%, respectively) over control. However, a lower increase in POX activity was recorded in Mylepelian, Kitchener and Vellaikolamban (46.19%, 51.10% and 56.66%, respectively). There was significant variation in terms of SOD activity, among the genotypes under different levels of salinity (0 mM, 25 mM, 50 mM and 100 mM). Activity of SOD was in the range of 14.24 to 18.35 units mg^{-1} of protein under control (0 mM), (Fig. 5). Maximum increase in SOD activity was recorded in Turpentine (22.22%) and minimum in Mylepelian (11.06%) at 100 mM level. Salinity at each level influenced the CAT activity in all genotypes (Fig. 6). CAT activity increased at 25 mM and 50 mM from (2.82% to 4.05%) in Mylepelian, whereas, in Turpentine it increased from 13.73% to 24.71%. Higher CAT activity was recorded in Turpentine (40.35%), Deorakhio (36.46%) and Olour (34.59%) against 9.73% in Mylepelian at 100 mM salt concentration. Activity of PPO increased with increase in salt concentration (Fig. 7). Maximum increase in PPO activity (122.05%, 105.49% and 105.22%) was noted at 100 mM salinity stress in the leaves of Turpentine, Deorakhio and Olour genotypes respectively, while, minimum increase (38.57%, 48.01% and 50.43%) was recorded in Mylepelian, Kitchener and Vellaikolamban, respectively. Similar trend of marginal increase in PPO activity was examined in 25 mM and 50 mM salinity stress-imposed plants. Generally, stress condition triggers generation of ROS such as O_2-, OH, $_1O^2$ and H_2O_2 which translocate the stress signals to different plant parts activating enzymatic and non-enzymatic antioxidant machineries, which subsequently stand against this adverse condition. POX, SOD, CAT and PPO are part enzymatic antioxidants defense system which has crucial role for mitigating the adverse effects of biotic and abiotic stresses. SOD an antioxidant enzyme, plays important role in dismutation of superoxide anion radicals and scavenging activity of cell against ROS under stress

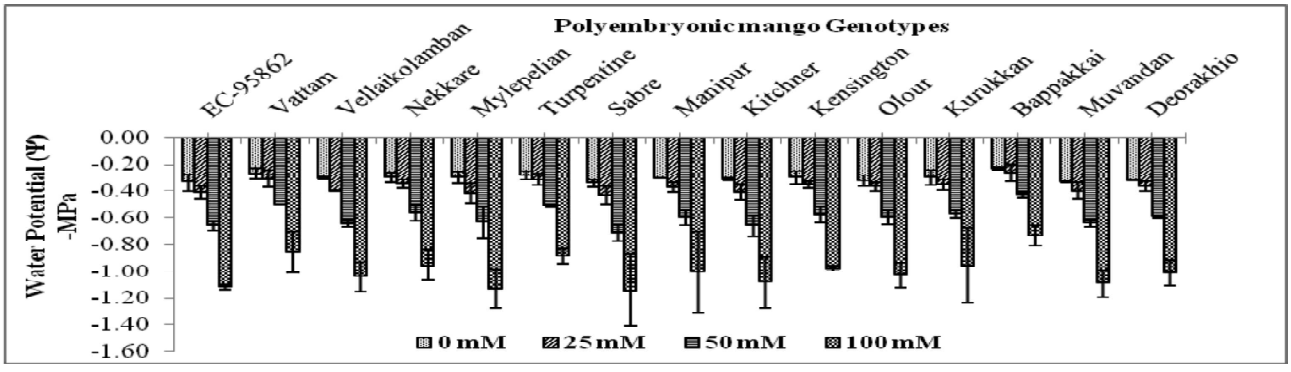

Fig. 4 : Changes in POX activity of polyembryonic mango genotypes under varying levels of salinity

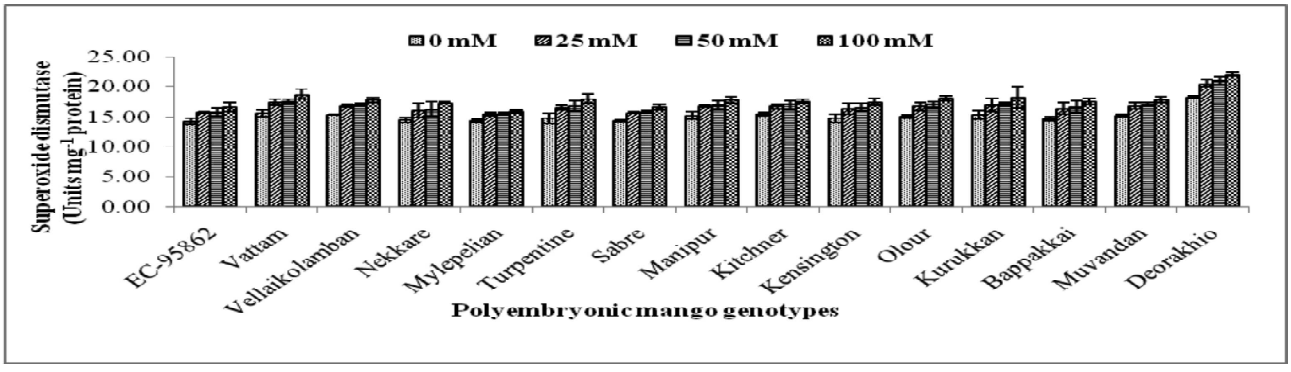

Fig. 5 : Changes in SOD activity of polyembryonic mango genotypes under varying levels of salinity

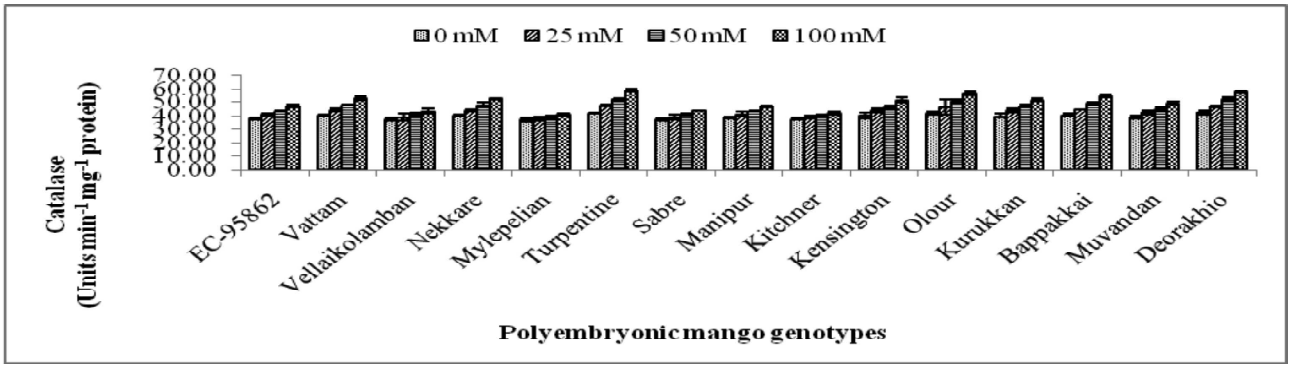

Fig. 6 : Changes in CAT activity of polyembryonic mango genotypes under varying levels of salinity

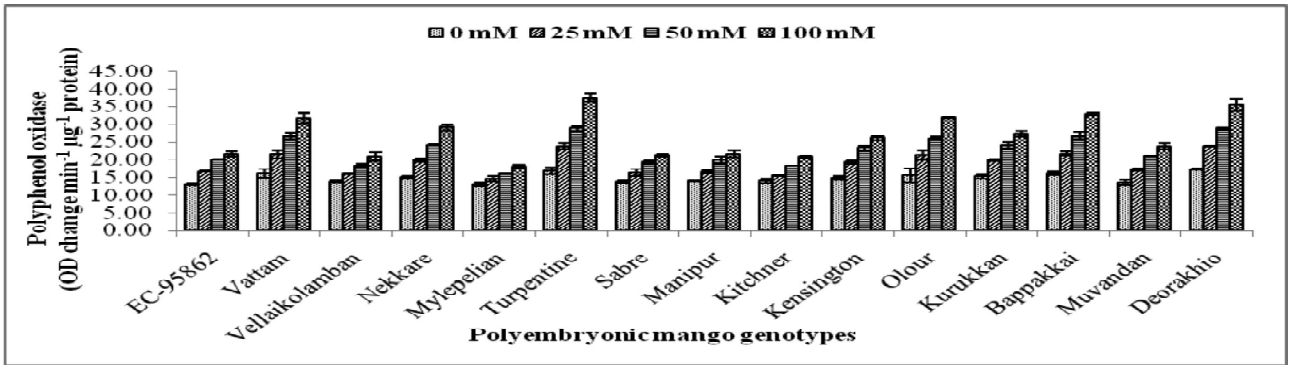

Fig. 7 : Changes in PPO activity of polyembryonic mango genotypes under varying levels of salinity

conditions. The increase in POX activity under salinity induced stress in rootstock-scion combinations of mango genotypes also reported by Dayal *et al*. (2014). The results are also in support of the findings of Rahnama and Ebrahimzadeh (2005) where the activity of SOD increased in salt tolerant cultivars of the potato. Increase in CAT activity plays a defence role against the accumulation of H_2O_2 under stressed condition by overcoming potential damage to leaf tissues. The genotypes depicted the higher percent of CAT activity under increasing salinity stress found better tolerance nature. Results yielded similar findings as that of Pandey *et al*. (2014) who showed increase in CAT activity with higher level of salinity in seven mango rootstocks. The higher activity of PPO also found under salinity stress which helps to imparts defense mechanism against salinity stress in mango (Abd-Allatif, 2015). With consideration to overall enzymatic study the activity of CAT, SOD, POX and PPO increased with salinity stress among all the mango genotypes. Maximum *per cent* increase over control was observed in seedlings of tolerant genotypes such as Turpentine, Deorakhio and Olour over the sensitive ones (Mylepelian, Kitchener and Vellaikolamban), in which minimum activities of the same antioxidant enzymes were noticed.

CONCLUSION

The polyembryonic mango genotypes under varying levels of salinity depicted that Turpentine, Olour, Deorakhio, Bappakkai and Vattam were found to respond with less reduction in RWC, chlorophyll 'a'and total chlorophyll content, and with more increase in leaf epicuticular wax, carbohydrate, proline, CAT, SOD, POX and PPO and less increase in lipid peroxidation (MDA). However, there were no significant differences in chlorophyll 'b' content and leaf Ψ at each level of salinity across the all genotypes. Physio-biochemical parameters such as proline content, antioxidant enzymes activity RWC and leaf ECW were the prominent biochemical markers for assessment of the salt tolerance of mango genotypes. Based on the overall performance pertaining to physio-biochemical changes under different levels of salinity, the genotypes in decreasing order of salt tolerance are Turpentine, Deorakhio, Olour, Bappakkai, Vattam, Nekkare, Kurukkan, Kensington, Muvandan, EC-95862, Manipur, Sabre, Vellaikolamban, Kitchener and Mylepelian. Among these genotypes, Turpentine,

Deorakhio, Olour and Bappakkai were performed better in response to their physio-biochemical behavior at higher level of salinity which could be potential rootstocks for salt sensitive commercial mango cultivars.

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Original Research Paper

Optimization of explants, media, plant growth regulators and carbohydrates on callus induction and plant regeneration in *Citrus jambhiri* Lush.

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ABSTRACT

Callus induction was attempted from the four explants *viz*. root, cotyledon, epicotyl, and leaf segments excised from *in vitro* raised seedlings of *C. jambhiri.* Among various MS media supplementations with growth regulators and carbohydrates, the maximum (95.50%) and the earliest (8.30 days) callogenesis was obtained in epicotyl segments, when cultured on MS medium supplemented with NAA (10.0 mgl^{-1}) + BAP (1.0 mgl^{-1}) + sucrose (8%). The modified MS (macro and micro-nutrients reduced to half) fortified with BAP (5.0 mgl^{-1}) + GA_3 (3.0 mgl^{-1}) recorded maximum shoot regeneration (43.10%) from callus, with an average of 5.30 shoots per callus after 35.50 days of culturing. However, prolonged exposure to GA_3 resulted in thin elongated shoots and leaves. The age of the callus substantially influenced the plant regeneration frequency. The potency of the callus to regenerate decreased significantly with an increase in the age of the callus. Shoot regeneration was recorded maximum (43.43%) in 60 days old calli, followed by 90 days old (30.48%) calli, whereas it was minimum (10.46%) in 150 days old calli. The maximum (79.50%) shoot proliferation was recorded in MS medium supplemented with BAP (1.0 mgl^{-1}) + Kin (0.5 mgl^{-1}) with an average of 5.06 shoots per culture. The MS medium fortified with NAA (1.0 mgl^{-1}) + IBA (1.0 mgl^{-1}) induced maximum (77.33%) rooting, with an average of 3.19 roots per shoot after 13.4 days of culturing. Rooted plants were hardened and survived the best (83.6%) on the potting mixture consisting of cocopeat + vermiculite + perlite (2:1:1).

Keywords : Callus, carbohydrates, *Citrus jambhiri*, explants, redifferentiation

INTRODUCTION

Rough lemon (*Citrus jambhiri* Lush.) is a commonly used citrus rootstock that is highly polyembryony and native to India (Wu *et al*., 2018). High vigour, profuse and deep root system, well adaptation to different agroclimatic zones, and resistance to viruses makes it a suitable rootstock for grafting scion of mandarins, sweet oranges, lime, lemons, and grapefruits in many countries (Russo *et al*., 2021). However, *Citrus jambhiri* is highly susceptible to *Phytophothora* fungus and yields moderate fruit quality. The genetic improvement through conventional plant breeding approaches is a difficult task because of its long juvenility, perennial habit, nucellar polyembryony, heterozygosity, etc (Salonia *et al*., 2020). Biotechnological tools such as genetic engineering, tissue culture-induced variations from somatic cells, and *in vitro* mutagenesis are alternate promising approaches for the genetic improvement of citrus genotypes (Kayim and Koc, 2006). *In vitro* manipulations through these techniques require an efficient and reproducible protocol for the production of callus and subsequent regeneration of plants through somatic embryogenesis (Moniruzzaman *et al*., 2021). The young and old embryogenic callus can be used for genetic transformation and induction of somaclonal variations, respectively. Earlier, embryogenic cultures were developed from reproductive organs *i.e.* isolated nucellar embryos or fertilized ovules (Maheshwari and Rangaswamy, 1958; Litz *et al*., 1985), abortive (Bitters *et al*., 1970), unfertilized (Button and Bornman, 1971), and undeveloped ovules (Carimi *et al*., 1998). But the availability of this reproductive tissue as explant at the right stage is only for a limited period. So to continue the tissue culture and transformation experiments throughout the year callus induction and shoot regeneration protocol from some alternate explant should be developed (Moniruzzaman *et al*., 2021). Gill *et al.* (1995) reported the embryogenic

potential of leaf, epicotyl, cotyledon, and root segments of *in vitro* grown seedlings of *C. reticulata*. Shoot regeneration from callus is very low in rough lemon (Raman *et al*., 1992). Although several *in vitro* propagation protocols are reported in citrus rootstocks (Hiregoudar *et al*., 2005; Singh and Rajam, 2009), few are available for rough lemon (Chaturvedi *et al*., 2001; Kumar *et al.,* 2011; Kaur 2018). A somatic embryo is a bipolar structure of single cell origin from which the whole organism develops and contains similar genetic information in all the somatic cells. Callus derived from different plant parts varies in their response to regenerate plants and its response is genotype-dependent. The culture media components and cultural conditions play an important role in the establishment of *in vitro* cultures (Rattanpal *et al*., 2011).

There are two pathways of shoot regeneration in plants *i.e.* direct and indirect regeneration. Direct regeneration produces more number of shoots per explant and induces fewer somaclonal variations among regenerants which is required for genetic transformation studies. Indirect regeneration (through the callus phase) is required for *in vitro* mutagenesis studies and induction of somaclonal variations. This study was conducted to optimize the tissue culture protocol to be used routinely by researchers for rough lemon (*C. jambhiri* Lush.). It aims to identify the best explant from *in vitro* raised seedling and plant growth hormones their concentration and combination for callus induction and callus maintenance, shoot regeneration from callus and rooting, and subsequent hardening of regenerated shoots.

MATERIALS AND METHODS

Preparation of explants

Explants, *viz.,* epicotyl, leaf, cotyledon, and root segments were collected from 2-3 week old *in vitro* grown seedlings of rough lemon. For growing seedlings, the seeds from mature fruits were excised aseptically using forceps and a scalpel under a laminar airflow cabinet.

Callus induction

Explants were cultured on Murashige and Skoog (MS) medium fortified with different concentrations and combinations of plant growth hormones including naphthalene acetic acid (NAA), indole butyric acid (IBA), benzyl amino purine (BAP), kinetin (Kin.), and carbohydrates (sucrose, maltose, and glycerol).

Culture conditions

The pH of the medium was adjusted to 5.8 and agar was added 0.8% w/v. Culture tubes/flasks after inoculation were incubated in the growth chamber by maintaining a 25 ± 2° C temperature with 16/8 hours day/night regime with 2500-3000 lux light intensity supplied through white fluorescent tubes.

Regeneration of calli

The calli induced on different media were put onto regeneration media consisting of MS salts with different combinations of benzyl amino purine (BAP) and gibberellic acid (GA_3).

Rooting and hardening of plantlets

The regenerated shoots were then transferred to a rooting medium. The rooted hardened plantlets were transplanted in the root trainer trays in the glasshouse The data were analyzed according to a completely randomized block design (CRD).

RESULTS AND DISCUSSION

Nature of explants

Response of various explants *viz*., root, cotyledon, leaf, and epicotyl segments to supplements added to medium are presented in Table 1. The explants exhibited swellings within 3-4 days of culturing and a little callusing was evident within 10 days of culturing on the callus induction media. Among the four explants studied, the maximum mean callus induction was recorded in epicotyls segments (69.19) which were significantly higher as compared to cotyledon (52.88), leaf (40.38) and root (13.70) segments, irrespective of the culture media used. M_4 medium induced the mean maximum (55.84%) callus irrespective of the explant used, whereas, M_1 media induced the minimum (34.36) per cent of callus. A maximum of 79.43 per cent of callus was induced in epicotyl segments on M_4 media while a minimum of 7.89 per cent was recorded in root segments on M_1. The explants cultured on M_1 and M_2 initiate callogenesis in 14-16 days, but after 20 days, they develop profuse rooting on their surface but it was not so in M_3 and M_4 media.

Researchers have reported successful induction of callus from various explants *viz*., from unopened

Table 1 : Effect of different media supplements on callus induction (%) in *C. Jambhiri* raised *in vitro* with different explants

Treatment	Media composition	Root Segments	Cotyledon	Leaf segments	Epicotyl segments	Mean
M_1	MS+NAA (7.5 mgl^{-1}) + Kin. (0.2 mgl^{-1})	7.89* (16.22)	43.56 (41.27)	29.20 (32.70)	56.79 (48.89)	34.36 (34.77)
M_2	MS+NAA (10.0 mgl^{-1}) + Kin. (0.2 mgl^{-1})	16.05 (23.50)	65.60 (54.08)	41.10 (39.86)	75.40 (60.25)	49.70 (44.42)
M_3	MS+NAA (7.5 mgl^{-1}) + BAP (1.0 mgl^{-1})	9.50 (17.80)	30.14 (33.26)	40.90 (39.74)	65.16 (53.80)	36.40 (36.15)
M_4	MS+NAA (10.0 mgl^{-1}) + BAP (1.0 mgl^{-1})	21.37 (27.46)	72.22 (58.19)	50.34 (45.17)	79.43 (62.92)	55.84 (48.44)
	Mean	13.70 (21.24)	52.88 (46.7)	40.38 (39.36)	69.19 (56.47)	-

*Values in parenthesis are *Arc sine* transformed values. CD at 5% : Medium (A): 1.75; Explant (B) : 1.75; A x B : 3.50

flower buds (stigmas and ovaries), *in vitro* seedlings (leaf, nodal and root segments), seeds (cotyledon and nucellar tissues) (Ali and Mirza, 2006; Savita *et al*., 2010, 2014) and epicotyl segments (Kumar *et al*., 2011; Kaur, 2018). Raman *et al*. (1992) observed callus initiation on MS with NAA (10 mgl^{-1}) and Kin (0.2 mgl^{-1}) in 7-10 days in *C. limon* and *C. jambhiri.* Callus induction frequency was higher in cultures derived from stem segments as compared to leaf and root segments. Similar behavior of different explants for callus formation was observed in *C. madurensis* (Grinblat, 1972) and *C. limon* cv. Pant lemon (Singh and Rana, 1997). Stem segments *viz*. shoot tip, epicotyl and hypocotyl were highly responsive for induction and proliferation of callus. Dhatt and Grewal (1997) reported the highest percent callusing from the stem segments in Mosambi and rough lemon, but from cotyledons in Baramasi lemon and Kinnow. Various reasons attributed to such differences in callus formation are endogenous hormone balance (Snijman *et al.,* 1977), medium, and genotype (Yeoman and Forche, 1980).

Explant excised from mature seedlings induced less callusing as compared to 2-3 weeks old seedling explants. Similar observations have also been reported in *C. aurantifolia* by Raman (1990). Furthermore, succulent nodal segments derived from fast-growing shoots induced more calli as compared to hard and slow-growing shoots. During culturing, mature tree explants derived calli, proliferated less as compared to seedling derived calli, which may be due to the maturity of tissue or secondary growth (Mukhopadhyay and Bhojwani, 1978). The root segments excised from the hypocotylar ends induced comparatively more callus than from the distal ends towards the root tip. This response may be due to optimum endogenous levels of auxins and cytokinins in this region.

Effect of carbohydrates on callus induction

The dedifferential response of epicotyl segments to varying concentrations of carbohydrates *i.e*. sucrose, maltose, and glycerol is depicted in Table 2. Out of various combinations of growth regulators studied, M_4 [MS + NAA (10 mgl^{-1}) + BAP (1.0 mgl^{-1})] emerged best (Table 1) and was supplemented with various concentrations of carbohydrates, to record their effect on the enhancement of callus induction (Table 2).

An increase in supplementation of sucrose from 3.0 to 10.0 per cent enhanced the callus initiation. Sucrose concentrations of 8.0 and 10.0 per cent were at par and were significantly better than all other treatments. In treatments from C_5 to C_8 the sucrose in the MS medium was completely replaced with the maltose at a varying concentration of 3, 6, 8, and 10 per cent. It could be perceived that the addition of 8 per cent of maltose only gave 10.0 percent callus initiation, whereas, other concentrations of maltose don't induce callogensis. Supplementation with glycerol at 2.0-3.0 percent in addition to 3 per cent sucrose doesn't induce callus in any of the explants. In the combination of sucrose and maltose, C_{10} [sucrose (3%) + maltose (5%)] induced 82.50 per cent callus in epicotyl segments. While C_9 and C_{11} induced only 65.15 and

Table 2 : Effect of various levels of carbohydrates on callus induction in *Citrus jambhiri* using epicotyl segments as explants

Treatment	Sucrose (%) (w/v)	Maltose (%) (w/v)	Glycerol (%) (v/v)	Callus induction (%)	Days for callus initiation
C_1 (Control)	3	-	-	79.43 (63.19)*	10.5
C_2	6	-	-	83.40 (66.21)	9.5
C_3	8	-	-	95.50 (78.89)	8.3
C_4	10	-	-	93.33 (76.60)	8.4
C_5	-	3	-	0.00 (0.00)	-
C_6	-	6	-	0.00 (0.00)	-
C_7	-	8	-	10.00 (18.39)	12.0
C_8	-	10	-	0.00 (0.00)	-
C_9	3	3	-	65.15 (53.83)	12.0
C_{10}	3	5	-	82.50 (65.63)	10.0
C_{11}	3	7	-	60.30 (50.94)	10.0
C_{12}	3	-	2	0.00 (0.00)	-
C_{13}	3	-	3	0.00 (0.00)	-
CD at 5%				6.88	2.9

*Values in parenthesis are *Arc sine* transformed values. MS + NAA (10mgl^{-1}) + BAP (1 mgl^{-1}) and agar @ 0.75 % (w/v) were added in all treatments

60.30 per cent callogensis, respectively. The addition of sucrose took 8.3-9.5 days to initiate callus, with a minimum of 8.3 days in C_3 followed by 8.4 days in C_4. All other treatments took 10 to 12 days for callus initiation.

The callogensis in sucrose supplements alone was the earliest and with maximum proliferation from 10 to 20 days in the first cycles. It had more bulgings on the surface and was less green than with maltose addition. With supplementation of maltose alone, less growth occurred and it was steady. A combination of maltose and sucrose (C_{10}) induced more green colour, whereas, sucrose and glycerol (2-3%) gave only swellings in the explants at cut ends. Carbohydrates play an important role in controlling the browning phenomenon in tissue culture. They control the activity of polyphenol oxidase and peroxidase enzymes which convert the phenolic substances (produced during browning) to pestilent compounds (Khosroushahi *et al*., 2011). In our study, higher concentration of sucrose results in less browning of cultures. In contrast to other plant species reduction in sucrose results in an increase in embryogenesis (Kochba *et al*., 1978). We find the optimum concentration of sucrose *i.e.* 8.0 per cent is best suitable for callus proliferation. The role of glycerol in embryogenesis is cultivar-dependent in citrus (Kayim and Koc, 2006). The addition of sucrose (0.15 M) to the basal medium promotes better growth and organization of *C. sinensis* callus followed by glucose, fructose, lactose, galactose, maltose, and sorbose (Button, 1978). Sucrose 3.0 per cent was more effective than 5.0 per cent sucrose or 2.0 per cent glycerol to induce callus in Satsuma mandarin on MT medium (Yun *et al.* 2006). Kayim and Koc (2006) reported that the best result of embryo formation from the callus of Clementine mandarin was obtained with 4.0 and 5.0 per cent glycerol concentrations.

Effect of various MT media modifications on callus induction

The effect of various MT media (Murashige and Tucker, 1969) modification on callus induction is presented in Table 3 and C_1/M_4 [MS + NAA (10.0 mgl^{-1}) + BAP (1.0 mgl^{-1})] was considered as control. The MT media was supplemented with the best combination of growth regulators and sucrose discussed in Tables 1 and 2. Moreover, in the best combination, MS was replaced with MT. It is apparent from Table 3 that MT_3 induced maximum (75.33%) callus initiation followed by MT_4 (70.00%) and MT_2 (65.00%) among different MT media modifications. MT_3 was at par with C_1 (control) in the callus

Table 3 : Effect of various MT media modifications on callus induction in *Citrus jambhiri* using epicotyl segments as explant

Treatment	Media composition	Callus Induction* (%)	Days for callus initiation	Degree of growth **	Remarks
C_1	MS+NAA (10 mgl^{-1}) + BAP (1 mgl^{-1}) + Sucrose (3%)	79.43 (63.20)*	10.50	+++	light green, less compact and globular surface
MT_1	MT+NAA (10 mgl^{-1}) + Kin. (0.1 mgl^{-1}) + Sucrose (8%)	40.00 (39.18)	20.20	++	small, creamish & rooting appeared
MT_2	MT+NAA (10 mgl^{-1}) + Kin. (0.2 mgl^{-1}) + Sucrose (8%)	65.00 (53.78)	14.20	++	profuse rooting after 20 days
MT_3	MT+NAA (10 mgl^{-1}) + BAP (1 mgl^{-1}) + Sucrose (8%)	75.33 (60.28)	12.00	+++	small and creamish
MT_4	MT+NAA (10 mgl^{-1}) + BAP (1 mgl^{-1}) + Sucrose (3%) + Maltose (5%)	70.00 (56.86)	11.50	+++	light greenish and compact
MT_5	MT+NAA (10 mgl^{-1}) + BAP (1 mgl^{-1}) + Sucrose (3%) + Glycerol (1%)	0.00 (0.00)	-	-	-
MT_6	MT+NAA (10 mgl^{-1}) + BAP (1 mgl^{-1}) + Sucrose (3%) + Glycerol (2%)	0.00 (0.00)	-	-	-
CD at 5%		6.52	4.17	-	-

*Values in parenthesis are *Arc sine* transformed values

**Degree of growth; + = poor; ++ = fair; +++ = good; ++++ = excellent

induction. MT supplementation with glycerol (1-2%) did not induce callus but just the swellings on the cut end of the explant. Considering the days for callus initiation, MT_2 (14.20), MT_3 (12.00), and MT_4 (11.50) were at par with control. The callus induction was delayed (20.20 days) in the MT_1 media, MT_4 took the least number of days (11.50) followed by MT_3 (12.00) and MT_2 (14.20) to induce callus in various combinations of MT with growth regulators and carbohydrates. It was noted that profuse rooting appears in MT_2 and MT_1 after 25 days of culturing.

MT medium may enhance callus induction due to more availability of vitamins in its composition. Hagagy *et al*. (2001) reported the highest callus induction in *C. jambhiri* when nucellar embryos were cultured on MT medium fortified with 2, 4-D (4.0 mgl^{-1}). Callus line maintained on MT medium supplemented with sucrose (5%) when transferred to MT + Maltose (150 µM) gave rise to globular embryos in *C. sinensis* and *C. limonia* (Tomaz *et al*. 2001). Handaji *et al*. (2005) reported maximum callus induction in *C. reticulata* ovules cultured on MT+Kin (1.0 mgl^{-1}).

Regeneration from callus cultures

Shoot regeneration on different media

The effect of various MS media modifications on shoot regeneration in 60 days old callus culture is presented in Table 4. Calli was induced on MS+NAA(10 mgl^{-1}) + BAP (1 mgl^{-1}) + sucrose (8.0%) and afterward transferred to regeneration media. Significantly higher (43.10%) regeneration was observed in calli cultured on Modified MS supplemented with BAP (5.0 mgl^{-1}) +GA_3 (3.0 mgl^{-1}) as compared to all other treatments. Modified MS fortified with BAP (5.0 mgl^{-1}) showed 26.80 percent regeneration, whereas the addition of GA_3 (5.0 mgl^{-1}) to the same induced 30.50 per cent regeneration.

Modified MS supplemented with sucrose (6.0%) + Gelrite (0.35%) induces 5.12 per cent shoot regeneration in 60-day old calli. Half strength MS

Table 4 : Effect of various MS media modifications on shoot regeneration in 60 days -old callus culture of *Citrus jambhiri*

Treatment	Media composition	Shoot regeneration* (%)	Shoots/callus (Average)	Days to regeneration
MR_1	½ MS basal**	0.00 (0.00)	-	-
MR_2	½ MS basal + BAP (5.0 mgl^{-1})	0.00 (0.00)	-	-
MR_3	Modified MS*** + BAP (5.0 mgl^{-1})	26.80 (31.10)	4.80	38.40
MR_4	Modified MS + BAP (5.0 mgl^{-1}) + GA_3 (3.0 mgl^{-1})	43.10 (40.99)	5.30	35.50
MR_5	Modified MS + BAP (5.0 mgl^{-1}) + GA_3 (5.0 mgl^{-1})	30.50 (33.49)	3.90	40.60
MR_6	Modified MS + BAP (5.0 mgl^{-1}) + GA_3 (7.0 mgl^{-1})	0.00 (0.00)	-	-
MR_7	Modified MS + Sucrose (6%) + Gelrite (0.35%)	5.12 (12.90)	1.00	34.00
MR_8	½ MS + BAP (5 mgl^{-1}) + Agar (0.9%)	0.00 (0.00)	-	-
MR_9	½ MS + BAP (5 mgl^{-1}) + Agar (1.0%)	0.00 (0.00)	-	-
CD at 5%		3.53	0.71	5.58

*Values in parenthesis are Arc sine transformed values, ** All five stocks reduced to half, *** Macro and micronutrients (first stock) reduce to half.

basal with supplementation of different concentrations of BAP did not give any regeneration response. Shoot induced on MR_3 was of normal morphology, whereas, the addition of various concentrations of GA_3 to MR_3 medium gave rise to the thinner shoots with elongated leaves. It is evident from Table 4 that a higher (5.30) number of shoots per callus were obtained on MR_4 medium which was at par with MR_3 (4.80) and both were significantly better than all other treatments. MR_5 gave rise to 3.9 shoots per callus, whereas, it was only 1.0 in MR_7.

Calli cultured on MR_7 was the earliest (34.00 days) to re-differentiate but did not differ significantly from MR_3 (38.40 days) and MR_4 (35.50 days). The Callus on MR_5 medium was late to regenerate, as it took 40.60 days to induce shoots in 60 days old calli.

The constitution of the medium used plays a vital role in the process of redifferentiation (Vasil and Hildebrandt, 1965; Reinert and Bocks, 1968). The presence of cytokinins was found essential for shoot regeneration, which pertains to the reason that more synthesis of nucleic acids and proteins is required in the process of organogenesis. The role of cytokinins in shoot regeneration in *Citrus* has been well documented (Bhansali and Arya, 1978, Gill, 1992 and Raman *et al*., 1992). Shoots were regenerated from the callus culture of citrus cultivars Mosambi, Baramasi lemon, and Kinnow upon transfer to half-strength MS medium supplemented with BAP (5.0 mgl^{-1}) (Dhatt and Grewal, 1997). Rashad *et al*. (2005) reported maximum shoot induction in *C. sinensis* cv. Mosambi, when calli were cultured on MS media supplemented with BAP (2.0 mgl^{-1}). Regeneration was best on MS + GA_3 (2.0 mgl^{-1}) + Adenine sulphateS (2.5 mgl^{-1}) (Khan and Nafees, 2005) and MS + NAA (10.74 µM) in Kinnow (Singh *et al*., 2006). Maximum indirect shoot regeneration response (70%) was observed on MS medium supplemented with BA (3.0 mgl^{-1}) in rough lemon (Ali and Mirza, 2006).

The capacity for *in vitro* regeneration varies considerably among different explants taken from the same plant. There are natural differences in the plant's ability to synthesize endogenous cytokinins and respond to external levels of cytokinins which play a significant role in the capacity for *in vitro* regeneration. It is irrelevant to increase the cytokinin

levels if the tissue is not responsive to external phytohormones. The calli derived from BAP-containing media turned brown and necrotic in appearance. This change could be due to enzymatic activity rather than hormonal toxicity as the callus was still regenerating. Similar results were also reported by Hill and Schaller (2013).

Effect of callus age on shoot regeneration

The effect of callus age on shoot regeneration is presented in Fig. 1. It is evident from the data that regeneration decreased with the increase in the callus age from 60 to 150 days. Redifferentiation was significantly higher (43.43) in 60 days old callus, whereas it was only 10.46 per cent in 150 days old callus. In 60 days old callus, the days to regenerate were significantly lower (35.42) than in 90 (52.31), 120 (87.58), and 150 (119.53) day-old calli. The effect of callus age on shoot regeneration and days to regenerate was significant. GA_3 initiated and enhanced regeneration in old callus as it may become dormant in the long-term maintenance by culturing.

It can be concluded that, besides, the media composition, the plant regeneration frequency also varied with the age of sub-cultured calli. The decrease in morphogenetic potential with the increase in callus age has earlier been reported, which may be due to karyotypic changes and altered hormonal balance within cells or tissue or sensitivity of the cells to growth substances (Yeoman and Forche, 1980). Raman (1990) reported no-shoot regeneration in sub-cultured calli of acid lime after 120 days. In contrast to this, Gill (1992) reported that the young calli (50-75 days old) exhibited significantly lower plant regeneration as compared to the older calli (125-150 days old). Hao et al. (2004) maintained the callus of Red Marsh grapefruit *in vitro* by slow growth culture method for one year and it survived with a significant weight increment over that period with fair regeneration.

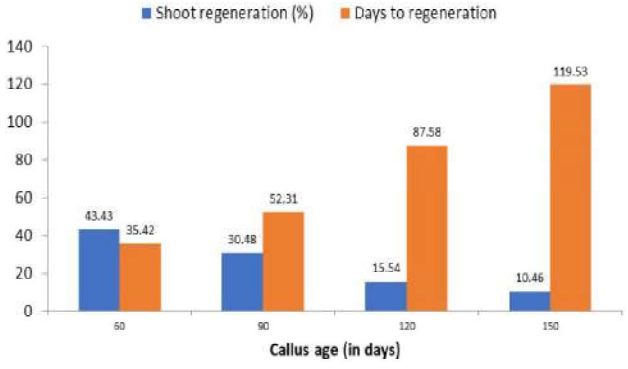

Fig.1 : Effect of callus age on shoot regeneration in *Citrus jambhiri* cultured on Modified MS + BAP (5 mgl^{-1}) + GA_3 (3 mgl^{-1})

Multiplication of shoot cultures

Data pertaining to the effect of various modifications of MS salts on shoot proliferation is presented in Fig. 2. Significantly higher percentage of shoot

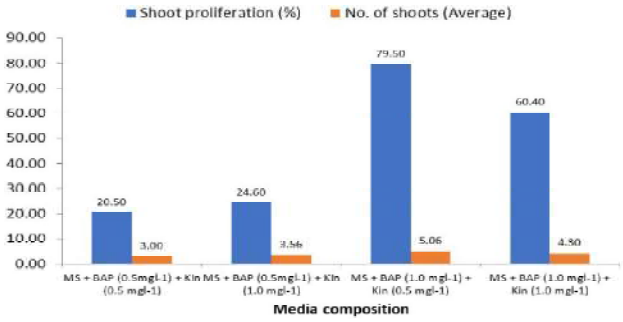

Fig. 2 : Effect of different media on shoot proliferation (Callus derived) in *Citrus jambhiri*

proliferation (79.50) was observed in cultures on MS + BAP (1.0 mgl^{-1}) + Kinetin (0.5 mgl^{-1}). MS supplemented with BAP (0.5 mgl^{-1}) and Kinetin (0.5 mgl^{-1}) resulted in the minimum (20.50%) shoot proliferation and mean number of shoots (3.00). Shoot proliferation was 60.4 per cent when it was cultured on MS + BAP (1.0 mgl^{-1}) + Kinetin (1.0 mgl^{-1}). The Maximum number of shoots (5.06) were noted in MS fortified with BAP (1.0 mgl^{-1}) and Kinetin (0.5 mgl^{-1}). The effect of medium composition on the number of shoots was not significant. In acid lime, six shoots per explant were achieved on MS+ BAP (5.0 mgl^{-1}) by Ramsunder *et al.* (1998). Similarly, El-Saway *et al.* (2006) reported maximum shoot proliferation and the maximum number of shoots and shoot length on MS+BAP (0.5 mgl^{-1}) + NAA (0.5 mgl^{-1}) in three *C. sinensis* cultivars and *C. jambhiri.*

Induction of rooting *in vitro*

In vitro regenerated shoots of more than 1.5 cm height were culled out from shoot clump and culture onto the rooting media. The response of plantlets regenerated from calli to rooting on different media is presented in Fig. 3. Among the four media studied for rooting response, the per cent rooting was maximum (77.33) on MS + NAA (1.0 mgl^{-1}) + IBA (1.0 mgl^{-1}), whereas, it was minimum (26.30) on MS medium supplemented with NAA (0.5 mgl^{-1}) and IBA (0.5 mgl^{-1}).

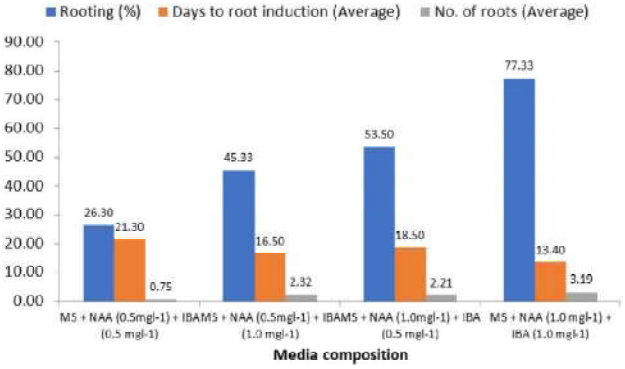

Fig. 3 : Effect of different media on rooting of callus derived shoots of Citrus jambhiri

Fig. 4 : In vitro raised seedlings of rough lemon

A) On basal MS medium, B) Surface topography of embryogenic callus of rough lemon on C_3 [MS + NAA (10 mgl^{-1}) + BAP (1 mgl^{-1}) + Sucrose 8%(w/v)], C) Embryoids formation and germination on callus surface on modified MS + BAP (5.0 mgl^{-1}) + GA_3 (3.0 mgl^{-1}), D) Redifferentiation in 60 days old rough lemon callus on modified MS + BAP (5.0 mgl^{-1}) + GA_3 (3.0 mgl^{-1}), E) Plants of Citrus jambhiri in soil after hardening

The root initiation from the *in vitro* derived shoots of rough lemon started within 13-21 days on various modifications of MS medium. Rhizogensis was significantly earlier (13.4 days) on MS + NAA (1.0 mgl^{-1}) + IBA (1.0 mgl^{-1}), whereas, it was delayed on all other media, with maximum duration of 21.3 days on MS+NAA (0.5 mgl^{-1}) + IBA (0.5 mgl^{-1}). From the perusal of data, it is clear that the media studied had a significant influence on days to root induction.

Maximum (3.19) number of roots per shoot was observed on MS medium fortified with NAA (1.0 mgl^{-1}) and IBA (1.0 mgl^{-1}), whereas, minimum (0.75) was noted in MS + NAA (0.5 mgl^{-1}) + IBA (0.5 mgl^{-1}). However, the number of roots per shoot on the remaining two media was at par with each other. Auxins have a major role in rhizogenesis, which reflects from the multiplication of meristematic cells, their elongation, and differentiation into root primordia. The root length and thickness and the number of roots produced per shoot depend upon the concentration and combination of auxins used in the rooting medium. The rooting response was maximum in *C. sinensis* cv. Mosambi when shoots were cultured on MS + NAA (1.5 mgl^{-1}) (Rashad *et al.* 2005) and on MS + NAA (0.75 mgl^{-1}) + IBA (2.0 mgl^{-1}) (Das *et al.* 2000). Gill and Gosal (2002) found that the average number of roots per shoot was maximum on MS + NAA (2.0 mgl^{-1}) in *Pectinifera* rootstock. Similarly, Parkash (2003) observed the earliest and maximum (62.00%) rooting in *C. jambhiri* on MS + NAA (1.0 mgl^{-1}) + IBA (1.0 mgl^{-1}).

Hardening

Rooted plantlets were taken out of culture jars, medium adhering the roots was washed under running tap water, and plantlets were placed in a plastic stray lined with the wet cotton layer of about 0.5 cm Plantlets were placed in the tray in such a way that roots touched the wet cotton layer. The tray was covered with the transparent poly sheet to retain the moisture and kept at room temperature in the hardening room. The hardened plants had 83.6% survival in polyhouse when transplanted in root trainer trays with the potting mixture consisting of cocopeat + vermiculite + perlite (2:1:1).

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Original Research Paper

Standardization of sterilization protocol for explants and its suitability for direct organogenesis in tuberose cv. Arka Vaibhav

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ABSTRACT

A study was carried out to standardize the sterilization protocol for different explants (terminal stem scale, immature flower bud and tepal segment) and to select the suitable explant for the direct organogenesis of tuberose cv. Arka Vaibhav. The highest survival per cent (100) and uncontaminated cultures (0.00) of terminal stem scale explant was observed in pre-treatment with overnight soaking of terminal stem scale in the solution comprising carbendazim (0.1%), chlorothalonil (0.05%) and myristyl trimethyl ammonium bromide (cetrimide) (0.05%) and subsequently surface sterilization with 70% ethanol (1 min), 4% sodium hypochlorite (10 min) followed by 0.1% $HgCl_2$ (15 min). The explant immature flower bud recorded the highest survival per cent (100) and maximum aseptic cultures in the treatment T_1 comprised of 1.0 drop Tween-20 + 70% ethanol (30 sec) and 1% sodium hypochlorite (3 min). Pre-treatment of tepal segment explant in 0.1% carbendazim (30 min) solution followed by surface sterilization with combination of 1.0 drop Tween-20 + 70% ethanol (30 sec) followed by 1% sodium hypochlorite (3 min) registered 91.66% of survival with the minimum contamination (10%) in the treatment. Among the three explants used, the terminal stem scale was found suitable for direct organogenesis with early greenness (5.72 days) and highly responsive to shoot induction (100%) in MS medium supplemented with 4 mg/L BAP + 0.1mg/L IAA. Other two explants *viz.,* immature flower bud and tepal segment failed to respond for direct organogenesis by shoot induction instead produced profuse callus.

Keywords : Aseptic culture, direct organogenesis, explants, surface sterilization

INTRODUCTION

Tuberose (*Agave amica* (Medik.) Thiede & Govaerts.) is an important bulbous flower crop valued for its pleasant fragrance and widely grown for loose flower as well as cut flower purpose. The commercial cultivation of tuberose in India is confined to West Bengal, Karnataka, Maharastra, Tamil Nadu, Haryana, Punjab, Gujarat, Rajasthan, Andhra Pradesh including Assam. It is cultivated in an area of about 21.77 ('000 ha) with loose flower production of 117.14 ('000 metric tons) and cut flower production of 102.25 lakh numbers of cut stems (Anon., 2023). Tuberose concrete and absolute are highly priced in international market and their extraction has been established as export oriented agro-industry in India. The consumer preference for natural products increased the market demand for the tuberose floral extract which intern upsurge the planting material requirement. Tuberose is propagated through bulbs and rapid multiplication of quality planting material through conventional propagation is highly time consuming.

Micro-propagation is a tool to augment the supply of planting material in a short period of time, need less space and time for large populations, and provides the opportunity to keep the plant material disease-free. Contamination is a major problem in the selection and preparation of explant and may lead to significant loss of cultures (Pandey *et al*., 2009; Mir *et al*., 2012). Contamination in tissue culture can originate from two sources, either through carryover of microorganisms from the surface of the explant or from within the tissue itself (endophytic micro-organisms). Although, in meristem culture, depending on meristem size most of the microorganisms can be eliminated, whereas, in leaf, petiole and stem explants, the infection would carry over to the cultures (Leifert and Cassells, 2001). Bacterial contamination was the major limitation in the sterilization process (Ray and Ali, 2016). In most of the studies, sodium hypochlorite or mercuric chloride

($HgCl_2$ 0.1%) has been used for sterilization. In some cases, treatment with fungicides such as carbendazim and chlorothalonil has been used and for inhibition of growth of bacteria in plant tissue culture antibiotics were used (Taha *et al*., 2018). Standardization of rapid multiplication techniques through tissue culture in tuberose is essential for the large scale production and distribution of quality planting material to the farmers. Keeping the above in view, present study was carried out to standardize the surface sterilization for different explants and to find the right explants for the direct organogenesis of tuberose cv. Arka Vaibhav.

MATERIAL AND METHODS

The present investigation was carried out in the Division of Flower and Medicinal Crops, ICAR-Indian Institute of Horticultural Research, Bengaluru during the year 2019-2020 using tuberose commercial cultivar Arka Vaibhav (double type). The variety Arka Vaibhav produces multi-whorled flower on the spikes having cut flower value. Three different explants *viz*., terminal stem scale (5 mm), immature flower bud (10-15 mm) and tepal segments (10-15 mm) were used to standardize the surface sterilization and to identify the best responsive explant for direct organogenesis (Fig. 1 & 2). As the terminal stem scales were obtained from the soil, in order to avoid heavy contamination due to soil borne diseases, seven different treatments were designed using completely randomised block design (CRD) and replicated thrice for the production of aseptic culture of terminal stem scales with pre-treatment followed by surface sterilization. The immature flower buds were separated from the spike and tepal segments were obtained from freshly opened flowers. Six different treatments were followed for the surface sterilization of immature flower bud and tepal segment explants using CRD with three replications. The parameters such as contamination per cent and survival per cent were recorded. To study the suitability of different explants for the direct organogenesis of tuberose cv. Arka Vaibhav, an

a. Terminal stem sacle (0.5-1.5 cm) b. Immature flower bud (10-15 mm) c. Tepal segment (10-15 mm)

Fig. 1 : Different explants with different sizes used to initiate the culture before inoculation into culture media

A B C

Fig. 2 : Terminal stem scale explants of tuberose cv. Arka Vaibhav during and after surface sterilization in LAF

experiment was conducted for direct organogenesis in CRD with six treatments and three replications using three different explants *viz*., terminal stem scale, immature flower bud and tepal segment. The explants were cultured on MS medium containing different combinations of growth regulators (auxins @ 0.1 mg and cytokinin @ 2.0, 4.0 mg). The observations on days to complete greenness and shoot induction per cent response were recorded. The collected data were subjected to statistical analysis using OPSTAT software.

RESULTS AND DISCUSSION

Establishment of aseptic culture for terminal stem scale explants

The data presented in Table 1 revealed that pre-treatment of terminal stem scale explants with the treatment (T_6) comprising of overnight soaking with 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristyl trimethyl ammonium bromide (cetrimide) and surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min) resulted in 0.00% contamination, while, the control treatment with distilled water wash for 30 minutes recorded the maximum contamination of 100.00%.

The per cent survival of explant significantly varied among the different treatments (Table 1). The maximum survival (100%) of terminal stem scales was recorded in T_6 treatment with the overnight soaking of terminal stem scale explants in 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristy trimethyl ammonium bromide (cetrimide) followed by surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min), followed by T_5 treatment with the overnight soaking of terminal stem scale explants in 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristyl trimethyl ammonium bromide (cetrimide) and surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) which recorded 96.66% of explants survival. The lowest per cent survival of explant was observed in control (40%).

Table 1 : Effect of surface sterilization on terminal stem scale explant of tuberose cv. Arka Vaibhav

Treatment	Contamination of explant (%)	Survival of explant (%)
T_0: Distilled water wash for 30 min (control)	100.00 (90.00)	40.00 (39.13)
T_1 : Overnight soaking with 0.05% carbendazim	86.66 (68.83)	50.00 (44.81)
T_2: Overnight soaking with 0.05% carbendazim + 0.05% chlorothalonil	83.33 (66.12)	63.33 (52.84)
T_3: Overnight soaking with 0.1% carbendazim + 0.05% chlorothalonil + 0.05% myristyl trimethyl ammonium bromide (cetrimide)	46.66 (43.06)	76.66 (61.11)
T_4: T_3 + 70% ethanol (1 min)	33.33 (35.20)	93.33 (77.69)
T_5: T_3+ 70% ethanol (1 min) + 4% sodium hypochlorite (10 min)	13.33 (21.14)	96.66 (83.85)
T_6: T_3 + 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min)	0.00 (0.00)	100.00 (90.00)
Mean	51.90 (46.34)	74.28 (64.23)
SEm±	2.82	5.49
CD (p=0.05)	8.54	16.66
CV (%)	9.40	12.80

Values in the parenthesis are angular transformed values

The success of micro-propagation mainly depends upon the initiation of aseptic culture and hence, the explants were initially pre-treated with fungicide and bactericide solution to reduce considerable amount of microbial load. The terminal stem scale explants obtained from underground parts of field grown plants are readily exposed to soil borne pathogens, therefore, decontamination of explants become a difficult task. The pre-treatment of terminal stem scale explants with combination of fungicides and bactericide solution is extremely necessary before surface sterilization with sterilizing agents. Krishnamurthy *et al*. (2001) reported that combined use of carbendazim (1000 ppm) and citrimide (500 ppm) helps in maximum control of *in vitro* culture contaminations in tuberose. Kanchana *et al*. (2019) and Copetta *et al*. (2020) also reported similar sterilization procedures in tuberose micropropagation. Decontamination of explants obtained from underground parts has been reported (Gajbhiye *et al.*, 2011). Similarly, Aslam *et al*. (2013) also reported the suitability of $HgCl_2$ as an ideal surface sterilant for disinfection of underground plant parts in lilum. The fungicidal and bactericidal compounds have to be used at non-phytotoxic levels to get the desired results.

Establishment of aseptic culture for immature flower bud and tepal segment explants

The per cent contamination of immature flower bud and tepal segment was significantly influenced by different treatments (Table 2). The results revealed that the per cent contamination of explants was 0.00 % in the treatment T_1 comprising of 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 1% sodium hypochlorite

Table 2 : Effect of surface sterilization on immature flower bud and tepal segment explant of tuberose cv. Arka Vaibhav

Treatment	Immature flower bud		Tepal segment	
	Contamination of explant (%)	Survival of explant (%)	Contamination of explant (%)	Survival of explant (%)
T_0: Distilled water wash for 15 min (control)	76.66 (61.11)	23.33 (28.84)	86.66 (68.83)	16.67 (23.85)
T_1: 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 1% sodium hypochlorite (3 min)	0.00 (0.00)	100.00 (90.00)	73.33 (58.98)	28.33 (32.13)
T_2: 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (3 min)	13.33 (21.14)	86.66 (68.64)	56.66 (48.83)	35.66 (36.58)
T_3: 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (5 min)	26.66 (30.98)	73.33 (58.91)	36.66 (44.98)	43.33 (41.14)
T_4: 0.1% carbendazim (30 min) + 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min)	10.00 (6.14)	88.33 (70.09)	10.00 (6.14)	91.66 (73.37)
T_5: 0.2% carbendazim (30 min) + 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min)	21.66 (27.70)	75.00 (59.98)	23.33 (28.07)	50.66 (45.37)
Mean	24.71 (24.51)	74.44 (62.74)	47.77 (42.64)	44.38 (42.07)
SEm±	2.80	1.36	6.53	3.04
CD (P=0.05)	8.65	4.19	20.11	9.36
CV (%)	20.50	3.16	23.12	11.80

Values in the parenthesis are angular transformed values

(3 min) followed by 10.00 % in treatment T_4 comprising of 0.1% carbendazim (30 min) + 1.0 drop Tween-20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min), while, the control treatment recorded the maximum per cent contamination of explants (76.66%). Significant difference was recorded among the treatments for the per cent survival of explants. The per cent survival of explants was recorded maximum (100%) in T_1 treatment consisting of 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 1% sodium hypochlorite (3 min), followed by T_4 treatment containing 1.0 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.1% carbendazim (30 min) showing 88.33% of explant survival. However, minimum survival of explants was recorded in control (23.33%).

Pre-treatment is not required for the immature flower bud as the explants are not readily in contact with any soil borne pathogens. In the present study, sequential application of 1 drop Tween-20 + 70 % ethanol for 30 seconds and 1 % sodium hypochlorite (NaOCl) for 3 minutes were found to be optimum for obtaining contamination free cultures. The bactericidal action of NaOCl is due to HOCl (hypochlorous acid) and OCl^- (hypochlorite ion) ions, which are highly effective with less phytotoxic effect. The level of surface sterilants, their exposure time and combination have great influence on the culture establishment. Further, the increase in concentration and prolonged exposure to sterilant led to the death of explants and excessive contamination of cultures. The probable reason may be due to metal contamination proving phytotoxic for the survival of the explants. The results are in accordance with the findings of Rather (2010) in peony. The phytotoxicity of sterilant mainly depend upon the nature, age and type of plant parts. The efficacy of these sterilizing agents on bulbous explants has been earlier reported by Aslam *et al*. (2013) who mentioned that the combination of chlorox (sodium hypochlorite) and mercuric chloride ($HgCl_2$ 0.1%) helps to reduce bacterial contamination in *in vitro* culture. Mercuric chloride has been one of the most used sterilant, while, sodium hypochlorite is also efficient for tuberose (Krishnamurthy *et al*., 2001; Mishra *et al*., 2005). Chen *et al.* (2005) also reported minimum contamination of cultures using NaOCl and ethanol for unopened flower buds of Narcissus.

Significant differences were observed among the treatments for the per cent contamination of tepal segment explants (Table 2). Sterilization of tepal segment explants with T_4 treatment consisting of 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.1% carbendazim (30 min) recorded less contamination (10%), followed by T_5 treatment consisting of 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.2% carbendazim (30 min) which recorded 23.33% contamination and maximum contamination (86.66%) was observed in control treatment. The per cent survival of tepal explants significantly differed among the six treatments studied and the maximum (91.66%) tepal explants survived in T_4 treatment comprising of 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.1% carbendazim (30 min), followed by T_5 treatment containing 1 drop Tween- 20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) + 0.2% carbendazim (30 min) which recorded 50.66% explant survival, while, lowest survival of explants (16.67%) was observed in control treatment. This might be due to the optimum concentration and duration of exposure to sterilizing agents which resulted in minimum contamination and maximum survival. Combined use of chemicals greatly contributed to the contamination free culture than the individual sterilizing agents. The results are in agreement with the findings of Kadam *et al*. (2010) who reported that pre-treatment of explants with 0.1% each of carbendazim, mancozeb and 200 mg/L of 8-HQC for 2 h recorded the maximum culture survival in petal segment (87.5%) with minimum fungal and bacterial contamination in tuberose. Mishra *et al*. (2005) in tuberose, Bora and Paswan (2003) in heliconia and Dilta *et al*. (2000) in lily reported that ethanol and sodium hypochlorite found effective for surface sterilization, however, Sangavai and Chellapandi (2008) found that combination of ethanol (70%) and sodium hypochlorite (10 min) resulted in 88.35% aseptic culture in tuberose.

Suitability of explants for the direct organogenesis

Days taken to complete greenness of explants such as terminal stem scale, immature flower bud and tepal segment were significantly differed (Table 3). The treatment T_5 consisting of MS + 4 mg/L BAP + 0.1 mg/L IAA recorded minimum days to complete greenness in all the three explants *viz*., terminal stem scale (3.60 days), immature flower bud (4.67 days) and tepal segment (5.83 days). This might be due to

Table 3 : Effect of different media combinations on days to complete greenness in different explant of tuberose cv. Arka Vaibhav

Treatment	Days to complete greenness		
	Terminal stem scale	Immature flower bud	Tepal segment
T_0 - Half MS media	7.40	8.00	10.00
T_1 - Basal MS media	6.20	7.67	8.33
T_2- Basal MS + 2.0 mg/L BAP	5.40	6.33	7.67
T_3 - Basal MS + 4.0 mg/L BAP	4.27	6.00	6.67
T_4 - Basal MS + 2.0 mg/L BAP + 0.1 mg/L IAA	4.40	5.33	6.00
T_5 - Basal MS + 4.0 mg/L BAP + 0.1 mg/L IAA	3.60	4.67	5.83
Mean	5.21	6.33	7.42
SEm±	0.13	0.36	0.25
CD (P=0.05)	0.39	1.11	0.76
CV (%)	4.24	9.84	5.73

higher concentration of cytokinin's (BAP @ 4.0 mg/L) inducing early bud emergence. The half MS medium which is devoid of growth regulators exhibited maximum number of days to complete greenness in all the three explants such as terminal stem scale (7.40 days), immature flower bud (8.00 days) and 10.00 days in tepal segment. Earlier findings reported that the days taken for greenness in tuberose decreased with increasing concentration of BAP. However, in the present study, a different trend was observed wherein minimum days taken for complete greenness of explants with 4 mg/L BAP, which indicated that there is no antagonistic effect of higher levels of BAP for all the types of explants used. These results are in conformity with the findings of Jyothi *et al*. (2008) in tuberose.

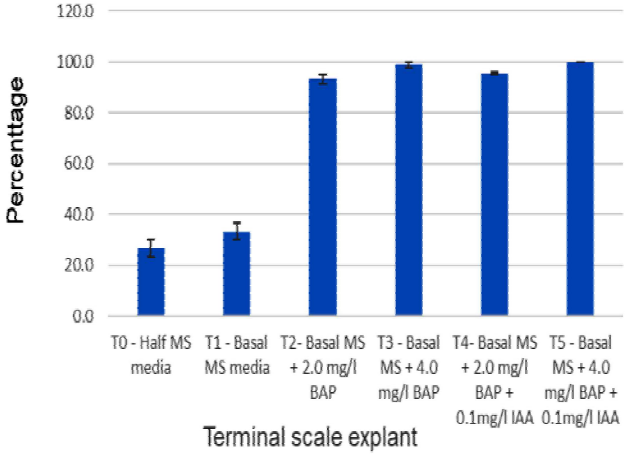

Fig. 3 : Influence of different media combinations on per cent response to shoot induction in tuberose cv. Arka Vaibhav

Among the three different explants used for the study, terminal stem scale explant alone responded very well for the shoot induction and other two explants like immature flower bud and tepal segment has not responded for shoot induction even after repeated inoculation on different combination of media. Media treatments differed significantly with respect to per cent response to shoot induction and culture establishment (Fig. 3). The results revealed that the maximum per cent response of 100% shoot induction was observed in terminal stem scale explants cultured in the T_5 media comprised of full MS + 4 mg/L BAP + 0.1 mg/L IAA, followed by T_3 media consist of MS + 4 mg/L BAP which recorded 98.88% of response to shoot induction. The least response of 78.87% was observed in control with half MS media.

The appropriate ratio of auxins and cytokinin's in the plant system has major role in culture establishment and the finest balance determines the direct and indirect organogenesis. Among the different explants studied for *in vitro* regeneration response, only terminal stem scale explants responded very well for direct shoot formation (Fig. 4). As it is the sole propagating part of tuberose which contain more totipotent cells and meristematic cells resulted in high response for direct organogenesis. Surendranath *et al*. (2016) in tuberose also reported that the axillary buds responded for shoot induction with the response rate of 95.00% and 92.50% in Arka Prajwal and Arka Suvasini, respectively.

Fig. 4 : Successful established cultures of terminal stem scale explant

CONCLUSION

The terminal stem scale explant pre-treated with 0.1% carbendazim + 0.05% chlorothalonil + 0.05% cetrimide and surface sterilization with 70% ethanol (1 min) + 4% sodium hypochlorite (10 min) + 0.1% $HgCl_2$ (15 min) resulted in contamination free aseptic culture. Pre-treatment of immature flower bud and tepal segment with 1 drop Tween-20 + 70% ethanol (30 sec) + 1% sodium hypochlorite (3 min) and 0.1% carbendazim (30 min) + 1 drop Tween-20 + 70% ethanol (30 sec) + 2% sodium hypochlorite (10 min) recorded contamination free culture. The study concluded that the terminal stem scale explant is the best suited and most viable explant compared to immature flower bud and tepal segment for direct organogenesis in tuberose.

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Original Research Paper

Efficient *in vitro* plantlets regeneration from leaf explant of *Haworthia retusa*, an important ornamental succulent

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ABSTRACT

This study was conducted to establish an efficient *in vitro* plantlet regeneration protocol using the *ex vitro* leaves as explants for *Haworthia retusa*. Leaf tissues were cultured on liquid full-strength Murashige and Skoog (MS) medium supplemented with 2.0 mg/L indole 3-butyric acids (IBA) for callus induction, followed by sub-cultured to solid medium for callus proliferation. Callus was then transferred to a fresh medium supplemented with 6-benzyl amino adenine (BA) for shoot development. The result showed that the maximum rate of shoot regeneration (100%), number of shoots per explant (43), and shoot height (9.4 mm) were recorded on the solid MS medium supplemented with 1.0 mg/L BA and 30 g/L sucrose. IBA improved rooting, whereas, NAA (naphthaleneacetic acid) causes calli to form at the base of the shoots. The half-strength MS medium supplemented with 0.5 mg/L IBA provided the best rooting response for the shoot. This medium formulation resulted in the highest rooting rate (100%) and the highest mean root number (5 roots/explant). The result of the present study would be helpful for the mass propagation of commercially important *H. retusa*.

Keywords : 6-benzyl amino adenine, *Haworthia retusa,* indole 3-butyric acid, leaf explants, micropropagation, plantlet regeneration

INTRODUCTION

The genus *Haworthia* includes succulent plants belonging to the Asphodelaceae family and is cultivated commercially as ornamentals. Some rare species are quite valuable but grow slowly and are difficult to propagate (Bayer, 1982). *Haworthia* species are small and have beautiful shapes and colors. The leaves are often arranged in the shape of flowers and used for decorating offices, apartments, restaurants, and hotels. They are also used as a meaningful gift because of their appealing shape, color, and ability to adapt to low moisture conditions. Traditionally, *Haworthia* species can propagate from seed, leaf-cutting, and offsets division with low multiplication efficiency. Currently, micropropagation is a potential technique to enhance multiplication efficiency in many succulents such as *Kalanchoe pinnata* (Jaiswal and Sawhney, 2006), *Aloe polyphylla* (Bairu *et al.*, 2007), *Pelecyphora aselliformis* (Badalamenti *et al.*, 2016), *Cotyledon orbiculata* (Kumari *et al.*, 2016), and *Aloe adigratana* Reynolds (Niguse *et al.*, 2020). Limited information is available on *Haworthia* micropropagation (Lizumi and Amaki, 2011; Liu *et al.*, 2017; Kim *et al.*, 2017; Kim *et al.*, 2019; Chen *et al.*, 2019). Different explants such as inflorescences, flower buds, and leaves of *Haworthia* species are used for regeneration, however, the leaf would be a more desirable explant for micropropagation due to its availability. Adventitious shoots can be regenerated directly from leaf tissue (Kim *et al.*, 2017) or indirectly through leaf-derived callus (Liu *et al.*, 2017; Chen *et al.*, 2019). In addition, somatic embryogenesis is a suitable method for *in vitro* plantlet regeneration in *Haworthia* species (Kim *et al.*, 2019). *Haworthia retusa* is a popular succulent plant with a short rosette of thick, triangular leaves. Propagation of *H. retusa* is possible by collecting offsets or leaf or stem cuttings. However, conventional propagation methods are difficult for large-scale plant production in slow-growing succulents. Therefore, an efficient alternative method for the propagation of *H. retusa* is required. In the present study, we establish an efficient micropropagation protocol using the leaf explants through organogenesis and embryogenesis in *H. retusa* to meet the growing market demand for this species.

MATERIALS AND METHODS

Plant material

H. retusa plants were grown in a greenhouse at the Institute of Tropical Biology, VAST, Vietnam. The leaves of *H. retusa* were excised from healthy plants and were surface sterilized first with 70% ethanol for 30 seconds and then with 0.1% $HgCl_2$ for 10 min, followed by washing with sterilized distilled water four times. The surface-sterilized leaves were cut into 2.0 x 3.0 mm pieces for subsequent experiments after removing the injured leaf.

Callus induction and proliferation

The surface-sterilized leaf explants (0.1 g fresh weight) were inoculated in flasks containing 50 mL of liquid MS (Murashige and Skoog, 1962) medium supplemented with 30 g/L sucrose. To induce callus formation, IBA was added to the medium at different concentrations of 1.0, 2.0, and 3.0 mg/L. These flasks were incubated on the shaker at 100 rpm. The IBA concentration at which the explant obtained the highest dry weight will be used to induce callus formation and proliferation in subsequent experiments. For the callus proliferation, the response explants in liquid shaking medium were transferred to solid MS medium supplemented with 30 g/L sucrose and IBA at the same concentration appropriate for callus induction. Calli were then used for shoot regeneration.

Shoot formation from callus derived from leaf explants

The callus (0.1 g FW) derived from the leaf of *H. retusa* were cultured on the MS medium supplemented with BA or KIN at different concentrations of 0.5, 1.0, 1.5, and 2.0 mg/L for shoot formation. After 6 weeks of cultured, the rate of shoot formation (%), the number of shoots/explants, and the shoot length (mm) were observed.

Root induction and plantlets formation

For root induction, six weeks old *in vitro* shootlets (1.5 cm) were cultured on different media such as full-strength MS, half-strength MS (½MS), full-strength SH (Schenk and Hildebrant, 1972), and half-strength SH (½SH) supplemented with 30 g/L sucrose. After four weeks of culturing, the rate of root formation (%), number of roots/explant, and root length (mm) were observed.

In addition to enhancing root formation, exogenous auxins such as IBA and NAA were added to the culture medium. The medium was used here as the previously determined optimal medium, and exogenous auxins were added to the medium before being autoclaved at different concentrations of 0.5, 1.0, 1.5, and 2.0 mg/L. Plantlets were collected after six weeks of culture.

Medium and culture conditions

All media were adjusted to pH 5.8 with NaOH 1N or HCl 1N, and plant growth regulators (PGRs) were added to the media before autoclaving (Hirayama, Japan) at 121°C and 101 kPa for 15 min. The chemicals and PGRs were analytical grades (Duchefa Biochemie, the Netherlands). The cultures were maintained in light at an intensity of 45 µmol m^{-2} s^{-1} under a 12h photoperiod at a room temperature of 24 ± 2°C.

Statistical analysis

The experiments were arranged in a completely randomized design (RCD) with three replications, 10 flasks per treatment. All data were analyzed statistically using the Statgraphics software (version 18.0) and the graph was drawn by Microsoft Office Excel 2010 Software. Significant differences among the treatments were determined using LSD's multiple range test at $p<0.05$. The results were expressed as the mean±SE of the repeated experiments.

RESULT AND DISCUSSION

Callus induction and proliferation

IBA is a synthetic growth regulator of the auxin group that plays a vital role in stimulating callus or root formation. This has been demonstrated in the present experiment, the leaf explants induced to form callus and adventitious roots in the culture medium supplemented with IBA at concentrations of 1.0 - 3.0 mg/L, and they did not trigger morphogenesis in the IBA-free MS medium. The highest total dry weight of the explants obtained in each culture flask was 0.52 g/flask at the concentration of 2.0 mg/L IBA after four weeks of cultured (Fig. 1, Fig. 2a). In the shaking liquid medium, the explants were directly exposed to the medium with a large area because they were immersed in the medium. Therefore, leaf fragments cultured in a liquid shaking medium absorbed nutrients better than in a solid medium, resulting in explants that

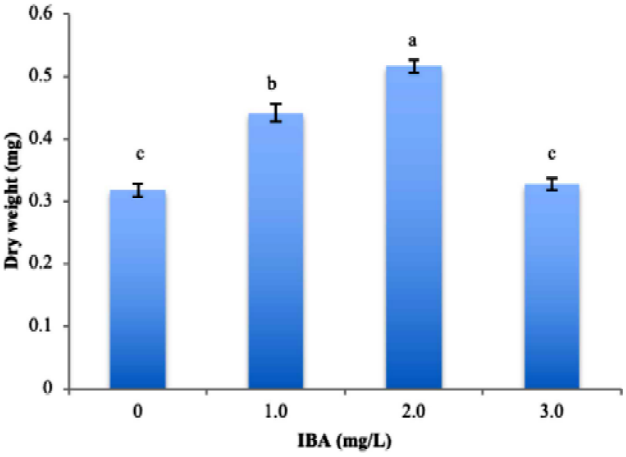

Fig. 1 : The total dry weight of the explants was obtained in each flask at different concentrations of IBA after 4 weeks of culture. Bars with different letters are significantly different at $p < 0.05$. Data are means ± SE.

respond early to the medium. In some previous studies, callus induction from the inflorescence explants of *H. obtuse* x *comptoniana* 'Sansenjyu' (Chen *et al.,* 2019); *H. splendens* and five commercial *Haworthia* cultivars which included *Haworthia* 'Natalie', 'Musin', 'Tiffany x Fertenon B Com', 'Baeckbong', 'White Wolf' (Reshma *et al.,* 2020), or the stem segments of *H. cymbiformis* (Haw.) (Lizumi and Amaki, 2011), or the leaf explant of *H. retusa* (Kim *et al.,* 2017, 2019) were performed in solid medium. In this study, leaf explants have cultured on a solid medium; however, this explant did not induce callus formation after 4 weeks of culture. Therefore, a liquid shaking culture system was used for the callus-induced culture.

After that, the response explants in this liquid shaking medium were transferred to the solid MS medium supplemented with 30 g/L sucrose and 2 mg/L IBA for callus formation (Fig. 2b). The callus was then used to induce shoot regeneration in the next experiments.

Shoot formation from callus derived from leaf explants

After six weeks of culture, all explants in the medium culture added BA enhanced shoot formation, with the proportion of shoot induction being 100%. The number of shoots per explant and shoot height increased with increasing concentration of BA from 0.5 to 1.0 mg/L, then they gradually decreased as the concentration of BA increased to higher concentrations (1.5 and 2.0 mg/L). The highest number of shoots, shoot height and the rate of shoot regeneration are recorded in the experiment using MS medium supplemented with 1.0 mg/L BA (43 shoots per explant, 9.4 mm, and 100%, respectively) (Table 1). In contrast, callus did not trigger shoot development in the MS medium without BA. According to Malik *et al.* (2005), the increase in BA concentration beyond the optimal level led to a decrease in shoot height and number of shoots. This was probably because exposure of explants to higher BA concentrations during the induction phase may have led to the accumulation of cytokinins, which inhibited further shoot growth. Liu *et al.* (2017) showed that multiplication adventitious shoots of *H. turgida* were induced from calli on MS medium using BA. The

Table 1 : Effect of BA and KIN on the shoot regeneration from callus derived from the leaf of *H. retusa* after 6 weeks of culture

BA (mg/L)	KIN (mg/L)	Rate of shoot regeneration (%)	Number of shoots/explant	Shoot height (mm)
0.5	-	100 ± 0.0^{a}	31.7 ± 1.8^{b}	8.3 ± 0.3^{b}
1.0	-	100 ± 0.0^{a}	43.0 ± 1.2^{a}	9.4 ± 0.4^{a}
1.5	-	100 ± 0.0^{a}	25.1 ± 1.8^{c}	7.3 ± 0.3^{c}
2.0	-	100 ± 0.0^{a}	22.2 ± 1.4^{d}	7.2 ± 0.5^{c}
-	0.5	44.3 ± 2.5^{e}	5.4 ± 0.7^{f}	5.1 ± 0.3^{d}
-	1.0	64.3 ± 2.5^{d}	7.1 ± 0.5^{ef}	5.2 ± 0.4^{d}
-	1.5	75.0 ± 1.7^{c}	8.3 ± 0.3^{e}	5.3 ± 0.2^{d}
-	2.0	78.7 ± 3.1^{b}	8.2 ± 0.4^{e}	5.2 ± 0.4^{d}
P	*	*	*	

*Means in the same column that is followed by different letters are significantly different ($p \leq 0.05$) using LSD's multiple range test

greatest induction ratio of shoot regeneration and several shoots was 76.6%, and 25.7, respectively. In the study of Lizumi and Amaki (2011), *H. cymbiformis* (Haw.) was propagated via thin cell layer (TCL) culture. The result showed that only stem transverse-TCL explants induced adventitious shoots. The maximum number of regenerated shoots was 24.0 per explant, and the percentage of shoot formation was 28.6% on the MS medium supplemented with 0.1 mg/L BA. Chen *et al.* (2019) established a shoot regeneration system for *Haworthia* from callus derived from inflorescence explants. The best shoot proliferation rates were on media with 1.0 mg/L BA and 0.0 - 0.4 mg/L NAA (65.57 - 81.01%) under a light intensity of 45 μmol $m^{-2}s^{-1}$. In another study, the effect of BA on adventitious shoot initiation of *Haworthia* 'Natalie', 'Musin', and 'Tiffany x Fertenon B Com' from inflorescence-derived callus were conducted. The highest number of shoot multiplications (20.8 ± 0.29) was observed for 'Tiffany x Fertenon B Com' on a medium containing 1.4 mg/L BA (Reshma *et al.*, 2020). The results obtained in our study showed a significant improvement in the percentage of shoots and the number of shoots formed compared with some previous studies.

In addition, many somatic embryos formed and developed into shoots when callus clusters were further sub-cultured on solid MS medium supplemented with 2.0 mg/L BA. Numerous globular somatic embryos were visible around the surface of the callus after 2 weeks of culture (Fig. 2c). After that, these somatic embryos continuously developed into shoots (Fig. 2f). This suggests that the shoot formation of *H. retusa* can be followed by two pathways; i) shoots formed through the callus; ii) shoots formed through the somatic embryo. This result can be explained during the liquid-shake culture period in the medium supplemented with auxin stimulated the formation of pre-embryonic cells, and then the explants transferred to the medium without auxin, or adding cytokinin enhanced somatic embryogenesis. This is also one of the novelties of this study; propagation via somatic embryos can produce this plant on a large scale. However, this issue needs to be further investigated to confirm the appropriate auxin and cytokinin concentrations and the corresponding embryonic stages in this species. Previous reports have shown that auxin is a plant hormone that is indispensable for the initiation of somatic embryogenesis, and cytokinin is often combined with auxin in a culture medium to enhance somatic embryo formation in most plants, including *Harworthia* species (Kim *et al.,* 2019; Reshma *et al.*, 2020). According to Kim *et al.* (2019), the somatic embryogenesis of plants depends on various types of cytokinins and auxins as well as their optimal concentration. This finding shows that adventitious shoot regeneration by somatic embryogenesis is effective in *H. retusa* micropropagation. Because *Harworthia* shoot regeneration is a time-consuming procedure, using this method can reduce time and improve the efficiency of *H. retusa* micropropagation.

Besides BA, KIN plays a vital role in the shoot morphogenesis of *Harwothia* species. For example, the shoot and callus induction percentages were affected by kinetin (KIN) and BA in *H. splendens* and 'White Wolf' (Reshma *et al.*, 2020). However, in the present study, the percentage of shoot induction, the number of shoots per explant, and the shoot height obtained in the treatments using KIN were lower compared with BA (Table 1). The positive effect of BA on shoot formation may be attributed to the ability of plant tissues to metabolize BA more readily than other plant growth regulators or to the ability of BA to induce the production of natural hormones, such as zeatin, within the tissue (Malik *et al.*, 2005).

Root induction and plantlets formation

Effect of culture medium on the root induction of *H. retusa*

Vitamins and nutrients in the culture medium play a significant role in the growth of *in vitro* plants. After 4 weeks of culture, *H. retusa* shoots induced roots on full-strength MS, half-strength MS, full-strength SH, and half-strength SH medium without plant growth regulator. On a half-strength MS medium, the highest rate of rooting explant (89.3%), number of roots/ explant (4.7), and root length (7.4 mm) were recorded (Table 2). Plants form roots to acquire water and minerals. Nutrient deficit acts as a powerful stimulant for root induction. The half-strength MS is to induce some level of nutrient stress, this resulted in the best root induction of *H. retusa* shoots. Whereas MS and SH are nutrient-rich media that are not suitable for the rooting of *H. retusa;* therefore, root induction on these media was lower than the half-strength MS medium. In addition, using a half-strength MS medium

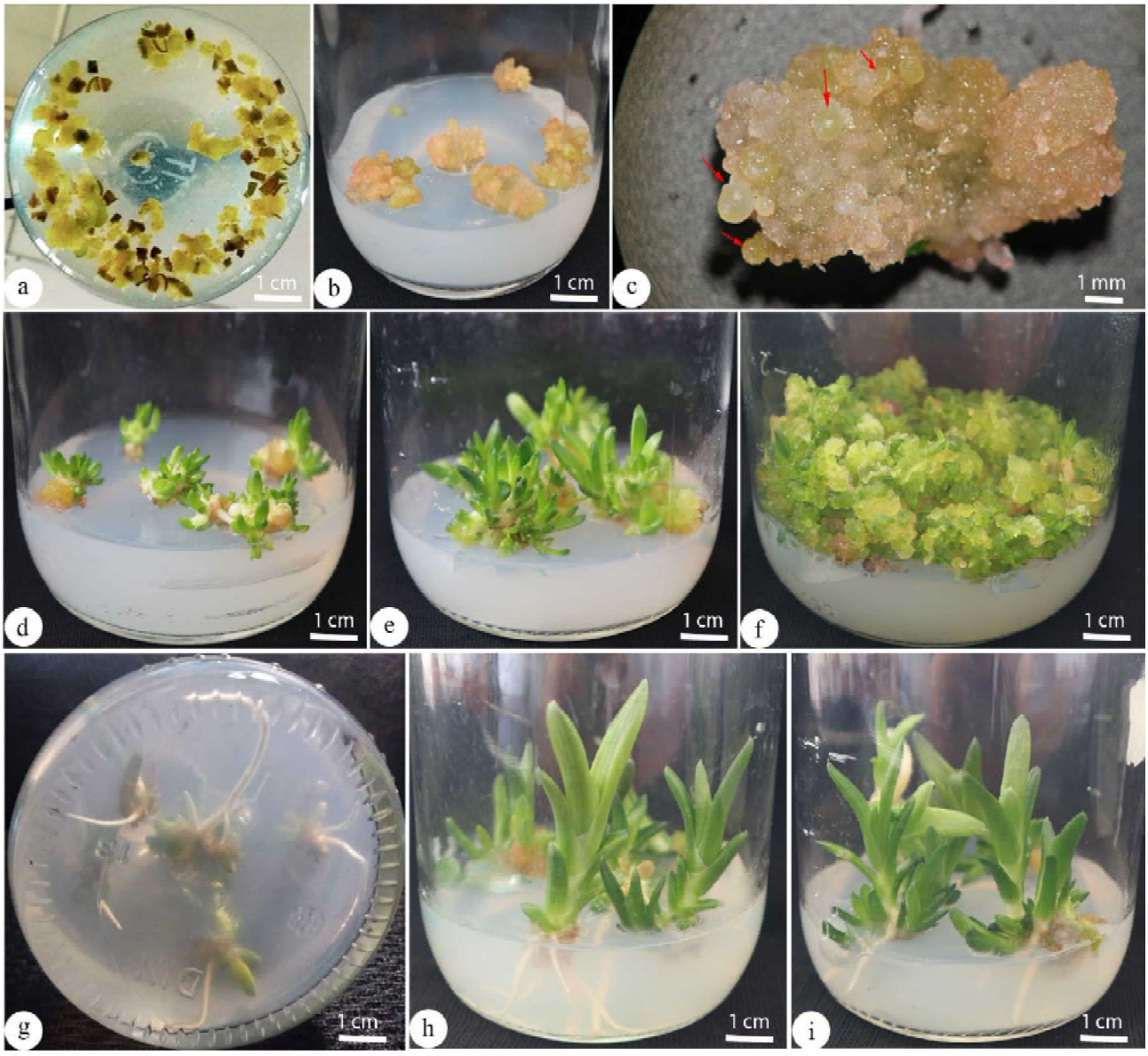

Fig. 2 : The *in vitro* plantlet regeneration protocol of *H. retusa* from leaves. a: leaf fragments induced to form a callus and adventitious roots in liquid shaking medium (MS + 2.0 mg/L IBA); b: callus proliferation on a solid medium (MS + 2.0 mg/L IBA); c: Somatic embryogenesis on the surface of callus. Arrows showed globular stage somatic embryo; d, e: shoots formation on MS medium supplemented 2.0 mg/L KIN (d) and 1.0 mg/L BA (e); f: somatic embryos developed into shoots; g, h, i: root induction and plantlet.

Table 2 : Effect of culture medium on the root induction of *H. retusa* after 4 weeks of cultured

Mineral medium	Rooting of explants (%)	Number of roots/explant	Root length (mm)
MS	82.0 ± 2.6^{b*}	3.3 ± 0.3^{b}	6.3 ± 0.1^{b}
½MS	89.3 ± 1.6^{a}	4.7 ± 0.4^{a}	7.4 ± 0.2^{a}
SH	70.3 ± 2.5^{c}	3.1 ± 0.3^{b}	6.4 ± 0.2^{b}
½SH	65.7 ± 4.0^{c}	1.8 ± 0.2^{c}	5.8 ± 0.3^{c}
P	*	*	*

*Means in the same column that is followed by different letters are significantly different ($p \leq 0.05$) using LSD's multiple range test

for the rooting stage also improves the acclimatization and survival rate of plantlets when they are transferred to the greenhouse because it reduces the difference in nutrient concentration between *in vitro* and soil conditions. Similarly, as in our experiments, the half-strength MS medium is effective in root induction in various species including *Cereus jamaica* (Monostori *et al.*, 2012), *Mentha spicata* (Fadel *et al.*, 2010), *Portulaca pilosa* (Chen *et al.*, 2020); *Rhus coriaria* (Amiri and Mohammadi, 2021).

Effect of IBA and NAA on the root induction of *H. retusa*

The results showed that IBA was more efficient in root induction than NAA after six weeks of culture (Table 3). When NAA (0.5 - 2.0 mg/L) was used to induce roots, a mass of callus was produced on the shoot and callus formation increased with increasing concentration of NAA. Callus production resulted in decreased rooting frequency, therefore, the percentage of rooting explants in treatments using NAA was lower than the control. The number of roots/explant and root length were not significantly different between NAA treatments. In contrast, callus formation was not observed in the IBA-supplemented treatments, and shoots induced roots with a high frequency. In *in vitro* culture, the addition of auxin at appropriate concentrations to the culture medium stimulated root formation, but higher concentrations stimulated callus formation. The auxin activity of NAA was higher than IBA, which was the reason for the shoots of *H. retusa* induced roots on the medium containing IBA and induced callus when NAA was used in this experiment.

In the treatment with 0.5 mg/L IBA, the percentage of rooting explant reached 100%, and it gradually reduced with higher IBA concentrations (1.0 - 2.0 mg/L). On a half-strength MS medium containing 0.5 mg/L IBA, the highest proportion of rooted explants, number of roots per explant, and root length were found among all treatments evaluated (Table 2). Previous studies showed that shoots of *Harwothia* species such as *H. attenuata* (Richwine *et al.*, 1995) and *H. turgida* (Liu *et al.*, 2017) formed roots on a medium supplemented with auxin. IBA has also been reported to be suitable for rooting in succulent species such as *Aloe adigratana* Reynolds (Niguse *et al.*, 2020); *Haworthia* 'Sansenjyu' (*H. obtuse* x *H. comptoniana*) (Chen *et al.*, 2019), *Haworthia* 'Natalie', 'Musin', 'Tiffany x Fertenon B Com' (Reshma *et al.*, 2020), *Stenocereus thurberi, Carnegiea gigantea* and *Pachycereus pringlei* (Pérez-Molphe-Balch *et al.*, 2002). In addition, it was observed that shoots produced several secondary shoots along with rooting when they were cultured on media for root formation (Fig. 2h, i).

Table 3 : Effect of IBA and NAA on the root induction of *H. retusa* after 6 weeks of culture

IBA (mg/L)	NAA (mg/L)	Rooting of explant (%)	Number of roots/explant	Root length (mm)
0	-	91.3 ± 3.2^{c*}	4.8 ± 0.2^{a}	10.3 ± 0.4^{a}
0.5	-	100.0 ± 0.0^{a}	5.0 ± 0.2^{a}	10.6 ± 0.8^{a}
1.0	-	98.0 ± 2.0^{ab}	4.7 ± 0.3^{a}	10.4 ± 0.3^{a}
1.5	-	95.3 ± 2.5^{b}	4.3 ± 0.2^{b}	10.2 ± 0.3^{a}
2.0	-	91.7 ± 1.2^{c}	4.1 ± 0.1^{b}	10.5 ± 0.3^{a}
-	0.5	33.7 ± 2.1^{d}	1.7 ± 0.2^{c}	3.7 ± 0.2^{b}
-	1.0	27.0 ± 1.0^{e}	1.7 ± 0.1^{c}	3.7 ± 0.3^{b}
-	1.5	21.3 ± 1.5^{f}	1.6 ± 0.2^{c}	3.6 ± 0.2^{b}
-	2.0	17.0 ± 2.6^{g}	1.7 ± 0.3^{c}	3.5 ± 0.4^{b}
P		*	*	*

Means in the same column that is followed by different letters are significantly different ($p \leq 0.05$) using LSD's multiple range test.

CONCLUSION

This study established an efficient plantlet regeneration system by indirect somatic embryogenesis through callus derived from the leaf tissues in a time span of 16-18 weeks. It included 5-6 weeks for callus induction and proliferation, six weeks for shoot regeneration, and 5-6 weeks for rooting and plants.

The time for this procedure can be shortened by 5-6 weeks for subsequent regeneration procedures because of the available source of callus and shoots. The process can be commercially exploited for the large-scale production of *H. retusa* plants.

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Original Research Paper

Effect of hot water treatments on physiological and biochemical changes in mango cv. Banganapalli during storage at ambient temperature

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ABSTRACT

Mango fruits majorly suffers from anthracnose and fruit fly infestations during storage, transportation and marketing. Hot water treatments (HWTs) at specific levels have shown to control the incidence of these important threats. Application of HWT not only act as a quarantine measure, but also maintains the quality and enhance the marketability of fruits, even at room temperature (RT), leading to its vast applicability in local / international markets. In this study, post harvest application of HWTs (48°C for 60 min and 55°C for 10 min) in mango cv. Banganapalli recorded reduced ethylene production rate, physiological loss in weight, improved sugar content, ascorbic acid, total carotenoids, phenolics and antioxidants compared to control. Combination of HWTs (48°C for 60 min followed by 55°C for 10 min) resulted in degradation of some quality parameters compared to individual HWT and control.

Keywords : Antioxidants, hot water treatments, mango cv. Banganapalli, phenols, quality

INTRODUCTION

Mango (*Mangifera indica*) is climacteric fruit with high respiration rate and have limited shelf life under ambient conditions. Sensitivity of fruits to decay, low temperature injury, perishability of fruits due to ripening and softening affects the handling, transport and storage potential of mangoes (Hoa *et al*., 2002). Mango cv. Banganapalli is one of the major export cultivars in India (Rao and Rao, 2008). Increasing consumer demand for quality, safety, variety, seasonal availability and consistency are creating opportunities as well as possible barriers for Indian small and marginal mango farmers. Some of the major issues restricting the international trade and domestic transport of mango fruits are fruit fly infestation, disease incidence, sap burn, non-uniform ripening, chilling injury development during cold storage, etc. (Sivakumar *et al*., 2011) and pesticide residue. For managing fruit fly and anthracnose (important storage disease), apart from implementing good agricultural practices (GAPs) in field, postharvest disinfestation/ quarantine treatments are mandatory for international exports. Hot water treatment (HWT) is one among many quarantine treatments used for mango exports. HWT is a highly efficient, non-chemical, environment friendly and low-cost method (Jacobi *et al*., 1995; Anwar and Malik, 2007), which can also be adapted in local markets for inter-state transportation, aiming major Indian markets.

There are recommended time-temperature combinations to disinfect mangoes from fruit fly infestation and anthracnose infection. Heat treatments disinfect the commodity by diminishing fruit fly eggs and maggots (Paul and Chen, 2000). When mango fruits are subjected to thermal treatments, the storage life may be further reduced as heat treatments were reported to enhance the ripening process. Hence, it is significant to know how the application of these thermal treatments at recommended levels affects the quality and storage life of mango fruits. The present investigation reveals, whether particular HWTs (quarantine and disinfection treatments) affect the quality and shelf life of mango cv. Banganapalli when stored under ambient conditions.

MATERIALS AND METHODS

The cv. Banganapalli fruits of green mature stage were harvested and procured from mango orchards of ICAR-IIHR and transported carefully to the laboratory. Fruits were sorted to discard damaged ones and uniform sized and matured fruits were selected. Fruits were separated into four lots, three for HWTs and one as control (T_4). Different HWTs *viz*., T_1: 48°C for 60 min (recommended quarantine HWT for control of fruit fly), T_2: 55°C for 10 min (recommended HWT for control of anthracnose disease) and T_3: a combination treatment of 48°C for 60 min followed by 55°C for 10 min (to control both fruit fly and anthracnose) were used. The experiments were conducted in a rectangular batch type hot water treatment plant with a capacity of 500 kg/batch, developed at ICAR-IIHR. Hot water treated fruits and control fruits (water washed and air-dried) were packed in corrugated fibre board (CFB) boxes with three replications, each containing approximately 4 kg fruits, and stored at room temperature (ambient temperature: 28.1° to 32.2°C with 45-50% relative humidity).

Measurement of physiological and biochemical parameters

Respiration rate was recorded using Checkmate O_2/ CO_2 analyzer and expressed as mg CO_2/ kg/ h and ethylene evolution was measured using ethylene analyzer and expressed as μl C_2H_4/ kg/ h (Rao and Rao, 2008), taking five fruits as five replications per treatment. Physiological loss in weight (PLW) was calculated cumulatively and expressed as percentage. Five fruits were selected at random from each treatment for quality attributes analysis. The pulp was extracted from fruits, grinded in a mixer grinder and then homogenized (specific quantity required for individual parameters) using IKA T25 digital ultra Turarax homogenizer before analysis. TSS was measured using hand refractometer calibrated to 25°C (Erma Inc., Tokyo, Japan). Parameters like acidity (%), ascorbic acid (mg/100 g) and sugars (%) were estimated using standard methods of analysis (AOAC, 2000). Five gram of mango pulp was grinded in pestle and mortar using a solution of petroleum ether and acetone (3:2) along with acid washed sand to extract the carotenoid pigments and the OD values were read in spectrophotometer at 452 nm using petroleum ether-acetone solution as blank. Total carotenoid content was then calculated with reference to the standard curve prepared with β-carotene and expressed as μg/100 g pulp. Total phenolic content in the pulp was determined by the method of Singleton *et al*. (1999) and was expressed as milligram of gallic acid equivalent per 100 g of fresh weight (mg GAE/100 g FW). Total antioxiant capacity was determined in terms of FRAP (ferric reducing antioxidant power), using the method of Benzie and Strain (1996) and values were expressed as mg acetic acid equivalent (mg AAE)/100g FW.

Statistical analysis: The effects of different treatments over the variables were evaluated by two-way analysis of variance based on a completely randomized design. Software WINDOSTAT 9.3 version was employed to analyze the analysis of variance at 5% significance level and statistical significance of differences between the mean.

RESULTS AND DISCUSSION

Respiration rate of all HW treated fruits was significantly higher than that of control fruits (Fig. 1). Heat treatment might have accelerated the physiological metabolism and hastened ripening in these fruits, which was slow in control. A hot water treatment of 54°±1°C for 5 minutes in Alphonso fruits accelerated ripening and resulted in higher respiratory climacteric (Laksminarayana, *et al*., 1974). At the last day of reading, highest respiration rate was observed in the combination treatment. The effect of temperature on the respiration rate can be directly related to chemical reactions where the rate of reaction increases exponentially with an increase in temperature (Wills *et al*., 1989). Ethylene production rate of hot water treated fruits and control fruits were measured upto 1 week under ambient condition (Fig. 2). Ethylene peak was seen on the sixth day, where control fruits showed a highest value followed by 48°C for 60 min + 55°C for 10 min, 55°C for 10 min and 48°C for 60 min treatments. Among heat treatments, combination treatment had highest ethylene production rate and quarantine HWT recorded lowest. Highest ethylene production in control fruits can be attributed to early onset of diseases like anthracnose and soft rot during storage, which was merely present in HW treated fruits. Yimyong *et al*. (2011) reported similar results in room temperature ripening of HW treated mangoes after low temperature storage. HWT eliminated ripening related ethylene rise and suppressed ethylene

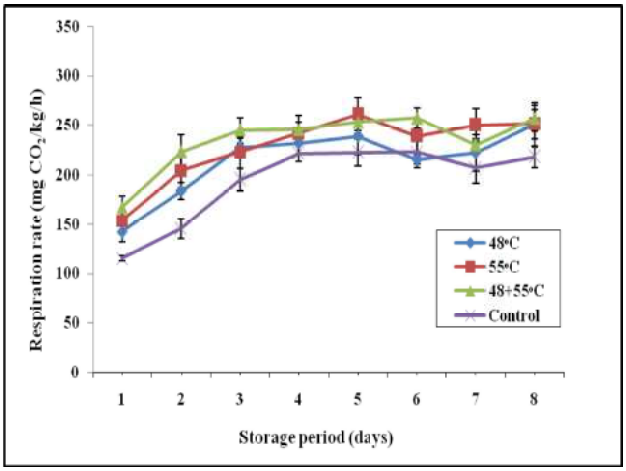

Fig. 1 : Effect of hot water treatments on respiration rate of mango cv. Banganapalli stored at RT

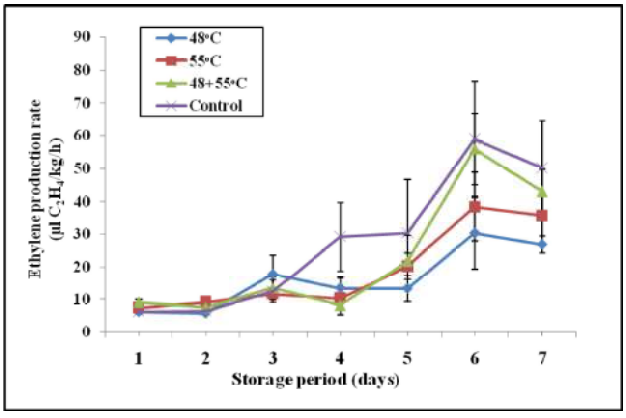

Fig. 2 : Effect of hot water treatments on ethylene production rate (µl C_2H_4/kg/h) of mango cv. Banganapalli stored at ambient temperature

production during subsequent storage at ambient temperature. PLW of fruits recorded upto 12 days of storage has been shown in Fig. 3. There was a gradual increase in fruits' PLW with storage duration, irrespective of the treatments. Initially, the loss in weight was higher in hot water treated fruits. This might be attributed to the stress developed in those fruits after subjected to heat treatment followed by ambient storage Further, the respiration rates of HW treated mangoes were also higher as depicted in Fig.1. PLW occurs in fruits due to many reasons, where membrane disruption associated higher rate of transpiration and water loss being one among them. The weight loss also depends on the temperature and duration of heat treatment (Perini *et al*., 2017; Vilaplana *et al*., 2018). After 9 days, the PLW in control fruits rapidly increased and at the end of storage highest PLW was observed in control fruits

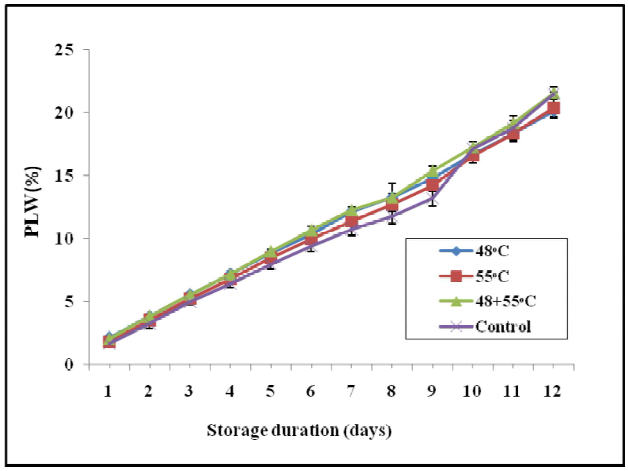

Fig. 3 : Effect of hot water treatments on physiological loss in weight of mango cv. Banganapalli stored at ambient temperature

and 48°C for 60 min + 55°C for 10 min treatment fruits. More damage due to secondary disease development was seen in control fruits at the end of storage. HWT effectively reduced disease and pest development in treated fruits and maintained marketability even after 1 week. HWT combined with inorganic salts solution dips effectively reduced disease incidence and enhanced quality and storability of fruits till consumer end (Dessalegn *et al*., 2013). Combination treatment, on the contrary, might have suffered more heat stress, leading to surface scald/ heat injury, providing inoculum for the development of diseases and hence, had maximum weight loss among HWTs.

Table 1 represents the changes in chemical attributes *viz*., TSS, acidity, ascorbic acid and total sugars with respect to the treatments. TSS was more and acidity was less in HWTs, when compared to control. This is because, HW treated fruits ripened faster than that of control resulting in higher TSS during initial storage period. There was no negative effect on the ascorbic acid content of treated mango fruits. Ascorbic acid was seen highest in T_3 and T_1. Being most unstable vitamin, ascorbic acid content is affected by pre and post harvest treatments, heat treatments, storage duration *etc*. (Khader and Lee, 2000). In this experiment, ascorbic acid content was maintained in HWTs and during storage duration. It is the length of high temperature exposure during storage which may cause the loss of vitamin C and high temperature during HW treatment is only for shorter duration and so there was minimum loss of vitamin C. Studies

Table 1 : Effect of different hot water treatments on TSS, acidity, ascorbic acid, total sugar of mango cv. Banganapalli stored at ambient temperature

Treatment	TSS (°B)			Mean (T)	Acidity (%)			Mean (T)	Ascorbic acid (mg/100 g)			Mean (T)	Total sugars (%)			Mean (T)
	5 days	7 days	10 days		5 days	7 days	10 days		5 days	7 days	10 days		5 days	7 days	10 days	
T_1: 48°C for 60 min	20.67	21.67	21.60	21.31	0.47	0.33	0.25	0.35	3.20	5.60	9.47	6.09	14.89	13.78	15.08	14.59
T_2: 55°C for 10 min	21.53	22.07	19.87	21.16	0.54	0.34	0.29	0.39	3.27	5.13	6.73	5.04	12.68	16.47	15.31	14.82
T_3: 48°C for 60 min + 55°C for 10 min	22.13	21.27	20.53	21.31	0.53	0.42	0.32	0.42	4.27	6.73	10.33	7.11	11.51	14.18	15.89	13.86
T_4: Control (without treatment)	17.83	19.87	19.47	19.06	0.63	0.53	0.50	0.56	3.87	5.60	7.00	5.49	12.84	15.05	15.98	14.62
Mean (D)	20.54	21.22	20.37		0.54	0.41	0.34		3.65	5.77	8.38		12.98	14.87	15.56	
	T	D	TxD		T	D	TxD		T	D	TxD		T	D	TxD	
F test	**	**	**		**	**	**		**	**	**		**	**	**	
S.E.m±	0.23	0.27	0.47		0.01	0.01	0.02		0.18	0.20	0.35		0.18	0.20	0.35	
CD at 5%	0.68	0.79	1.37		0.03	0.03	0.06		0.52	0.60	1.03		0.52	0.60	1.04	

Table 2 : Effect of different hot water treatments on total carotenoid content, total phenols and total antioxidant capacity of mango cv. Banganapalli stored at ambient temperature

Treatment	Total carotenoid content (µg/100g)			Mean (T)	Total phenols (mg GAE/100g)			Mean (T)	Total antioxidant capacity (mg AAE/100g)			Mean (T)
	5 days	7 days	10 days		5 days	7 days	10 days		5 days	7 days	10 days	
T_1: 48°C for 60 min	1197.9	2228.9	2139.7	1863.8	42.45	40.46	40.65	41.19	42.81	40.64	39.20	40.88
T_2: 55°C for 10 min	1236.7	2206.1	2368.5	1937.1	41.48	39.93	41.19	40.87	41.62	40.06	38.01	39.90
T_3: 48°C for 60 min + 55°C for 10 min	991.7	1674.8	2242.8	1636.5	40.54	37.42	42.66	40.20	37.63	36.07	34.31	36.00
T_4: Control (without treatment)	1261.0	2063.0	2190.5	1838.2	40.43	38.24	34.08	37.58	37.12	39.26	36.98	37.79
Mean (D)	1178	2043.2	2235.4		41.22	39.01	39.64		39.80	39.01	37.12	
	T	D	TxD		T	D	TxD		T	D	TxD	
F test	**	**	**		**	**	**		**	**	**	
S.E.m±	43.57	50.31	87.14		0.57	0.66	1.14		0.23	0.27	0.46	
CD at 5%	127.18	146.85	254.35		1.66	1.91	3.31		0.67	0.78	1.35	

(Djioua *et al*., 2009) reported the positive correlation between heat treatment and ascorbic acid content, stating, heat treatment did not affect but also maintained the ascorbic acid content.

HWTs maintained the total sugars in fruits during storage. Total sugars were highest in T_2 and lowest in T_3, which is the combination treatment (Table 1). Increased exposure to the heat in T_3 might have denatured the enzymes responsible for the synthesis of sugars. Papaya when immersed in hot water at 49°C for 90 min and 120 min acquired minimum sugar content due to the reduction of xylanal and polygalacturonase activity by reducing their synthesis (Benjamin *et al*., 2018).

Other important bio-chemical parameters on which the effect of HWTs studied includes total carotenoids, phenols and antioxidant capacity (Table 2). Total carotenoids were higher in T_2, followed by T_1 and then T_4. Here, the combination treatment, T_3 recorded minimum carotenoids, though it had a higher peel colour. There was an elevated outcome on total phenols and antioxidant capacity in HWTs than that of control. Less antioxidant activity was seen in T_3. The total antioxidant activity, levels of phenolic compounds and ascorbic acid were higher in heat treated-pomegranate (Mirdehghan *et al*., 2005) and strawberries due to the stimulation of protective enzymes against oxidation (Viente *et al*., 2006).

CONCLUSION

Hot water treatment is one of the promising methods to prevent fruit fly disinfestations and decay in mango during storage, though, there is concern regarding its effect on fruits' storage life and quality. In the present experiment, HWTs did not negatively affect the physiological and biochemical attributes. Recommended HWTs for fruit fly and anthracnose control, when applied alone enhanced the biochemical quality viz., higher sugar content and lower acidity, higher carotenoid content, phenolics and antioxidant capacity compared to control fruits. The combination of these treatments did not give any positive results in terms of quality. Standalone HWT can be recommended as a potential, safe and non-chemical treatment to manage diseases, fulfil quarantine requirements, improve quality and storage life, aiding to quality fruits supply in local as well as international markets.

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Original Research Paper

Post-harvest melatonin application reduced browning in minimally processed lettuce (*Lactuca sativa* L.) during low temperature storage

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ABSTRACT

The investigation was carried out to assess the effect of post-harvest dipping of minimally processed fresh cut lettuce with various concentrations (10, 100 and 1000 µmolL^{-1}) of melatonin on shelf-life and sensory quality of lettuce stored at 6±2ºC for 8 days. Melatonin treatment was found effective in maintaining freshness and sensory quality of lettuce during storage. Browning was reduced by 45% and visual quality index increased by 44.10% compared to control in 100 µmol L^{-1} melatonin treated samples on the 6th day of storage. Maximum total chlorophyll, total phenol and total antioxidants and least activity of browning related enzyme *i.e.,* peroxidase (POD) was observed in 100 µmol L^{-1} melatonin treated samples during storage. No significant variation was observed between 10 µmol L^{-1} melatonin treated and control samples. Browning index value had significant negative correlation with total chlorophyll, total phenol and total antioxidants whereas POD activity had significant positive correlation. It can be inferred from the present investigation that post-harvest treatment of 100 µmol L^{-1} melatonin extended shelf-life of minimally processed lettuce for 6 days by preserving phenols, chlorophyll, antioxidants and inhibiting POD activity.

Keywords : Browning, lettuce, melatonin, minimal processing, peroxidase, phenols

INTRODUCTION

Minimally processed vegetables, popularly known as ready-to-use or ready to eat or fresh-cut, are raw vegetables that have been sanitized, peeled, sliced, chopped or shredded and packaged to make them readily usable without decline in freshness and quality (Siddique *et al*., 2011). Since lettuce is having meager number of calories (10 kcal100^{-1} g FW), it is often advised for reducing obesity and also minimizing risk of cataracts, heart ailments, cancers and paralysis due to presence of ample amount of β-carotene and lutein contents (Mampholo *et al*., 2016). In recent years, demand for minimally-processed (MP) vegetables is increasing in India and projected to record 6.5% compound annual growth rate (CAGR) by 2026 due to their minimal processing, ready to consumption form and high dietary value. Lettuce is an important leafy vegetable usually consumed as salads. Currently, share of salads has enhanced in diet and, hotels, restaurants and catering services are demanding lettuce in ready to eat form. Lettuce is highly delicate and prone to surface browning through enzyme action. Minimally processed produce deteriorates more rapidly than whole produce because internal and outer tissues are exposed to external environment. Physical damage during the minimal processing elevate metabolic activities, respiration, biochemical conversion and microbial growth, that often result in dilapidation of texture, color, ûavour and affect visual quality as well as marketability of the product. Several chemical and physical treatments have been widely tried out to manage fresh-cut lettuce browning. However, most of the methods are commonly constrained by toxic nature, cost and potentially spoiling sensory properties and reduction in nutrient content of the produce.

Melatonin is a harmless biological molecule synthesized naturally in mitochondria and chloroplast of the plants (Tan *et al*., 2013). Melatonin works as an antioxidant and augments the post-harvest life of horticultural produce. Earlier, post-harvest treatment of melatonin had found effective in mitigating browning and extending shelf-life in strawberry (Aghdam and Fard, 2017), litchi (Zhang *et al*., 2018), peach (Gao *et al*., 2018), broccoli (Zhu *et al*., 2018) and cut anthurium flowers (Aghdam *et al*., 2019). The objective of this investigation was to assess the impact

of post-harvest melatonin treatment on tissue browning and storage quality of fresh-cut lettuce.

MATERIALS AND METHODS

Lettuce var. 'Grishma' grown under aeroponic conditions at vegetable hydroponic centre of ICAR-CISH, Lucknow was procured. Uniform size healthy heads were selected and wrapper foliage was removed and heads were sanitized with 100 ppm chlorine water. Then heads were cut into two halves with sanitized sharp stainless-steel knife. Thereafter, cut pieces were treated with aqueous solution of melatonin (CDH, New Delhi) (10 µmol, 100 µmol and 1000 µmolL^{-1}) by immersing them for 5 min and treated heads were air-dried to evaporate surface water. Dried cut pieces with sample size of 200 g were packed in zip-n-lock polypropylene bags. Packaged samples were stored at 6±2°C temperature for period of 8 days and observation was recorded on 0, 2, 4, 6 and 8 days of storage. Physical and biochemical observations were recorded in four samples for each treatment.

The appearance and browning index of lettuce were recorded as suggested by Tian *et al.* (2014). Five lettuce pieces evaluated for each treatment by 8 panelists. Overall visual quality (OVQ) was measured on a scale from 9 to 1, where 8- 9: excellent (completely devoid of brown spots) 6-8: good (minor defects; not objectionable) and less than 6: poor (moderately to excessive defects) quality. Salability limit was restricted to 6 OVQ rating. The browning index (BI) was calculated by using following formula: BI="(browning scale) × (number of lettuce pieces with that browning level)/ (total number of lettuce pieces). Sample rated BI more than 2.0 was considered unsuitable for marketing. Electrolyte leakage (EL) was measured by using the method of Aghdam *et al*. (2015). The total phenols were determined by the Folic-Ceocalteu method using tannic acid as standard. The total chlorophyll was determined using the equation: Total chlorophyll (µg/ml) = (20.2 x O.D. at 645 nm) + (8.02 x O.D. at 663 nm) as given by Arnon (1949). The antioxidants activity in lettuce was measured as ferric reducing antioxidant potential (FRAP) value (Bhattacherjee *et al*., 2014). The peroxidase enzyme activity was estimated as number of absorbance units per gram fresh weight of leaf. Experiment was designed in complete randomized block design (CRD) and data was analyzed by using Web Agri Stat Package 2.0 (WASP 2.0) statistical software.

RESULTS AND DISCUSSION

The emergence of brown spots on minimally processed (MP) lettuce leaves increased progressively in all samples during storage period irrespective of post-harvest treatment. During first 2 days of storage, browning index (BI) remained below the threshold limit (less than 2) in all samples. However, BI in control and 10 µmolL^{-1} melatonin treated samples was significantly higher compared to 100 and 1000 µmolL^{-1} melatonin treated samples (Fig. 1). After 2 days of storage, sharp elevation in BI was observed in control and 10 µmolL^{-1} melatonin treated lettuce and it exceeded threshold BI limit (less than 2) on 4th day during storage and with values 3.50 and 3.16, respectively. However, BI in 100 and 1000 µmolL^{-1} melatonin treated leaves was lesser than the threshold limit (below 2) *i.e*., 1.91 and 1.95, respectively on 6th day of storage. At 6th day of storage, 45% lower BI value was observed in 100 µmolL^{-1} melatonin treated samples compared to control. At 8th day of storage, the lowest BI (2.45) was noticed in 100 µmol melatonin treated samples followed by 1000 µmolL^{-1} melatonin treatment (2.51) whereas, significantly higher BI (3.82) was observed in control and 10 µmol melatonin treated lettuce. Similar outcomes were reported by Aghadam *et al*. (2015) in cut anthurium where 51% lower browning was noticed in 100 µmol melatonin treated flowers. Zhang *et al*. (2018) observed strong suppression of pericarp browning in litchi through post harvest melatonin treatment. Membrane damaged during minimal processing

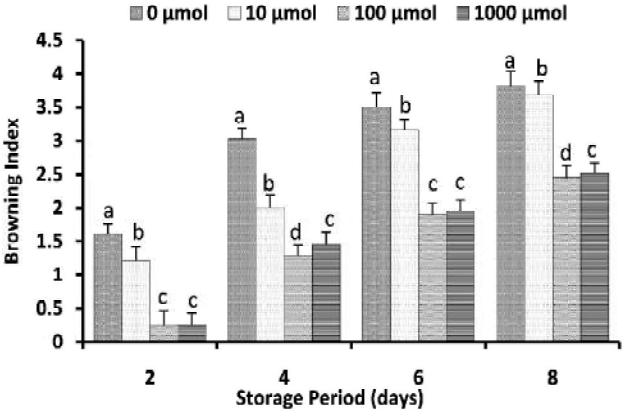

Fig. 1. Browning index score in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

operations caused loss of sub-cellular compartmentalization, leading to contact between browning inducing-enzymes (PPO and POD) and phenolic substrates, further leading to enzymatic browning in fruits and vegetable produce. In the current investigation, less tissue browning in treated samples compared to control might be due to suppression of phenol oxidizing enzymes by melatonin. This is supported by the significant positive correlation (r=0.915) between BI and POD activity at the end of storage period (Table 2).

Visual quality of MP lettuce was considerably retained by exogenous post-harvest dip treatment of 100 and 1000 µmolL^{-1} melatonin. On the 6th day of storage, Visual Quality Index (VQI) was significantly higher *i.e.,* 7.12 (more than threshold limit 6) in 100 µmolL^{-1} melatonin treated samples whereas, in control and 10 µmol melatonin treated lettuce VQI was calculated as 4.78 and 5.02, respectively. On the 8th day of storage, maximum VQI (5.26) was recorded for 100 µmol melatonin treated heads and minimum VQI (3.65) was observed in control (Fig. 2). In 100 µmol melatonin treated samples, VQI value was 44.10% higher than the control samples. No considerable visual quality difference was observed in 10 µmol melatonin treated lettuce and untreated samples. Similarly, 100 µmolL^{-1} melatonin treatments-maintained freshness in broccoli florets for 7 days storage period (Zhu *et al*., 2018). VQI demonstrated significant negative association with browning index (r= -0.945) and POD activity (r= -0.986) whereas, it displayed significant strong positive correlation with total chlorophyll (r=0.0.963), total phenol (r=0.794) and total antioxidants (r=0.961) (Table 2).

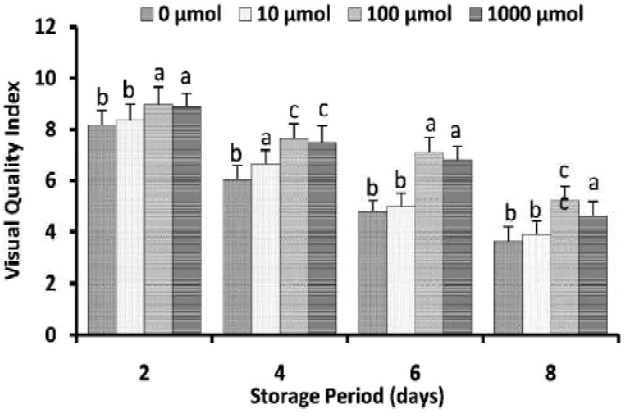

Fig. 2. Visual Quality Index (VQI) score in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

Electrolyte leakage (EL) is correlated with maintenance of membrane integrity during cold storage of fresh produce. An enhancement in EL has been used as an indicator of physiological damage in cell membrane during storage. EL of MP lettuce leaves increased in control as well as melatonin treated samples during storage. During initial two days of storage, non-significant difference was observed in EL among the treatments. However, on 4th, 6th and 8th day of storage considerably lower EL was noticed in lettuce dipped in 100 and 1000 µmolL^{-1} melatonin compared to control (Fig. 3). At the end of storage period, 55.77% enhancement in EL was noticed in 100 µmolL^{-1} melatonin treated samples whereas 97.92% elevation was noticed in control samples. Melatonin treatment slowed down the production of superoxide radicals (O_2^{-}) and hydrogen peroxide (H_2O_2) during post-harvest storage which resulted in protection of membrane structure and lower electrolyte leakage (Aghdam *et al*., 2015). Our results were in concomitant with findings of Aghdam *et al*. (2019) in melatonin treated anthurium cut flowers during cold storage.

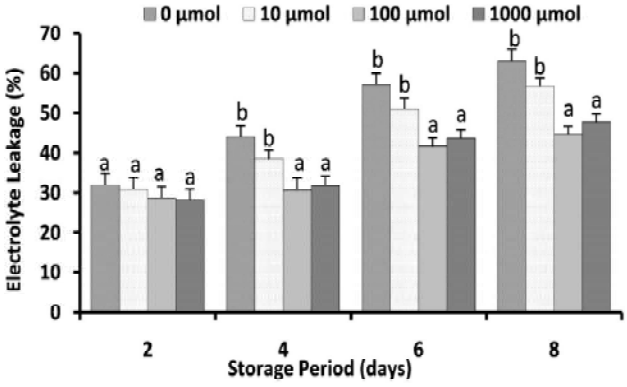

Fig. 3. Electrolyte leakage (%) in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

Chlorophyll in lettuce leaves is a crucial quality parameter with respect to salability and consumer acceptance. Total chlorophyll content decreased in MP lettuce during storage irrespective of post-harvest treatment. The rate of chlorophyll degradation was considerably delayed in 100 and 1000 µmolL^{-1} melatonin solution dipped samples whereas rapid chlorophyll loss was recorded in untreated and 10 µmolL^{-1} melatonin treated samples (Table 1). On the 8th day of storage, 71.61% and 68.16% chlorophyll loss was noticed in control and 10 µmolL^{-1} melatonin treated samples, respectively

Table 1 : Post harvest melatonin treatment effect on total chlorophyll, total phenols and total antioxidants of minimally processed lettuce during low temperature (6±2°C) storage for 8 (days)

Biochemical parameter	Post-harvest treatment	Storage period (D)					
		D_0	D_2	D_4	D_6	D_8	Mean
Total chlorophyll (mg/g FW)	Control	3.18[a]	2.90[b]	2.48[b]	1.34[c]	0.91[b]	2.16
	10	3.11[ab]	2.91[b]	2.67[b]	1.59[c]	0.99[b]	2.25
	100	3.15[ab]	3.11[a]	3.05[a]	2.45[b]	1.39[a]	2.67
	1000	3.12[b]	3.10[ab]	2.98[c]	2.89[a]	1.32[a]	2.68
	Mean	3.14	3.05	2.81	2.06	1.15	
Total phenols (TAE mg/g FW)	Control	0.117[a]	0.168[a]	0.211[a]	0.227[c]	0.236[b]	0.191
	10	0.118[a]	0.155[a]	0.206[b]	0.220[c]	0.250[b]	0.189
	100	0.116[a]	0.160[a]	0.237[ab]	0.253[b]	0.268[a]	0.206
	1000	0.117[a]	0.166[a]	0.251[c]	0.237[a]	0.263[a]	0.206
	Mean	0.117	0.162	0.226	0.234	0.254	
Total antioxidants (mmol/g FW)	Control	12.91[a]	15.73[b]	19.17[c]	22.39[b]	24.90[b]	19.02
	10	12.91[a]	16.12[b]	18.15[c]	23.37[b]	26.10[b]	19.33
	100	12.91[a]	18.31[a]	23.59[b]	26.25[ac]	29.29[a]	22.11
	1000	12.91[a]	19.54[a]	22.34[a]	27.12[a]	28.56[a]	22.09
	Mean	12.91	17.42	20.81	24.78	27.19	

*Means with same superscript are non-significant. TAE=Tannic acid equivalent

whereas in 100 and 1000 µmolL^{-1} melatonin treated samples 44.12% and 42.30% chlorophyll loss was observed. Similar, observation were reported by Zhu *et al*. (2018) who recorded 24.15% higher chlorophyll in 100 µmolL^{-1} melatonin treated broccoli compared to water treated samples on the 6th day of storage. During post-harvest storage of fresh produce chlorophyll, dilapidation occurs through the continuous reduction of chlorophyll binding proteins due to elevated action of chlorophylase enzyme (Arnao and Ruiz, 2008). It may be possible here that melatonin plays a role as antioxidant, prevents the accumulation of free radicals such as ROS and lipid radicals and thus delaying the chlorophyll degradation.

Changes in total phenol content occurred in MP lettuce throughout the storage period. Both treated and untreated samples displayed an enhancing trend with respect to total phenols. Lettuce dipped in 100 and 1000 µmolL$^{"1}$ melatonin demonstrated considerably increased levels of total phenol compared to untreated samples from day 4 to 8 days of storage, whereas no considerable variation was noticed between untreated and 10 µmol L^{-1} melatonin treated lettuce (Table 1). On the 8th day of storage maximum total phenols (0.268 TAE mgg^{-1} FW) was estimated in 100 µmolL$^{"1}$ melatonin treated samples followed by 1000 µmolL^{-1} melatonin treatment (0.263 TAE mgg^{-1} FW) with non-significant difference. Consistent with our findings, high total phenols retained in melatonin treated samples of litchi (Zhang *et al*., 2018), strawberry (Liu *et al*., 2018), peaches (Gao *et al*., 2018) and anthurium (Aghdam *et al*., 2019) during low temperature storage has been reported earlier. Damage occurs during minimal processing due to induced accumulation of phenols in both untreated and melatonin treated lettuce leaves. Phenolics are converted into quinone through oxidation by PPO and POD in the presence of oxygen which is responsible for browning in fresh produce (Pardossi and Tognoni, 2005). Significantly higher level of total phenols in samples treated with melatonin might be ascribed to the fact that melatonin is mitigating the action of phenol oxidizing agents such as peroxidase (POD) and polyphenol oxidase (PPO) enzyme.

Melatonin treatment significantly influenced the antioxidant activity of MP lettuce leaves during storage. Gradual enhancement in antioxidant activity was noticed during storage irrespective of post-harvest treatment. At the end of storage period, untreated lettuce leaves exhibited lowest (24.90) antioxidant activity as compared to melatonin pre-treated samples.

Among melatonin treated samples, maximum antioxidant activity (19.29) was observed in minimally processed lettuce leaves which were treated with 100 µmolL^{-1} melatonin followed by 1000 µmolL^{-1} melatonin (Table 1). However, non-significant difference was noticed in both the treatments. The findings are in concurrence with the previous reports that melatonin treatment preserved antioxidant level in strawberry (Liu *et al*., 2018), litchi (Zhang *et al*., 2018) and anthurium (Aghdam *et al*., 2019). Phenolics are well recognized for their antioxidant properties. In lettuce strong positive correlation (r=0.884, Table 2) was noticed among total phenols and total antioxidants during storage. Higher antioxidant activity of melatonin treated lettuce might be attributed to higher presence of phenol content accompanied by lower activity of peroxidase (POD) and polyphenol oxidase (PPO).

During storage of MP lettuce POD activity was enhanced in the tissues irrespective of post harvest treatments. The augmenting tendency of POD activity was considerably inhibited by melatonin pre-treatment (Fig. 4). During initial 2 days of storage non-significant variation was observed in POD activity in all treatments. However, it was rapidly enhanced afterwards but remains significantly low throughout the storage period in 100 and 1000 µmolL^{-1} melatonin treated lettuce compared to control (Fig. 4). On the 8th day of storage, POD activity in untreated and 10 µmolL^{-1} melatonin treated samples was 6.60, 6.42 times of the initial value, respectively whereas 100 and 1000 µmolL^{-1} melatonin treated samples had 5.05- and 5.43-fold POD activity compared to initial value. Previous researchers also reported lower POD activity in post-harvest melatonin treated broccoli florets

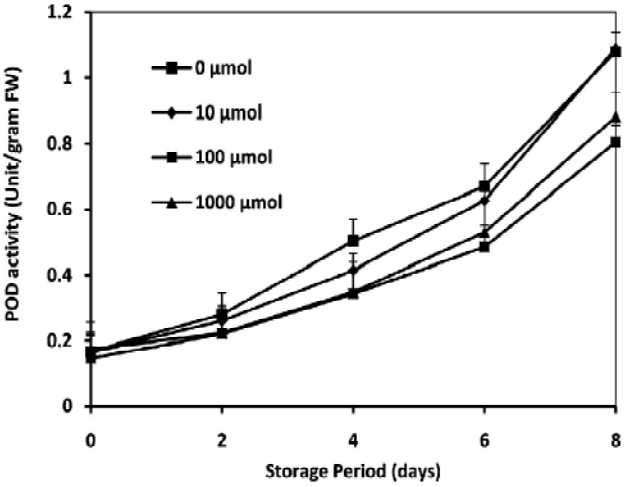

Fig. 4. Peroxidase (POD) activity in minimally processed lettuce treated with exogenous application of melatonin during 8 days storage at 6±2°C temperature.

(Zhu *et al*., 2018), litchi (Zhang *et al*., 2018) and peach (Gao *et al*., 2018). POD takes part in the oxidation of polyphenols into quinones that contributes in development of the brown pigments in minimally processed fresh produce. Slow increases in POD concentration coupled with elevated levels of total phenolics were noticed in treated lettuce signaled that melatonin slowed down the enzyme induced phenolic oxidation and inhibit brown color development in minimally processed lettuce. In present study, POD activity showed strong positive correlation (r=0.915, Table 2) with browning index.

The results of the present study concluded that post-harvest melatonin treatment proved effective in reducing browning, maintaining freshness and quality of minimally processed lettuce for 6 days during storage at 6±2 °C. Comparing to the higher dose (1000

Table 2 : Correlation between browning index and various physical and biochemical parameters recorded during storage of lettuce

Trait	Browning Index	Visual Quality Index	Electrolyte leakage	Total phenol	Total chloro-phyll	Total antioxi-dants	POD activity
Browning Index	1.000						
Visual Quality Index	-0.945	1.000					
Electrolyte Leakage	0.935	-0.891	1.000				
Total Phenol	-0.884	0.794	0.667	1.000			
Total chlorophyll	-0.886	0.961	-0.931	-0.615	1.000		
Total antioxidants	-0.997	0.963	0.921	0.889	-0.898	1.000	
POD activity	0.915	-0.986	0.918	-0.693	-0.993	0.931	1.000

μmolL^{-1}) and control, lower dose of melatonin at100 μmolL^{-1} was found highly beneficial for minimizing browning, quality retention and enhancing shelf life of minimally processed lettuce for 6 days.

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Original Research Paper

Dragon fruit peel extract mediated green synthesis of silver nanoparticles and their antifungal activity against *Colletotrichum truncatum* causing anthracnose in chilli

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ABSTRACT

Plant extracts have been used as reducing and stabilising agents to synthesise various metal-based nanoparticles due to their cost-effective and eco-friendly nature. In the present work, a green and environment-friendly method is adopted for synthesising silver nanoparticles (Ag NPs) using a biowaste of dragon fruit (*Hylocereus* spp.) peel aqueous extract at 80ºC in an alkaline condition. The Ag NPs were characterised through various analytical and microscopic techniques. The UV-Vis spectra of Ag NPs showed a characteristic peak between 400 - 410 nm. Transmission and scanning electron microscopic studies confirmed spherical monodispersed particles with an average size of 7 nm. Energy-dispersive X-ray spectroscopy (EDX) confirmed the presence of silver and silver chloride among the principal elements. The X-ray powder diffraction (XRD) spectra showed the crystalline nature of synthesised silver and silver chloride nanoparticles. The synthesised nanoparticles showed potential antifungal activity against *Colletotrichum truncatum* spores in both *in vitro* conidial germination and spread plate assays. The efficacy of the synthesised NPs confirmed that these NPs could be used as potential antifungal agents against *C. truncatum* to control anthracnose in chilli.

Keywords : Anthracnose, antifungal activity, chilli, *colletotrichum truncatum,* dragon fruit, green synthesis, silver nanoparticle

INTRODUCTION

Nanotechnology deals with materials in a size range between 1-100 nm. Materials display novel, unique features at this scale. These unusual properties have attracted researchers from different science streams, including agriculture and medicine, to work in the field of nanotechnology (Gowda and Sriram, 2020). Due to their small size, nanoparticles show a larger surface-to-volume ratio and behave differently than bulk materials. Nanoparticles are synthesized by top-down and bottom-up approaches. Metal nanoparticles are commonly synthesized by chemical and physical methods using various toxic, hazardous solvents and chemicals as reducing agents. Moreover, the chemical and physical methods require complex experimental conditions and costly instruments. Green synthesis of nanomaterials is gaining importance due to their nontoxicity, biocompatibility, low cost and eco-friendly synthesis. The plant acts as a potential nano factory since its extracts have been used as a reducing, stabilizing and capping agent for synthesizing nanomaterials. The application of nanotechnology to manage fungal diseases may lead to the development of new nano-based antifungal products, ushering in a new era of nano-fungicide discovery. Nano-based antimicrobial agents have many advantages over conventional agrochemical delivery systems as they possess increased solubility, bioavailability, sustained and targeted delivery, and even give protection from harsh environmental conditions (Chowdappa and Gowda, 2013).

Dragon fruit (*Hylocereus* spp.), well known as Pitaya, is a tropical fruit that belongs to the *Hylocereus* genus and family of cactus, Cactaceae. The fruits are triangular with wide scales. Dagon fruit originated from West Indies and Latin America (Hua *et al.*, 2018). It has gained considerable consumer interest due to its potent nutritional and medicinal benefits. It has been reported that red pitaya fruit is very rich in iron content, and consuming its juice during pregnancy increases hemoglobin and erythrocyte levels and treats anemia in pregnant women (Widyaningsih *et al.*,

2017). The colour of the fruit is due to the presence of Betalains, water-soluble nitrogen-containing pigments. Betalains are plant secondary metabolites that are reported to possess antioxidant, antitumor activities and other health benefits (Tenore *et al.*, 2012). Dragon fruit is usually eaten directly or made into juice. Therefore, fruit peel is the major biowaste. Researchers have explored ways to use dragon fruit peel biowaste as a constituent for microbial growth media (Putri *et al.*, 2017), biochar production (Hu *et al.*, 2020), and biosorbents to remove methylene blue dye from aqueous solution (Jawad *et al.*, 2018).

Researchers have proved that dried pitaya fruit peels are a good source of dietary fiber and pectin (Jiang *et al.*, 2020). The dragon fruit peel is reported to contain higher phenolic and antioxidant capacity than edible portions. It has more flavonoids, total phenols, and antioxidant activities than pulp (Abirami *et al.*, 2021; Nurliyana *et al.*, 2010). The metabolic content of dragon fruits varies based on the fruit's cultivars, colour, size and shape. Nurliyana *et al.* (2010) studied the antioxidant activity of pulps and peels of dragon fruits. They reported that the white dragon fruit peels contained higher phenolic content than the red dragon fruit peel, but the pulp of white dragon fruit had lower Total Phenolic Content (TPC) than the red dragon fruit. They found that peels from both varieties' of dragon fruit contained higher phenolic content and radical scavenging activity than their pulps. This suggests that dragon fruit peel extract (DPE) can be effectively used to synthesize nanoparticles since they are a good source of natural metabolites and antioxidants, which can serve as reducing and capping agents to produce nanomaterials.

The presence of a wide range of reducing phytochemicals in the peel extracts, such as phyllocactin, betanin, betacyanin, hylocerenin, terpenoids, flavonoids, polyphenols, sugars and alkaloids, etc., serve as an excellent source for the production of nanomaterials with various shapes, sizes, compositions, morphology, and crystallinity. Green synthesis of Ag NPs using various plants has been reported in the literature, but only limited studies targeted the biowaste of dragon fruit peel (Aminuzzaman *et al.*, 2019). This study uses an eco-friendly, cost-effective, green synthesis approach to prepare Ag NPs from dragon fruit peel. The synthesized Ag NPs were characterized using various analytical and microscopic tools. The synthesized nanoparticles were tested against *C. truncatum*, a fungus that causes anthracnose disease in chilli.

MATERIALS AND METHODS

Chemicals

Silver nitrate ($AgNO_3$, 99.9%) was purchased from Merck, India. Potato Dextrose Agar (PDA) and Sodium hydroxide were procured from HiMedia Laboratories, India. Whatman No.1 filter paper was purchased from Sigma-Aldrich, India. Deionised water was used throughout the experiment. Dragon fruits were collected from the ICAR-Indian Institute of Horticultural Research (IIHR) experimental farm in Bengaluru, India. All the chemicals were used as received without further purification.

Preparation of dragon fruit peel extract

Dragon fruits were collected from the experimental farm of the ICAR-IIHR, Bengaluru, India. The healthy dragon fruits were hand-picked at a ripe stage, washed once under running tap water, and thrice with deionised water, the peels were separated and oven dried at 60 ºC for two days to completely remove moisture. The dried peel was finely powdered, and the dragon fruit peel powder (1 g) was boiled with distilled water (25 ml) for an hour. The contents were centrifuged for 10 min at 5000 rpm, and the supernatant was collected and filtered using two layers of cheesecloth and Whatman paper (No.1). The extract was carefully collected and used for Ag NP synthesis.

Synthesis of nanoparticles

The Ag NPs were synthesised by mixing 1 ml of peel extract with 100 ml of $AgNO_3$ (1 mM) solution at 80 ±1 °C for 20 min with continuous stirring at 800 rpm in a magnetic stirrer. The pH was altered to 10 with NaOH (0.4 M). The synthesis of Ag NPs was monitored for reaction colour change to yellow. The reaction was continued for 30 min, and the contents were cooled and centrifuged for 30 min at 13000 rpm at 4 ºC. The precipitate was washed with Milli-Q water, re-centrifuged to eliminate any unbound polymers and organic matter and finally dried at 60 °C for 24 h to obtain the Ag NPs, which were used for further characterisation and antifungal studies.

Analytical measurements and characterization

The formation of Ag NPs was monitored by measuring the absorption peak of synthesised NPs in the range of 300-800 nm using a Thermo Scientific UV/VIS

Spectrometer (Genesys 10S UV-Vis). The size, shape and morphology of the nanoparticles were examined by Transmission electron microscopy (TEM) using Hitachi HT7700 (Tokyo, Japan) and field emission scanning electron microscopy (FESEM). For FESEM studies, a drop of the NPs (with an aqueous solution) was placed on an aluminium stub with double-sided copper or carbon tape. Then it was dried under ambient conditions for four hours. The samples were then desiccated for two days. In order to prevent the sample from being charged during the analysis, gold sputtering was done on the sample's surface. The samples were observed using a FESEM (Zeiss-Ultra 55 model; Carl Zeiss; Germany) equipped with an energy-dispersive X-ray (EDX) spectrometer. The XRD patterns of synthesised nanoparticles were recorded on a glass substrate using a Rigaku SmartLab X-ray Diffractometer operated at a voltage of 40 kV and a current of 30 mA with Cu Kα radiation (λ = 1.5404°A). The XRD pattern of nanoparticles was taken in the range of 5° to 90° in a fixed time mode at room temperature.

Spore germination inhibition assay

The spore germination inhibition assay was done to investigate the antifungal potential of the Ag NPs. Pathogenic *C. truncatum* isolates (NCBI accession number MW677960) from chilli were used for sporulation. *C. truncatum* was maintained on Potato Dextrose Agar (PDA) medium for seven days. The spores were collected by pouring sterile distilled water (5 ml) into the fully grown PDA plate and gently scraping the culture plate with a sterile loop to release the *C. truncatum* spores. The obtained spore suspension was sieved through two layers of sterile cheesecloth to remove any fragments of mycelia. Using a hemocytometer, the spore count of the resulting spore suspension was adjusted to 1.5 x 10^6 conidia/ml.

Aliquots of spore suspension (50 µl) were mixed with NPs (50 µl) of different concentrations (1, 0.5, 0.25, 0.125, 0.062, 0.031, 0.016, 0.008, 0.004, 0.002, 0.001, 0.0005, 0.00025 and 0.000125%) and added to the cavity slide well. The spores were also treated with different concentrations of 50 µl of extracts. Spores mixed with sterile distilled water were placed on the control slide. The slides were incubated for 24 h at 26±1 °C and 95% humidity. Spore germination was measured by counting the spores (germinated and non-germinated) in 10 randomly chosen fields using Zeiss bright field microscope (Axio Scope.A1, Gottingen, Germany) at 200X. When the germ tube length matched or exceeded the length of the conidia, the conidia were considered germinated. The percentage inhibition of spore germination was calculated using the formula, I = (C-T/C) x100, where I is the percentage inhibition of conidial germination in the test, C is the number of germinated conidia in control, and T is the number of conidia germinated in treated samples. The study was done with three replicates (Chowdappa *et al.*, 2014).

In-vitro* antifungal test of silver nanoparticles against *C. truncatum

The *in-vitro* evaluation of synthesised nanocomposites was done against *C. truncatum* on PDA through the spread plate method. Molten PDA medium was poured gently into a 90 mm Petri plate and left for solidification. 200 µl of *C. truncatum* spore suspension containing 2 X 10^6 conidia per ml was mixed with different concentrations of 200 µl of nanomaterials and dragon fruit peel extract (1, 0.5, 0.25, 0.125, 0.062, 0.031 and 0.015%) and incubated for an hour at 26 ºC. The spore suspension treated with sterile water was used as a control. The treated and control spores were then transferred to the Petri plate containing PDA and spread uniformly by gently rotating it with a sterile spreader and incubating at 26 ºC. The assay was carried out in triplicates, and colony growth was observed after 72 h.

RESULTS AND DISCUSSION

In this study, value-addition was done to dragon fruit (DF) peel biowaste by using it as a material for the bio-reduction of Ag NPs. Red dragon fruit peel containing white pulp was used to synthesise Ag NPs. A colour change to yellow confirmed the formation of Ag NPs. The synthesis of nanoparticles was observed by colour change (Kedi *et al.*, 2018). In this study, it was observed that when the $AgNO_3$ solution was mixed with peel extract, the colour of the solution turned brownish yellow, indicating the bio-reduction and formation of silver nanoparticles, as depicted in Fig. 1. There was no nanoparticle formation under ambient conditions and without adding NaOH. The Ag NPs formation was preliminarily observed by the reaction colour change to brownish yellow when the reaction pH was adjusted to 10 at a high temperature (80 ºC). The intensity of the change of reaction colour from light yellow to brownish yellow shows the increased production of Ag NPs by the metabolites of

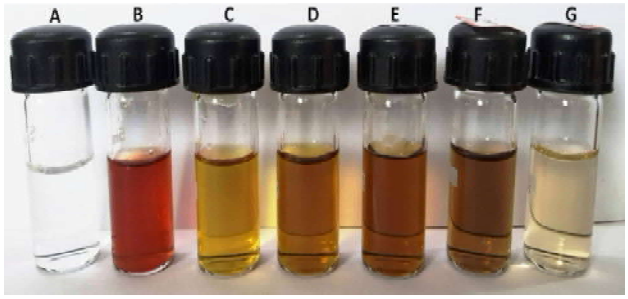

Fig. 1 : Formation of Ag NPs with dragon fruit peel
A) $AgNO_3$, B) Dragon fruit peel extract, C, D, E) Ag NPs synthesized with 0.25, 0.5 and 1 mM $AgNO_3$ at 80 ºC, F) Ag NPs synthesized at ambient temperature, G) Ag NPs synthesized at 80 ºC without NaOH

peel extract. Therefore, the formation of Ag NPs is greatly influenced by elevated temperature and basic pH.

The optical properties of Ag NPs were examined using UV-Vis spectroscopy from 300 to 800 nm. Ag NPs show strong SPR properties because of the collective oscillations of free electrons on the surface of metallic nanoparticles (Haes *et al.*, 2004). These oscillations change with particle size, showing the specific wavelength range in which particles absorb light in the visible spectrum. A larger particle causes a red-shift or a change in the absorption maximum towards higher wavelengths (Politano and Chiarello, 2009). Ag NPs show maximum absorbance at 420 nm (Chowdappa *et al.*, 2014). Fig. 2 depicts the absorption spectra of the synthesized silver nanoparticles. In this work, the maximum absorbance was observed at around 410 nm, which shows the blue

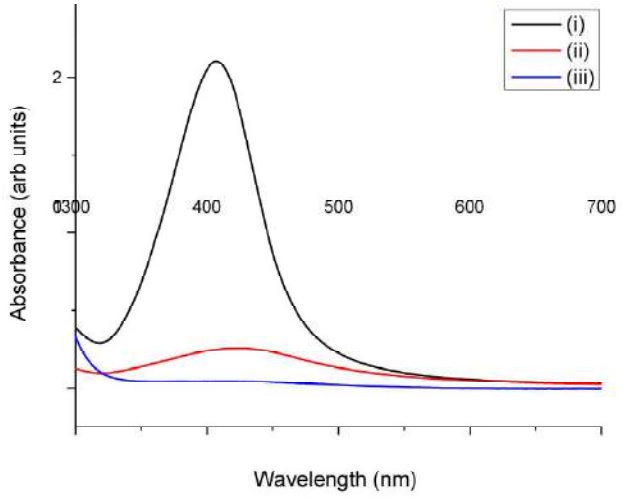

Fig. 2 : UV-VIS Spec observation of DPE-Ag NPs
(i) DPE-Ag NPs synthesised at 80 °C and pH 10, with 1 mM $AgNO_3$, (ii). Ag NPs synthesised at ambient conditions with NaOH, (iii) Ag NPs synthesised at 80 °C without NaOH.

shift and the formation of small-sized Ag NPs. The reaction pH significantly influences nanoparticle size, morphology and formation (Iravani and Zolfaghari, 2013). The synthesis was done under an alkaline condition (pH 10). Previous studies have reported that by changing the reaction mixture's pH, nanoparticle morphology and size could be manipulated (Velgosová *et al.*, 2016). pH alters the electrical charges of biomolecules, thereby influencing the growth of the nanoparticles by affecting their stabilising ability and the amount of nanoparticle synthesis (Mulvaney, 1996).

The morphology and size of the synthesised Ag NPs were examined using TEM and FESEM. The average size of synthesised Ag NPs was 7 nm (Fig. 3). The SEM image of DF peel extract showed a bulky structure, whereas the SEM image of DF peel extract treated with $AgNO_3$ showed spherical Ag nanoparticles formation (Fig. 4). The elemental analysis of Dragon fruit peel extract showed the presence of elements in

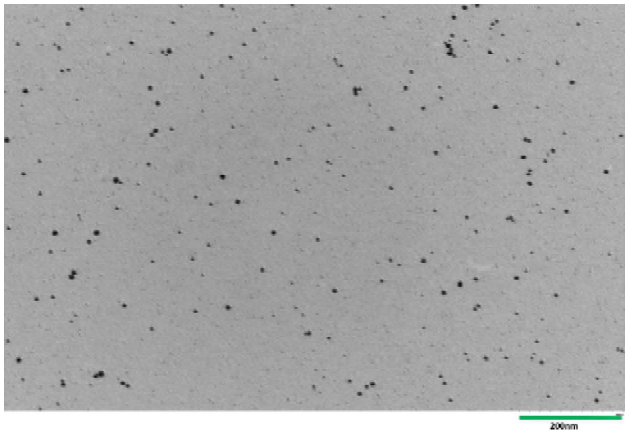

Fig. 3 : TEM of Ag NPs synthesized with dragon fruit peel extract

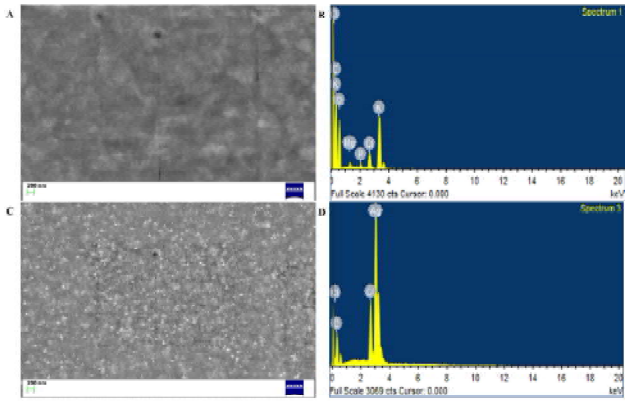

Fig. 4 : SEM micrograph of nano particles
a) Dragon fruit peel extract aggregates, b) EDX of DF peel extract, c) DPE-Ag NPs (1 mM $AgNO_3$), and d) EDX spectra of DPE-Ag NPs.

the peel extract. In contrast, the EDX spectra of Ag nanoparticles synthesised using Dragon fruit peel extract showed a prominent silver and chlorine peak, confirming the formation of silver nanoparticles (Fig. 4). No other peaks have been detected, showing the presence of pure silver in the nano formulation. EDX study shows the presence of chloride ions in the Dragon fruit peel extract, which confirmed the formation of Ag and AgCl nanoparticles. The possible reason for forming AgCl NPs was due to the interaction of the chloride ion in the peel extract and the silver ion from the metal precursor. The EDX of the peel extract confirmed the presence of chlorine. Similar findings were reported by Devi *et al.* (2016). The organic constituents present in the fruit peel extract stabilised AgNP formation.

XRD analysis was done to determine the crystal structure of the Ag NPs. The XRD pattern of dragon fruit peel extract and synthesised nanoparticles were depicted in Fig. 5. The diffraction pattern displayed well-resolved diffraction peaks illustrating crystalline peaks at 2θ values of 38.1°, 44.3°, 64.4°, 77.4°, and 81.5°, which corresponds to the standard face-centred cubic (fcc) crystal lattice planes of Ag (111), (200), (220), (311), and (222) respectively (JCPDS file: 04-0783). Apart from the distinct peaks, the presence of the fcc phase of silver chloride was also observed at 2θ values of 27.8°, 32.2°, 46.2°, 54.8°, 57.4°, 67.4°, 74.4°, 76.7°, and 85.7° that can be assigned respectively to the (fcc) structure planes of AgCl (111), (200), (220), (311), (222), (400), (331), (420) and (422) (JCPDS file: 31-1238). The absence of any other peaks shows the purity of synthesised Ag and AgCl NPs. XRD study of the synthesised Ag NPs showed the formation of sharp and intense diffraction peaks, confirming the crystalline nature of NPs (Awwad *et al.*, 2015). Pattern recognition revealed the production of pure Ag and AgCl crystals. Similar findings were reported in the literature with different plant extracts (Kedi *et al.*, 2018; Siddiqui *et al.*, 2013). The AgCl NPs might have been formed due to the crystallisation of the bioactive components from the DF peel extract (Philip *et al.*, 2011). Ag is the primary material in the composite, as the Ag NP peaks are much stronger than those of the AgCl NPs.

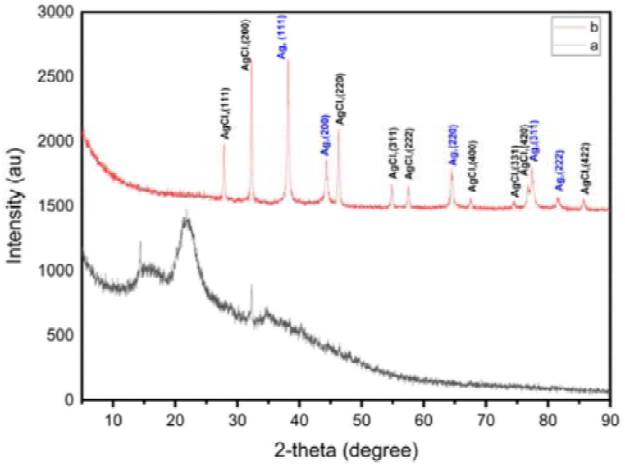

Fig. 5 : XRD pattern of nano formalations
a) Dragon fruit peel extract and b) DPE-Ag NPs

Evaluating the antifungal efficacy of Ag NPs on fungal spore germination is essential because it serves as a primary tool for the preliminary screening of nanomaterials against fungal pathogens (Lopez-Meneses *et al.*, 2018). The antifungal activity of Dragon fruit Peel extract and DPE-Ag NPs (1mm $AgNO_3$) were evaluated by studying its impact on the conidial germination of *C. truncatum* at different concentrations. The Ag NPs effectively reduced the conidial germination of *C. truncatum* more than its counterparts, as shown in Fig. 6. Ag nanoparticles at 1%, 0.5%, 0.25%, 0.125%, 0.062%, 0.031%, 0.015%, 0.008%, 0.004%, 0.002%, and 0.001% concentrations showed 100% inhibition of conidial germination whereas growth was observed below 0.001% concentrations. In addition, conidial germinations were observed in all the concentrations of dragon fruit peel extract (Fig. 7). Normal conidial germination was observed in water. Increased conidia

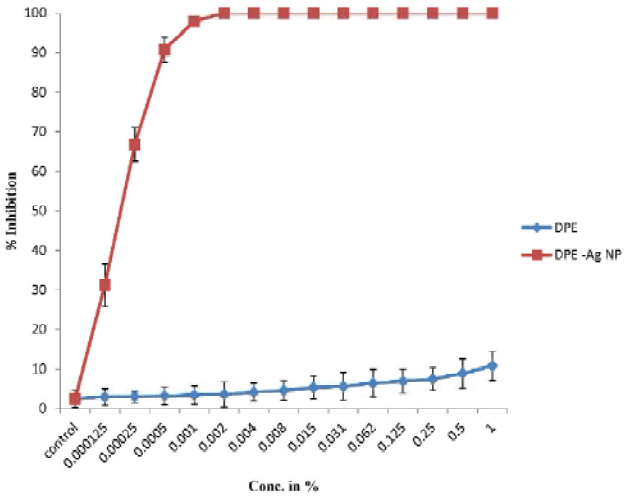

Fig. 6 : Effect of dragon fruit peel extract and DPE-Ag NPs on conidial germination of *C. truncatum* after 24 h of incubation

growth inhibition was observed with the increasing concentration of Ag nanoparticles. Similar inhibitory potential of Ag NPs against *Colletotrichum* was reported by other groups (Chowdappa *et al.*, 2014; Lamsal *et al.*, 2011).

Treating *C. truncatum* conidia with nanoparticles showed structural changes and complete inhibition of conidial germination. Interestingly, nanoparticle treatment also prevented appressoria formation in *C. truncatum,* even at low concentrations. It is apparent from Fig. 7 that normal conidial germination and also appressorial formation is found in conidial spores treated with DF peel extract and control. Melanin production is an important factor in the development of the appressoria structure. The inhibition of appressoria structure in the Ag NP treatment might be due to the reduction of melanin content in *Colletotrichum* by nanoparticles (Wei *et al.*, 2017). Lin *et al.* (2020) reported the inhibition of Anthracnose disease due to reduced melanin synthesis-related genes by Ag nanoparticles. Our previous report also suggested the efficacy of nanoparticles in the management of *Colletotrichum* sp. (Gowda and Sriram, 2020). The inhibition of conidial germination by Ag nanoparticles might be due to damage to cell structure permeability and leaking of the cellular content. Several studies have reported the enhanced efficiency of Ag NPs against *Colletotrichum* species

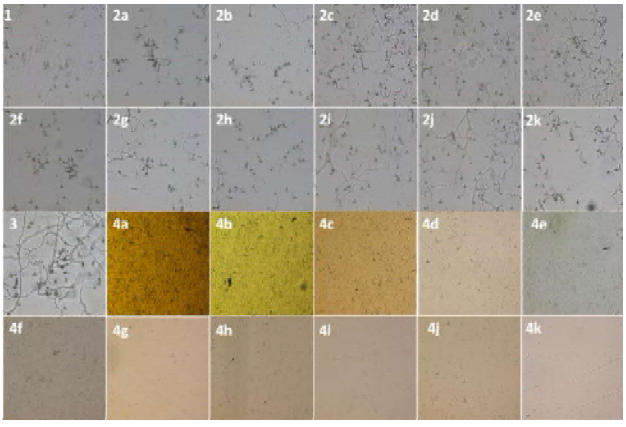

Fig. 7 : Effect of dragon fruit peel extract and DPE-Ag NPs on conidial germination of *C. truncatum*.

1) Spores, 2a-2k) Conidia treated with DF Peel extract 1%, 0.5%, 0.25%, 0.125%, 0.062%, 0.031%, 0.015%, 0.008%, 0.004%, 0.002% and 0.001% concentrations respectively. 3) Conidia treated Water (control), and 4a-k) Conidia treated with DPE-Ag NPs 1%, 0.5%, 0.25%, 0.125%, 0.062%, 0.031%, 0.015%, 0.008%, 0.004%, 0.002% and 0.001% concentrations respectively. Images were recorded at 200 × magnification.

(Aguilar-Mendez *et al.*, 2011; Lamsal *et al.*, 2011). Thus, Ag NPs synthesized by a green, cost-effective approach have a great potential to use as an excellent antifungal agent in controlling spore-forming fungal pathogens.

When evaluating the antifungal efficacy of the synthesised Ag NPs on the colony growth of the *C. truncatum*, it was found that the Dragon fruit peel extract alone did not show an inhibitory effect on *C. truncatum* in a spread plate assay. However, the green synthesised Ag NPs exhibited a potential inhibitory effect on forming fungal colonies compared to the control and DF peel extract. It was evident from Fig. 8 that no colony growth was observed in the PDA

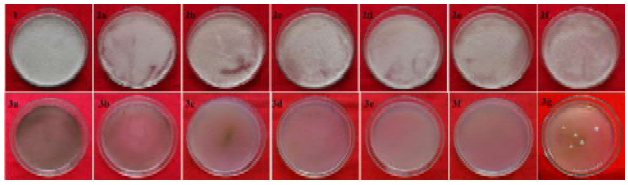

Fig. 8 : Spread plate assay of conidia growth of *C. truncatum* 1. Water, 2a-f-. Dragon fruit peel extract 1, 0.5, 0.25, 0.125, 0.062, and 0.031, respectively. 3a-g. DPE-Ag NP 1, 0.5, 0.25, 0.125, 0.062, 0.031, 0.015%, respectively.

plates treated with nanoparticles from 1 to 0.031%, whereas growth was seen at 0.015% concentration. In contrast, colony formation was observed in control and dragon fruit peel extract of all the tested concentrations. The fungistatic effect was increased with increasing concentrations of Ag NPs. Due to their small size, high surface area and stability, the synthesised Ag NPs can effectively kill the fungal spores, thereby giving potential antifungal activity. It was also shown that lower concentrations of Ag NPs would be sufficient to kill microbes as they efficiently penetrate microbial cells (Samuel and Guggenbichler, 2004). Ag NPs interact with molecules to disrupt the transport systems and stop cellular functions, including metabolism. Silver ions react with oxygen and form reactive oxygen species, which destroy lipids, nucleic acids and proteins, inhibit ATP production and kill the pathogen (Hwang *et al.*, 2008; Morones *et al.*, 2005). The application of Ag NPs to manage plant fungal pathogens has led to the development of a new class of potential silver-based antifungal agents. With an increase in the application of Ag NPs as a potential antifungal agent, studies need to be done to know their mechanism of action and potential toxicity to animals (Lamsal *et al.*, 2011).

CONCLUSION

The application of Nanotechnology to plant disease management has revolutionised the field of agriculture because of its unique properties. Green and eco-friendly synthesis of Ag NPs offers significant advantages over conventional disease management strategies by being cost-effective and highly efficient at low doses. Silver and silver chloride nanoparticles synthesised in this study exhibited significant inhibition against *C. truncatum* spores even at low doses; hence could be considered as a potential antifungal agent against *Colletotrichum* infections in chilli.

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Original Research Paper

Screening for resistance to gummy stem blight, powdery mildew and cucumber green mottle mosaic virus in bottle gourd [*Lagenaria siceraria* (Mol.) Standl.]

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ABSTRACT

Investigations were carried out to identify the source of resistance in 67 bottle gourd genotypes for gummy stem blight, powdery mildew and cucumber green mottle mosaic virus (CGMMV) diseases, under natural field epiphytotic conditions. The genotypes BG-95 (105.13), BG-114-1 (131.04), BG-114-3 (208.81) and BG-77-6-1 (221.80) were resistant for gummy stem blight with low AUDPC values, while, BG-125-5 (232.22), BG-6-3 found (250.00), BG-125-4 (307.78), BG-8-1 (308.89), BG-125-2 (311.11) and BG-124-2 (423.33) resistant with low AUDPC values for powdery mildew. Further, the two genotypes such as IIHR-19 and BG-131 showed field level resistance against CGMMV. The selected genotypes based on field evaluation were subjected for artificial screening under glass house conditions. The genotypes, recorded consistent resistant reactions were BG-114-3, BG-77-6-1 and BG-95 for gummy stem blight disease and BG-6-3, BG-8-1, BG-125-4 and BG-125-2 for powdery mildew. The stable and durable source of resistance identified for gummy stem blight and powdery mildew in bottle gourd genotypes will hasten the process of developing resistance varieties in bottle gourd.

Keywords : Bottle gourd, CGMMV, gummy stem blight, powdery mildew

INTRODUCTION

Bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] is widely cultivated cucurbitaceous vegetable throughout India and also in various parts of Asia. Its economical part is fruit which is harvested in young stage and it is available in the market throughout the year. Fruits are rich source of minerals and vitamins and also have medicinal value (Thakur *et al.,* 2015). In India, production of bottle gourd during 2021-22 was 3742.71 (000 MT) with an area of 1.58 mha. Major bottle gourd producing states in India are Bihar, Uttar Pradesh, Madhya Pradesh, Haryana and Chhattisgarh (Anona, 2022). Powdery mildew, gummy stem blight, anthracnose and cucumber green mottle mosaic virus (CGMV) are important diseases limiting bottle gourd production in India (Vasudeva and Nariani, 1952; Ullasa and Amin, 1986; Nayak *et al.,* 2017; Kousik *et al.,* 2018; Dombrovsky *et al.,* 2017).

Gummy stem blight is a devastating disease on cucurbits worldwide. In India, this disease is reported on muskmelon (Sudisha *et al.,* 2004), watermelon (Sohi and Om-Prakash, 1972), pickling cucumbers (Garampalli *et al.,* 2016) and ridge gourd (Bhat *et al.,* 2010). Cucurbit gummy stem blight is reported to be caused by *Didymella bryoniae* (*Stagonosporopsis cucurbitacearum*), *Stagonosporopsis caricae* and *Stagonosporopsis citrulli* (Stewart *et al.,* 2015). *Stagonosporopsis caricae* and *S. citrulli* were associated with gummy stem blight epidemics in gherkin cucumber (*Cucumis sativus*) in Karnataka and *Didymella bryoniae* on *Sechium edule* and *Citrullus lanatus* (Sohi and Om-Prakash, 1972). Two pathogenic fungi *Golovinomyces cichoracearum (Erysiphe cichoracearum*) and *Podosphaera xanthii (Sphaerotheca fuliginea)* are reported to cause powdery mildew disease (Cohen *et al.,* 2004). In India, *Podosphaera xanthii* is reported to cause powdery mildew disease on bottle gourd (Nayak *et al.,* 2017).

Few attempts were made by Indian researcher to identify resistance sources against gummy stem blight and powdery mildew, however, no stable resistance sources could be identified (Maheshwari *et al.,* 2012; Bhardwaj *et al.,* 2018). Hitherto, no attempts have been made for identification of resistance against CGMV in bottle gourd in India. In this context, there is a need to identify stable sources for resistance against these diseases for the development of bottle

gourd varieties/hybrids with good horticultural and resistance traits.

With this background, field experiments were conducted with an objective to identify gummy stem blight, powdery mildew and CGMMV resistance sources in bottle gourd breeding material.

MATERIALS AND METHODS

The experiments were conducted at ICAR-Indian Institute of Horticultural Research, Bengaluru, India during of 2017-2018 and 2018-19. The experimental site was located at an altitude of 890 m above mean sea level with coordinates of 13° 7'N, 77° 29'E. The site receives an average annual rainfall of 757 mm. In both the years the plots were located at same sites to ensure the build of soil inoculums. Based on PDI calculated, genotypes were categorized as: 0 = immune; 1-10% = resistant; 11-25% = moderately resistant; 26-50% = moderately susceptible; 51-75%=susceptible; and >75%= highly susceptible as described by Bhardwaj *et al.* (2018).

Plant material

Bottle gourd genotypes comprising of 67 inbred lines were evaluated during *kharif* season for gummy stem blight, *rabi* season for powdery mildew and CGMMV during summer of 2017-18 and 2018-19. The field screening experiment was laid out in randomized block design with two replications. Eighteen plants of each entry under study were transplanted on raised beds with polythene mulching at a spacing of 1.60 m × 0.9 m. Standard package of practice was followed to raise the crop under open field condition.

Disease assessment and identification

Observations on disease incidence and severity were recorded at weekly interval from the date of first initiation of diseases. Diseases were identified based on microscopic examination of symptomatic leaf and plant samples (n=50) at periodic intervals and morphological characterization.

Powdery mildew (PM) severity was assessed based on assessment key of 0-10 scale, where 0=no visible symptoms, 1=very sparse mycelia growth on leaves with very few to no visible conidia (0 to 3%), 2=4 to 6% of leaf area covered with PM and sparse development of conidia, 3=7 to 12% leaf area covered with abundant conidia, 4=13 to 25%, 5 = 26 to 50%, 6= 51 to 75%, 7=76 to 87% leaf area covered with abundant conidia, 8= 88 to 94%, 9= 95 to 97% and 10= 98 to 100% leaf area covered with abundant conidia and leaf dying or dead (Kousik *et al.,* 2008 & 2018). For artificial inoculation, spores from powdery mildew infected bottle gourd leaves were dusted on plants at two leaves stage. Dusting was made twice at one day interval. Disease rating was done on 0-10 scale (as described above) starting from one week after inoculation. Total of nine observations were made at 3 days interval.

Gummy stem blight scoring was done on 5 leaves and stem each in 5 plants using 0-9 rating scale as per Gusmini *et al*. (2005), where, 0=no symptoms, 1=yellowing on leaves (suspect of disease only), 2=moderate symptoms (<20% necrosis) on leaves only, 3=slight symptoms (21–45% necrosis) on leaves only, 4=severe symptoms (>45% necrosis) on leaves only, 5=some leaves dead, no symptoms on stem, 6=moderate symptoms (<20% necrosis) on leaves, with necrosis also on petioles and stem (<3 mm long), 7=slight symptoms (21–45% necrosis) on leaves, necrosis also on petioles and stem (3-5 mm long), 8=severe symptoms (45% necrosis) on leaves, necrosis also on petioles and stem (>5 mm long) and 9=plant dead.

In vitro screening for resistance against gummy stem blight

A highly virulent culture of *Staganosporopsis cucurbitacearum* from previous study of Mahapatra *et al*. (2020) was used. This culture was initially isolated from watermelon and had high virulence and aggressiveness on bottle gourd. The isolate was maintained by periodic culture on quarter strength potato dextrose agar media.

Virulence was maintained by periodic inoculation and reisolatiion from bottle gourd plants. Since, the isolate was poorly sporulating on different media assayed, mycelial plugs were used as inoculum in this evaluation. Briefly, 8 mm plugs were taken from margin of five days old actively growing culture on potato dextrose agar with a cork borer. Mycelium plugs were inoculated on to stem of five weeks old seedlings with 2 to 4 true leaves. Mycelial plugs were fastened with cello tape. Inoculated plants were kept in plant growth chamber with 12 hour light cycle set at temperature 28°C and relative humidity above 85%. Starting from five days after inoculation, up to 15 days, disease severity scoring was made by adopting

0-5 scale modified from Zhang *et al.* (1997), where 1=no damage, 2=expanding lesion on stem without girdling, 3=lesions with extensive girdling of stem, 4=withered stem, 5=dead seedling with stem shredding Area under disease progress curve was worked out as per equation (Wilcoxson *et al.,* 1975)

Per cent disease index (PDI) was calculated based on field scoring data by using the formula.

$$PDI = \frac{Sum\ of\ all\ disease\ rating}{Total\ no.of\ observations\ X\ Maximum\ disease\ grade}\ X\ 100$$

$$A = \sum_{i-1}^{K} \frac{1}{2(Si + Si - 1)} \times d$$

where, Si=disease severity at the end of week i, k = the number of successive evaluation of disease and d= interval between two evaluations.

In the present experiment, the 67 genotypes of bottle gourd were screened against CGMMV disease resistance under natural field conditions and with artificial screening under glasshouse conditions. Mechanical sap inoculation was carried out with Cucumber green mottle mosaic virus (G: Tobamovirus, F: Virgaviridae) infected leaf samples collected from ICAR-IIHR experimental plots. Viral sap was extracted with 0.05 M potassium phosphate buffer by grinding CGMMV infected leaves with an antioxidant 5 µl (β-mercapto ethanol). Viral sap was inoculated on 10-12 days old test seedlings of bottle gourd genotypes by smearing on the emerging leaves.

After 10 minutes, it was washed with distilled water. Inoculated plants were kept for observations under glass house conditions with insect proof net (20 mesh size) to prevent damage from possible glass house insects to the seedlings.

After 12-15 days of inoculation, symptomatic leaves were observed under Transmission Electron Microscopy (TEM) and captured the rigid rod virus particles indicating the presence of *Tobamovirus* particles. Further, symptomatic leaves were tested and confirmed with CGMMV specific immuno strips (Agdia^{R}). Test seedlings were assessed based on 0-5 scale (0: no symptoms, 1: initiation of greenish lesions and vein greening, 2: enlarged lesions and thickened vein greening, 3: blisters and mottling, 4: leaf distortion and 5: veinal distortion and loss of chlorophyll). Same assessment scale was used for field assessment of CGMMV severity.

RESULTS AND DISCUSSION

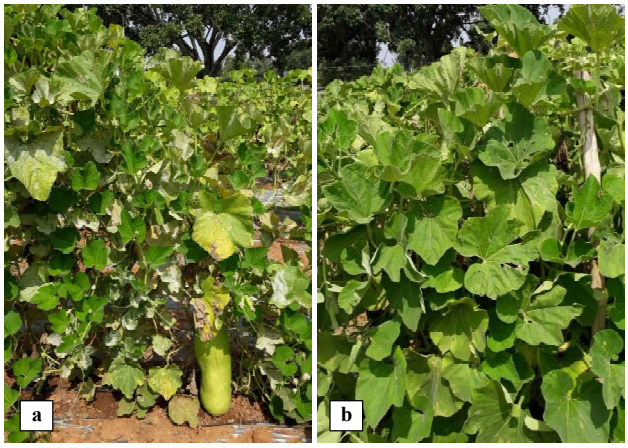
A total of 67 bottle gourd genotypes were subjected to field evaluation to identify source of resistance against powdery mildew, gummy stem blight and *CGMMV*. Among them, the genotypes; the genotypes BG-95 (105.13), BG-114-1 (131.04) BG-114-3 (208.81), and BG-77-6-1 (221.80) were found promising for gummy stem blight resistance with low AUDPC values and PDI (Table 1 and Fig. 1), whereas, BG-125-5 (232.22), BG-6-3 (250.00), BG-125-4 (307.78), BG-8-1 (308.89), BG-125-2 (311.11), and BG-124-2 (423.33) were found promising for powdery mildew with low disease index and AUDPC values (Table 2 and Fig. 2). The genotypes IIHR19 and BG131 were recorded field resistant to CGMMV (Table 3).

Table 1 : Bottle gourd genotypes field reaction against gummy stem blight

PDI (%)	Reaction	Genotype
0	Immune	-
1-10	Resistant	BG-95, BG-114-3 and BG-114-1
11-25	Moderately resistant	BG-77-6-1(221.80), BGAIC-6, BG-91, BG-108-2, BG-124-4, BG-124-2
26-50	Moderately susceptible	BG-23-5-6, BG-23-5-10, BG-136, BG-78, BG-135, BG-98, BG-98-3, BG-143, BG-67, BG-123-3-1, BG-62-1-1, BG-131, BG-114-46, BG-75-2, BG-108-1
51-75	Susceptible	BG-99, BG-47, BG-79, BG-18, BG-24, BG-108-2-3, BG-8-1, BG-139, BG-6, BG-125-5, BG-6-3, BG-141, BG-138, BG-118-3, BG-115-2, BGAIC-11, BG-125-4, BG-120-5, BG-125-2, BG-81, BG-104, BG-4, IIHR-22349, BG-61-10, BG-105, BG-23, BG-115-5b, BG-118-3-3, BG-62-1, BG-49, BG-49-5, BG-119-2, BG-122-5, BG-123-3, BG-44, BG-11, BG-39-112-1, BG-93, P.Lauki-8
>75	Highly susceptible	BG-64, BG-75, BG-108, IIHR-19

a) BG-64 (susceptible) b) BG-77-6-1(resistant)

Fig. 1 : Gummy stem blight disease reaction on bottle gourd genotypes



a) BG-11(susceptible) b) BG-124-2 (resistant)

Fig. 2 : Powdery mildew disease reaction on bottle gourd genotypes

Table 2 : Bottle gourd genotypes field reaction against powdery mildew disease

PDI (%)	Reaction	Genotype
0	Immune	-
1-10	Resistant	BG-6-3, BG-8-1, BG-125-4, BG-125-2, BG-124-2, BG-125-5
11-25	Moderately resistant	BG-64, BG-118-3, BG-114-3, BG-114-1, BG-115-2, BGAIC-11, BGAIC-6, BG-120-5, BG-81, BG-104, BG-4, IIHR-22349, BG-61-10, P.Lauki-8
26-50	Moderately susceptible	BG-105, BG-18, BG-23, BG-98, BG-6, BG-91, BG-115-5b, BG-118-3-3, BG-62-1, BG-49, BG-49-5, IIHR-19, BG-119-2, BG-122-5, BG-123-3, BG-44, BG-24,
51-75	Susceptible	BG-11, BG-39-112-1, BG-78, BG-67, BG-93, BG-95, BG-77-6-1, BG-108-2, BG-124-4, BG-23-5-6, BG-23-5-10, BG-136, BG-135, BG-98-3, BG-143, BG-123-3-1, BG-62-1-1, BG-131, BG-114-46, BG-75-2, BG-108-1, BG-99, BG-47, BG-79, BG-108-2-3, BG-139, BG-141, BG-138, BG-75, BG-108
>75	Highly susceptible	-

Table 3 : Reaction of bottle gourd genotypes against CGMMV under natural field conditions

Score	Reaction	Genotype
0	Immune	-
1	Resistant	IIHR-19, BG-131
2	Moderately resistant	BG-95, BG-62-1-1, BG-75-2, BG-124-4, BG-108-1, BG-67
3	Moderately	BG-108-2-3, BG-108-2, BG-98, BG-114 -46, BG-8-1, BG-114-1
4	Susceptible	BG-108, BG-78, BG-114-3, BG-24, BG-136, BG-143BG-118-3, BG-115-2, BGAIC-11, BG-125-4, BG-120-5, BG-125-2, BG-81, BG-104, BG-4, IIHR-22349, BG-61-10, BG-105, BG-23, BG-115-5b, BG-118-3-3, BG-62-1, BG-49, BG-49-5, BG-119-2, BG-122-5, BG-123-3, BG-44, BG-11, BG-39-112-1, BG-93, P. Lauki-8BG-64, BG-75, BG-77-6-1, BG-124-2, BG-18, BG-99, BG-139, BG-123-3-1, BG-141, BG-23-5-6, BG-47, BG-79
5	Highly susceptible	BG-138, BG-6, BG-125-5, BG-91, BG-98-3, BG-6-3, BGAIC-6, BG-135, BG-23-5-10

Table 4 : *In vivo* evaluation of promising genotypes with artificial inoculation against powdery mildew

Genotype	PDI**	AUDPC*	Class
BG-6-3	11.11	266.67	R
BG-8-1	13.58	300.00	R
BG-125-4	14.81	343.27	R
BG-125-2	17.77	378.83	R
BG-124-2	37.77	811.49	MS
BG-125-5	33.33	717.64	MS
BG-11 (susceptible check)	68.56	1222.83	S

*AUDPC calculated based on nine observations at 3 days interval
**PDI is average of two replications (n=20)
R= resistant, MS= moderately susceptible, S= susceptible

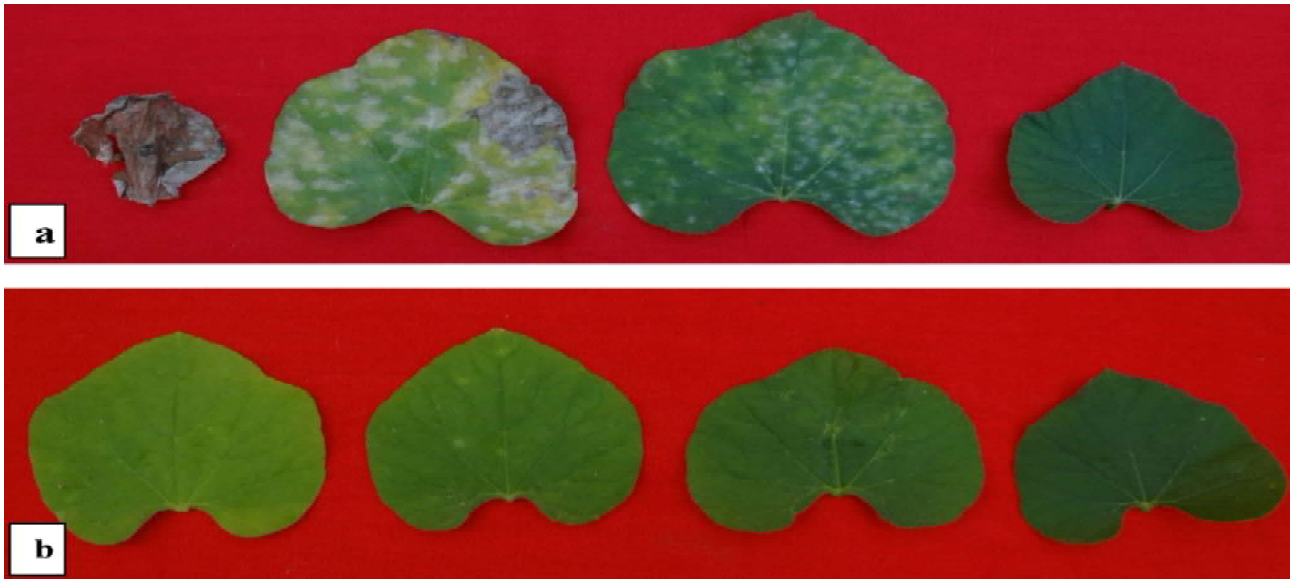


Fig. 4 : Powdery mildew disease reaction on bottle gourd genotypes *in vivo* screening with artificial inoculation, four leaves from left to right shows disease progress starting from basal leaf to young leaf a) BG-11 (susceptible), b) BG-6-3 (resistant)

Table 5 : Resistance reaction of bottle gourd genotypes against gummy stem blight under artificial screening

Genotype	Artificial inoculation	
	Per cent disease index	Resistant class
BG-114-3	9.45	R
BG-114-1	9.25	R
BG-77-6-1	10.00	R
BG-95	8.89	R
BG-98	41.67	MS
BGAIC-6	15.56	MR
Warad	54.45	S
BG-24	67.22	S
BG-18	65.56	S
Arka Bahar	48.34	MS
Pusa Naveen	71.67	S

Average of two replications, n=20, PDI based on modified 0-9 scale (Gusmini ýet *al.,* 2005); 0 = immune; 1-10% = resistant; 11-25% = moderately resistant; 26-50% = moderately susceptible; 51-75%=susceptible; and >75%= highly susceptible (Bhardwaj *et al.,* 2018)

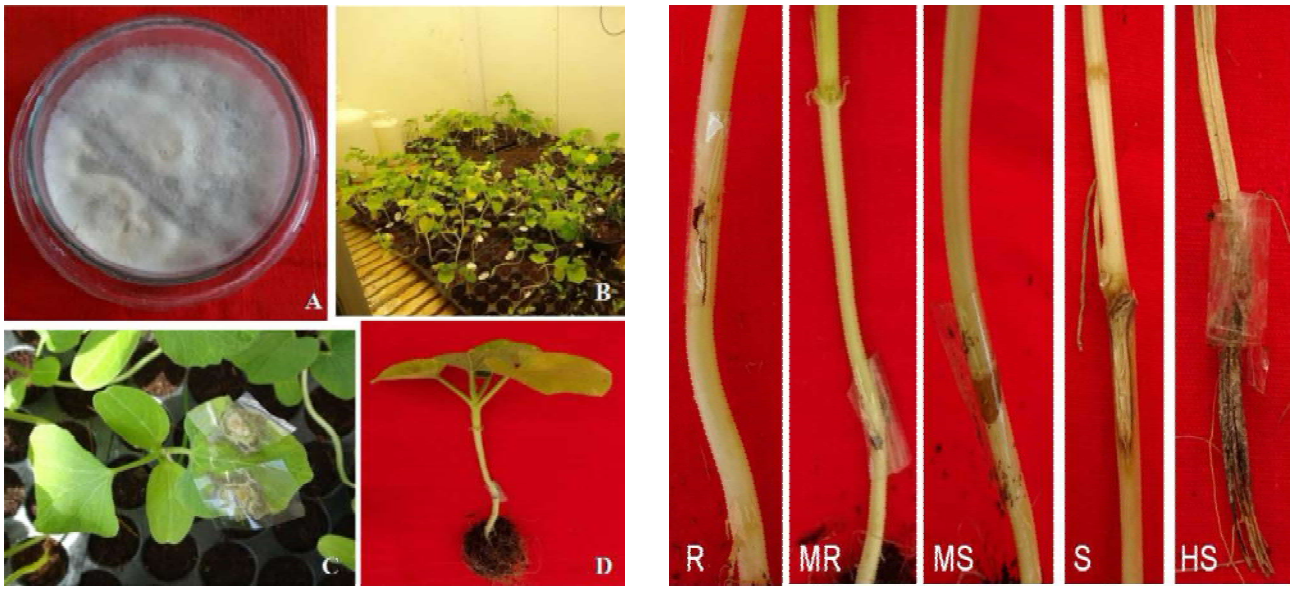


Fig. 5 : Screening method (A-D) and different disease response categories observed

Further, promising genotypes from field evaluation were subjected to *in vivo* screening for powdery mildew, gummy stem blight (3 resistant and 6 moderately resistant) and CGMMV with artificial challenge under glass house conditions. Under *in vivo* evaluation; out of six genotypes only BG-6-3 (266.67), BG-8-1 (300.0), BG-125-4 (343.27) and BG-125-2 (378.83) were found resistant for powdery mildew (Table 4 and Fig. 4). The genotypes BG-114-3, BG-77-6-1 and BG-95 were found resistant for gummy stem blight disease (Table 5 and Fig. 5), whereas, promising genotypes for CGMMV from field evaluation showed susceptible reaction under artificial screening.

CONCLUSION

The bottle gourd resistant genotypes identified could be utilized as potential source for incorporation of resistance in to bottle gourd breeding lines. Further, they can also be utilized as rootstocks for grafting in other cucurbits.

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Original Research Paper

Seasonal incidence, population dynamics and morphometric traits of exotic coconut whiteflies in southern Tamil Nadu

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ABSTRACT

Survey was conducted at fortnightly intervals to assess the intensity of damage caused by the invasive whiteflies in coconut in the southern districts of Tamil Nadu *viz.*, Thoothukudi, Tirunelveli, Tenkasi and Kanyakumari from December 2020 to August 2021. Among the four districts, Kanyakumari recorded the highest whitefly incidence (56.30%), whereas, Tenkasi showed the lowest infestation (48.83%). Two whitefly species *viz.*, rugose spiralling whitefly, *Aleurodicus rugioperculatus* Martin and bondars nesting whitefly (BNW), *Paraleyrodes bondari* Peracchi were observed in all the surveyed districts. The rugose spiralling whitefly nymphs and adult populations were found to be highest in Kanyakumari (49.46 nymphs/leaflet; 36.99 adults/leaflet) and lowest in Tenkasi (32.76 nymphs/leaflet; 26.71 adults/leaflet). Similarly, the population of bondars nesting whitefly nymphs and adults were highest in Kanyakumari (35.31 nymphs/leaflet; 34.84 adults/leaflet), whereas, the lowest nymphal population was observed in Tenkasi (22.79 nymphs/leaflet) and adult population in Thoothukudi (24.19 adults/leaflet). In morphometric analysis, length and breadth of egg (0.24 ± 0.03 mm and 0.13± 0.02 mm), nymphal (0.83 ± 0.08 mm and 0.38 ± 0.04 mm), pupal (1.08 ± 0.09 mm and 0.70 ± 0.09 mm), adult (female: 2.59 ± 0.09mm, 1.71 ± 0.14 mm; male: 2.27 ± 0.21 mm, 1.30 ± 0.05 mm) was recorded for *A. rugioperculatus* and egg (0.15 ± 0.02 mm and 0.08 ± 0.01 mm), nymphal (0.46 ± 0.02 mm and 0.36 ± 0.02 mm), pupal (0.59 ± 0.16 mm and 0.41 ± 0.09 mm), adult (1.09 ± 0.08 mm and 0.73 ± 0.07 mm) for *P. bondari*.

Keywords : Coconut, intensity of damage, morphometry, whiteflies

INTRODUCTION

Coconut (*Cocos nucifera*), palm of family Arecaceae is an important plantation crop grown in India and the southern states *viz.*, Kerala, Tamil Nadu, Karnataka and Andhra Pradesh constitute major area and production of coconut. In India, coconut is grown in an area of 2,150.89'000 ha with an annual production of 21,288.24 million nuts and productivity of 9897 nuts/ha (CDB, 2018-2019). In Tamil Nadu, coconut is cultivated in 4,38,935.20 ha with 49,474 lakh nuts and 11,271 nuts/ha production and productivity, respectively (CDB, 2019-2020). Most of the human population in India depends on coconut directly or indirectly for their livelihood. Coconuts possess high nutritive value including minerals, vitamin B, copper, iron along with proteins and antioxidants. They have several health benefits and it is a multipurpose tree, as the whole parts of coconut are used in one or the other way.

The coconut tree is infested by several insect pests throughout the year (Thampan, 1975). Recently, whiteflies pose serious threat to the coconut growers in the country. Rugose spiralling whitefly (RSW), *Aleurodicus rugioperculatus* Martin (Hemiptera: Aleyrodidae) originally known as gumbo limbo spiralling whitefly was reported first from coconut during 2004 in Belize, Central America (Martin, 2004), in South Florida, United States in 2009 (Stocks *et al.*, 2012), in Changanassery, Kottayam, Kerala during 2016 (Shanas *et al.*, 2016), Mangalore and Udupi of Karnataka in 2016 (Selvaraj *et al.*, 2017) and in Pollachi tract, Coimbatore district, Tamil Nadu, in August 2016 (Srinivasan *et al.*, 2016). A total of 118 hosts have been documented to be attacked by the RSW, including crops and weeds (Stocks *et al.*, 2012). They deposit creamy golden eggs in a spiral pattern on the underside of the leaves. When the nymphs hatch, they begin sucking the plant sap from the underside of the leaves, releasing honeydew that falls

on the upper surface of the fronds below them (Josephrajkumar *et al.*, 2016). The fungus *Capnodium* grows on the honeydew, giving it a charcoal black appearance that may be visible from distance (Chandrika *et al.*, 2016) that affects photosynthesis and in turn reduction in the quality of nuts.

Later in 2018, bondars nesting whitefly (BNW), *Paraleyrodes bondari* Peracchi was first identified in Kayamkulam, Kerala. It feeds on more than 25 host plants. (Chandrika *et al.*, 2018) which is also creating menace in the coconut gardens of Tamil Nadu recently. The nymphs and adults of *P. bondari* construct nesting chambers of woolly wax and the adults will be remaining on the nests for egg laying. The woolly wax nests will be seen on the under surface of the leaflets. Another invasive nesting whitefly, *Paraleyrodes minei* laccarino was observed in coconut gardens in larger areas along the Western Ghat coastal regions of Kerala and Karnataka since November 2018 (Sujithra *et al.*, 2019). Palm infesting whitefly, *Aleurotrachelus atratus* Hempel was first reported on ornamental areca palm in 2019 at Mysore and Mandya districts of Karnataka (Selvaraj *et al.*, 2019). At present, the whitefly complex in coconut pose serious threat to the growers as the under surface of leaves were totally covered with whiteflies and the sooty mould infestation dominates the upper surface. Coconut is an important crop in the southern districts of Tamil Nadu and the incidence of whiteflies can cause stress to the plant by removing nutrients and water. In addition to damaging the host plants, whiteflies also create a nuisance in the area of infestation. In this context, the present study was undertaken to assess the seasonal incidence and population dynamics of whitefly species in southern regions of Tamil Nadu and to study the morphometric parameters of exotic whiteflies of coconut.

MATERIALS AND METHODS

Surveys were conducted at fortnightly intervals in the southern districts of Tamil Nadu *viz.*, Thoothukudi, Kanyakumari, Tirunelveli and Tenkasi on five locations of each district from December 2020 to August 2021 to assess the incidence and population dynamics of whitefly species. The seasonal incidence and the population dynamics of coconut whiteflies was assessed on the under surface of 100 leaflets randomly on ten palms each in five locations. The intensity of damage was calculated using the following formula as suggested by Elango *et al.* (2019).

$$\text{Intensity of damage (\%)} = \frac{\text{No. of fronds infested / tree}}{\text{Total no. of fronds observed/ tree}} \times 100$$

The adult whiteflies were caged on potted coconut plants leaf for oviposition and freshly laid egg spirals were identified for *A. rugioperculatus* and the nests were observed for the eggs of *P. bondari*. The eggs were observed regularly and the immature stages of whiteflies were excised daily and measurements on eggs, nymphal stages, pupae and adults were made using LEICA S8 APO with image analyser. The data obtained on the intensity of damage and populations of *A. rugioperculatus* and *P. bondari* were statistically analysed using SPSS version 16.0 software.

RESULTS AND DISCUSSION

Survey on the incidence and population dynamics of coconut whiteflies in the four southern districts of Tamil Nadu revealed that among the different species of whitefly inhabiting coconut the two whitefly species *viz.*, rugose spiralling whitefly, *A. rugioperculatus* and bondar's nesting whitefly, *P. bondari* were prevalent in Thoothukudi, Tirunelveli, Tenkasi and Kanyakumari districts. The intensity of damage, nymphal and adult population of coconut whiteflies and their morphometric parameters are detailed here.

Intensity of damage (%) by coconut whiteflies

The distribution and severity of *A. rugioperculatus* and *P. bondari* in the southern districts of Tamil Nadu from December 2020 to August 2021 are presented in Table 1. The highest whitefly infestation (56.30%) was recorded in Kanyakumari followed by Tirunelveli (54.36%) and Thoothukudi (51.83%), whereas, Tenkasi district had the lowest infestation (48.83%). On considering the pest infestation in different months among the four districts, the highest infestation was observed in March 2021 (54.44%) followed by December 2020 (54.20%). The per cent infestation was found to be low during August 2021 (47.78%). The survey results on the intensity of damage (%) revealed that the mean per cent infestation of coconut whiteflies among the different months ranged from 47.78 to 54.44% and the mean infestation of coconut whiteflies in different districts revealed that the highest damage was recorded in Kanyakumari district

(56.30%) followed by Tirunelveli district (54.36%). Alagar *et al*. (2020) assessed the intensity of infestation of *A. rugioperculatus* during June 2018 to March 2020, the severity of *A. rugioperculatus* infestation was substantially higher in Tirunelveli (70.50%) and Kanyakumari (75.70%) districts, respectively. The study results are also in line with the findings of Selvaraj *et al*. (2016) and Sundararaj *et al*. (2017) who reported that the severity of infestation of *A. rugioperculatus* ranged from 40-60% in coconut.

Population of *A. rugioperculatus* nymphs

The population of *A. rugioperculatus* nymphs in four different southern districts of Tamil Nadu is given in Table 2. On considering the overall mean of different months, the descending order of the nymphal population of *A. rugioperculatus* is as follows: Kanyakumari (49.46 nymphs/leaflet) >Tirunelveli (44.01 nymphs/leaflet) >Thoothukudi (39.68 nymphs/leaflet) > Tenkasi (32.76 nymphs/leaflet). The nymphal population of *A. rugioperculatus* was found to be highest throughout the period of observation except April and June 2021 in Kanyakumari district and Thoothukudi district recorded highest population in April 2021 (51.24 nymphs/leaflet) and Tirunelveli district in June 2021 (46.11 nymphs/leaflet). The lowest population of 22.95 nymphs/leaflet was observed in Tenkasi district during August 2021. In the

Table 1 : Intensity of damage by coconut whiteflies in southern districts of Tamil Nadu

Location	Intensity of damage*(%)									Mean
	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	
Thoothukudi	53.24 (46.88)	50.59 (45.34)	52.30 (46.32)	54.27 (47.46)	51.69 (45.97)	53.89 (47.24)	50.46 (45.26)	52.46 (46.41)	47.59 (43.62)	51.83 (46.06)
Tirunelveli	55.10 (47.93)	54.91 (47.82)	54.78 (47.75)	55.84 (48.36)	55.55 (48.19)	54.62 (47.65)	54.57 (47.62)	53.76 (47.16)	50.15 (45.09)	54.36 (47.51)
Tenkasi	48.11 (43.92)	50.18 (45.10)	49.70 (44.83)	51.08 (45.62)	50.20 (45.12)	49.40 (44.66)	50.30 (45.17)	49.00 (44.43)	41.51 (40.10)	48.83 (44.33)
Kanyakumari	60.35 (51.07)	58.56 (49.93)	58.11 (49.67)	56.58 (48.79)	56.17 (48.56)	54.40 (47.53)	54.06 (47.34)	56.62 (48.81)	51.89 (46.08)	56.30 (48.64)
Mean	54.20 (47.45)	53.56 (47.05)	53.73 (47.14)	54.44 (47.55)	53.40 (46.96)	53.08 (46.76)	52.35 (46.34)	52.96 (46.70)	47.78 (43.72)	
SE(d)	District =0.387; Month =0.580; D×M = 1.159									
CD (*P*=0.05)	District = 0.767; Month = 1.150; D×M = 2.301^{ns}									

*Mean of five replications. Figures in parentheses are *arc sine* transformed values

Table 2 : Population of *Aleurodicus rugioperculatus* nymphs in southern districts of Tamil Nadu

Location	Population of *A. rugioperculatus* nymphs/leaflet*									Mean
	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	
Thoothukudi	44.99 (6.71)	35.15 (5.96)	32.98 (5.78)	42.39 (6.53)	51.24 (7.19)	43.46 (6.63)	33.37 (5.80)	40.04 (6.36)	33.48 (5.80)	39.68 (6.30)
Kanyakumari	62.70 (7.89)	49.83 (7.01)	53.83 (7.33)	55.30 (7.44)	45.15 (6.71)	50.73 (7.13)	45.13 (6.70)	42.80 (6.52)	39.65 (6.28)	49.46 (7.00)
Tirunelveli	50.62 (7.02)	41.07 (6.35)	47.21 (6.75)	40.21 (6.27)	46.38 (6.72)	50.37 (7.05)	46.11 (6.69)	34.95 (5.95)	39.15 (6.28)	44.01 (6.56)
Tenkasi	40.94 (6.41)	31.24 (5.60)	26.82 (5.23)	36.35 (6.07)	39.90 (6.35)	35.08 (5.96)	28.54 (5.38)	32.98 (5.78)	22.95 (4.83)	32.76 (5.73)
Mean	49.81 (7.00)	39.32 (6.23)	40.21 (6.27)	43.56 (6.57)	45.67 (6.74)	44.91 (6.69)	38.29 (6.14)	37.69 (6.16)	33.81 (5.80)	
SE(d)	District=0.133; Month =0.200; D×M = 0.398									
CD (*P*=0.05)	District =0.262; Month =0.394; D×M = 0.787^{ns}									

*Mean of five replications. Figures in parentheses are $\sqrt{x+0.5}$ transformed values

present study, it was observed that the mean population of *A. rugioperculatus* was prevalent throughout the study period and this is in tune with the findings of Elango *et al.* (2020) who studied the population dynamics of a novel exotic whitefly species, *A. rugioperculatus* and their natural enemies on five year old Chowghat orange dwarf coconut trees and found the population of *A. rugioperculatus* on coconut throughout the year, and the observation recorded on a weekly interval basis showed that the population of *A. rugioperculatus* increased from the first week of July 2018 (130.8 nymph/leaf/frond) to a maximum during the first week of October, 2018 (161.0 nymph/leaf/frond) and then decreased to a minimum during April, 2019 (Elango *et al.*, 2020).

Population of *A. rugioperculatus* adults

The adult population of *A. rugioperculatus* during the period of observation is presented in Table 3. The mean population of *A. rugioperculatus* adults varied from 29.50 to 34.60 adults/leaflet throughout the study period from December 2020 to August 2021. Considering the overall mean the highest population of *A. rugioperculatus* adults was recorded in Kanyakumari district (36.99 adults/ leaflet) and the lowest population in Tenkasi district (26.71 adults/ leaflet).

Population of *P. bondari* nymphs

The nymphal population of *P. bondari* was found to be less when compared to *A. rugioperculatus*. The mean nymphal population of *P. bondari* was high in Kanyakumari (35.31 nymphs/leaflet) followed by Tirunelveli (31.70 nymphs/leaflet), Thoothukudi (25.31 nymphs/leaflet) and Tenkasi (22.79 nymphs/leaflet). The *P. bondari* nymphs was found to be maximum in December 2020 (32.78 nymphs/leaflet) followed by February 2021 with 30.17 nymphs/leaflet. Among the months of observation, the least number of *P. bondari* nymphs was noticed during January 2021 (25.69 nymphs/ leaflet) (Table 4).

Population of *P. bondari* adults

The adults of *P. bondari* were found to be highest in Kanyakumari district similar to nymphs with a population of 34.84 adults/leaflet and followed by Tirunelveli district (30.80 adults/leaflet) and then by Tenkasi and Thoothukudi districts with a mean population of 25.05 and 24.19 adults/leaflet, respectively (Table 5). While considering the monthly mean, the adult population of *P. bondari* was highest in December 2020 with a population of 33.07/leaflet followed by May 2021 (30.18 adults/leaflet). The lowest population of 25.01 adults/leaflet was recorded in July 2021.

Morphometrics parameters of *A. rugioperculatus* and *P. bondari*

Egg

The rugose spiralling whitefly, *A. rugioperculatus* eggs were 0.24±0.03 mm length, 0.13±0.02 mm breadth and 0.67±0.07 mm diameter and the bondar's nesting whitefly, *P. bondari* eggs were 0.15±0.02 mm in length, 0.08±0.01 mm breadth and 0.37±0.06 mm diameter.

Table 3 : Population of *Aleurodicus rugioperculatus* adults in southern districts of Tamil Nadu

Location	Population of *A. rugioperculatus* adults/leaflet*									Mean
	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	
Thoothukudi	30.13 (5.51)	35.67 (5.99)	27.88 (5.26)	31.86 (5.67)	28.30 (5.35)	38.69 (6.25)	30.58 (5.56)	22.83 (4.74)	26.75 (5.20)	30.30 (5.50)
Kanyakumari	41.48 (6.47)	35.45 (5.99)	41.41 (6.47)	33.94 (5.86)	40.94 (6.43)	33.01 (5.78)	31.39 (5.65)	35.91 (6.03)	39.38 (6.31)	36.99 (6.11)
Tirunelveli	38.76 (6.26)	34.70 (5.92)	32.12 (5.70)	35.07 (5.95)	32.10 (5.69)	31.16 (5.61)	34.47 (5.90)	33.50 (5.82)	32.66 (5.74)	33.84 (5.84)
Tenkasi	28.04 (5.33)	25.20 (5.06)	29.95 (5.51)	25.57 (5.10)	27.92 (5.31)	25.83 (5.13)	23.62 (4.90)	25.77 (5.12)	28.45 (5.38)	26.71 (5.21)
Mean	34.60 (5.89)	32.76 (5.74)	32.84 (5.73)	31.61 (5.65)	32.32 (5.69)	32.17 (5.69)	30.02 (5.50)	29.50 (5.43)	31.81 (5.66)	
SE(d)	District=0.093; Month = 0.139; D×M = 0.278									
CD (*P*=0.05)	District =0.184; Month =0.276 ^{ns}; D×M = 0.551									

Table 4 : Population of *Paraleyrodes bondari* nymphs in southern districts of Tamil Nadu

Location	Population of *P. bondari* nymphs/leaflet*									Mean
	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	
Thoothukudi	29.72 (5.47)	22.11 (4.72)	24.99 (5.03)	28.08 (5.33)	26.55 (5.17)	21.54 (4.66)	27.45 (5.27)	22.21 (4.66)	25.18 (5.05)	25.31 (5.04)
Kanyakumari	40.58 (6.41)	32.07 (5.70)	39.31 (6.30)	31.23 (5.63)	37.79 (6.19)	31.04 (5.61)	34.46 (5.91)	37.70 (6.18)	33.63 (5.83)	35.31 (5.97)
Tirunelveli	35.90 (6.02)	28.97 (5.39)	32.02 (5.67)	33.02 (5.78)	32.41 (5.72)	30.75 (5.57)	31.31 (5.62)	29.43 (5.46)	31.54 (5.65)	31.70 (5.65)
Tenkasi	24.94 (5.03)	19.62 (4.48)	24.37 (4.98)	18.52 (4.36)	22.84 (4.83)	24.83 (5.03)	24.32 (4.97)	17.34 (4.19)	28.29 (5.36)	22.79 (4.80)
Mean	32.78 (5.73)	25.69 (5.07)	30.17 (5.50)	27.71 (5.28)	29.90 (5.47)	27.04 (5.22)	29.39 (5.44)	26.67 (5.12)	29.66 (5.47)	
SE(d)	District=0.097; Month = 0.146; D×M = 0.292									
CD (*P*=0.05)	District =0.193; Month =0.290; D×M = 0.579^{ns}									

Table 5 : Population of *Paraleyrodes bondari* adults in southern districts of Tamil Nadu

Location	Population of *P. bondari* adults/leaflet*									Mean
	Dec-20	Jan-21	Feb-21	Mar-21	Apr-21	May-21	Jun-21	Jul-21	Aug-21	
Thoothukudi	29.28 (5.44)	23.93 (4.92)	20.06 (4.49)	23.13 (4.84)	25.87 (5.12)	27.23 (5.26)	24.00 (4.94)	19.75 (4.42)	24.43 (4.98)	24.19 (4.94)
Kanyakumari	41.55 (6.47)	33.34 (5.81)	40.52 (6.40)	31.37 (5.64)	38.41 (6.24)	30.06 (5.53)	34.9 (5.95)	30.75 (5.58)	32.63 (5.75)	34.84 (5.93)
Tirunelveli	35.45 (5.98)	31.22 (5.62)	27.81 (5.30)	33.34 (5.81)	29.95 (5.51)	33.78 (5.85)	31.11 (5.61)	23.69 (4.90)	30.84 (5.59)	30.80 (5.57)
Tenkasi	25.99 (5.12)	21.42 (4.67)	25.93 (5.12)	19.21 (4.43)	23.73 (4.89)	29.64 (5.48)	23.88 (4.92)	25.86 (5.12)	29.80 (5.49)	25.05 (5.03)
Mean	33.07 (5.76)	27.48 (5.25)	28.58 (5.33)	26.76 (5.18)	29.49 (5.44)	30.18 (5.53)	28.47 (5.36)	25.01 (5.01)	29.43 (5.45)	
SE(d)	District=0.090; Month = 0.136; D×M = 0.271									
CD (*P*=0.05)	District =0.179; Month =0.269; D×M = 0.538									

*Mean of five replications. Figures in parentheses are transformed values

Nymph

The first instar nymphs of *A. rugioperculatus* were 0.35±0.04 mm length, 0.24±0.01 mm breadth, and 1.14±0.29 mm diameter, second instar nymphs were 0.58±0.04 mm length, 0.27±0.01 mm breadth and 1.27±0.19 mm diameter, third instar nymphs were 0.83±0.08 mm length, 0.38±0.04 mm breadth and 2.50±0.35 mm diameter and the fourth instar nymphs were 1.08±0.09 mm in length, 0.70±0.09 mm breadth and 2.93±0.28 mm diameter. The body measurements of *P. bondari* were 0.24±0.01 mm length, 0.16±0.02 mm breadth and 0.83±0.03 mm diameter for first instar nymphs, 0.35±0.04 mm length, 0.25±0.02 mm breadth, 0.90±0.03 mm diameter for second instar nymphs, 0.46±0.02 mm length, 0.36±0.02 mm breadth and 1.11±0.17 mm diameter for third instar nymphs and 0.59±0.16 mm in length, 0.41±0.09 mm in breadth and 1.67±0.41 mm in diameter for fourth instar nymphs, respectively. Fourth instar nymphs are considered as a pseudo pupal stage.

Adult

The length and breadth of adult male were 2.27±0.21 and 1.30±0.05 mm and the adult female were 2.59±0.09 and 1.71±0.14 mm. In bondar's nesting whitefly, *P. bondari* adult, the length and breadth were 1.09±0.08 and 0.73±0.07 mm, respectively (Table 6). The morphometric analysis on the developmental stages of *A. rugioperculatus* in coconut revealed that the present result is almost similar in length (mm) with the findings of Saranya *et al.* (2021).

Table 6 : Morphometric parameters of developmental stages of *Aleurodicus rugioperculatus* and *Paraleyrodes bondari*

Sl. No.	Parameter	Descriptions*		
		Length (mm)	Breadth (mm)	Diameter (mm)
A. rugioperculatus				
1	Egg	0.24 ± 0.03	0.13 ± 0.02	0.67 ± 0.07
2	Nymph			
	1st instar	0.35 ± 0.04	0.24 ± 0.01	1.14 ± 0.29
	2nd instar	0.58 ± 0.04	0.27 ± 0.01	1.27 ± 0.19
	3rd instar	0.83 ± 0.08	0.38 ± 0.04	2.50 ± 0.35
	4th instar	1.08 ± 0.09	0.70 ± 0.09	2.93 ± 0.28
3	Adult female	2.59 ± 0.09	1.71 ± 0.14	-
	Adult male	2.27 ± 0.21	1.30 ± 0.05	-
P. bondari				
1	Egg	0.15 ± 0.02	0.08 ± 0.01	0.37 ± 0.06
2	Nymph			
	1st instar	0.24 ± 0.01	0.16 ± 0.02	0.83 ± 0.03
	2nd instar	0.35 ± 0.04	0.25 ± 0.02	0.90 ± 0.03
	3rd instar	0.46 ± 0.02	0.36 ± 0.02	1.11 ± 0.17
	4th instar	0.59 ± 0.16	0.41 ± 0.09	1.67 ± 0.41
3	Adult	1.09 ± 0.08	0.73 ± 0.07	-

*Mean ± SD of 10 observations

CONCLUSION

From the present study it is concluded that the exotic whitefly species, *viz.*, RSW, *Aleurodicus rugioperculatus* and BNW, *Paraleyrodes bondari* were the prevalent whiteflies in southern tracts of Tamil Nadu in coconut. The population of these invasive species were found throughout the year.

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Original Research Paper

Production function analysis for vegetable cultivation in Kullu valley of Himachal Pradesh: Application of Cobb-Douglas production model

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ABSTRACT

Vegetable cultivation plays a vital role in the agricultural economy of India. Agriculture is the main occupation of the people of Himachal Pradesh. Vegetable cultivation is facing challenges in profitability and economical use of resources. But a limited research has been done on resource use efficiency and elasticity of production in tomato, cauliflower and peas which are the major vegetable crops grown in Kullu. The present study was carried out in Kullu valley in the year 2019-2020 and multi-stage random sampling technique was used to select sixty farmers from different panchayats and villages on the basis of area they had under these crops. The elasticity of inputs used in the production of vegetables was worked out by fitting Cobb-Douglas production function. The sum of elasticity coefficients in case of tomato (Σb_i = 1.22), cauliflower (Σb_i = 1.56) and pea (Σb_i = 1.31) were greater than unity which is statistically significant and shows increasing returns to scale. The ratio of marginal value product (MVP) to marginal factor cost (MFC) represented by value of r, was greater than unity in tomato for plant protection (8.38) and labour (1.05) which indicated their under-utilization. Value of plant protection (0.30) on the other hand was less than unity in cauliflower, which shows its over-utilization. In case of peas, values for fertilizer (-1.09), seed (-2.44) and FYM (0.87) showed these were over utilized. It is suggested that the farmers should be trained for judicious use of resources.

Keywords : Cobb-douglas, elasticity, panchayats, resource use efficiency

INTRODUCTION

India has been blessed with a wide range of climate and geographical conditions and is most suitable for growing various kinds of vegetable crops. Vegetables are important constituents of Indian agriculture and are grown in an area of 10353 thousand hectares with an annual production of 191769 thousand MT (National Horticulture Board, 2020). Vegetables with shorter duration and higher productivity have resulted in greater economic returns to farmers over the last two decades. Agriculture is the main occupation of the people of Himachal Pradesh. The total area under vegetable cultivation in the state was 8861 thousand hectares with a total production of 1776.02 thousand MT in the year 2019-2020 (National Horticulture Board, 2020). The major vegetables grown in the state are potato, tomato, pea, ginger, capsicum, cauliflower, french beans, radish, cabbage, okra, carrot, chilli, and spinach.

There are a number of problems associated with the vegetable production. Productivity of vegetable crops is unable to reach its optimum level. Low productivity may be attributed to poor infrastructure, poor irrigation, small and fragmented land holdings, and low investment capacity of the farmers, fragile ecosystem and inaccessibility to technology (GC and Hall, 2020). The perishable nature of the vegetables also results in inability on the part of producers to manage supply in assembling markets. Vegetable cultivation is also facing the challenge of profitability and economical use of resources. These parameters need to be validated time to time for policy making and for the farmers to take judicious farm decisions (National Commission on Farmers, 2006). Production function analysis expresses the relationship between the quantities of productive factors (such as labour and capital) used and the amount of product obtained (Britannica, 2022).

It can also be used to determine the cheapest combination of productive factors that can be used to produce a given output. Keeping in view the above facts, the study was conducted to study about the production function analysis for vegetable cultivation in Kullu valley of Himachal Pradesh.

MATERIALS AND METHODS

The study was conducted in Kullu valley of Himachal Pradesh, India. Multi-stage random sampling technique was used to select the respondents. At the first stage, two development blocks *viz.,* Kullu and Naggar were selected. At the second stage, five panchayats from each block were selected randomly. The panchayats selected from the Kullu block were Bajaura, Hatt, Jia, Mohal and Shamshi and the panchayats selected from the Naggar block were Badagran, Brann, Hallan-I, Hallan-II and Katrain. At the third stage, a list of farmers growing vegetables was prepared from the selected panchayats and a sample of six vegetable growers was taken assigning random number using simple random technique from each panchayat, thus, comprising a sample of 60 vegetable growers in total for final survey. Primary data was collected on a pre-tested and well-structured schedule by personal interview method from the selected respondents during the year 2019-2020 and were studied at Department of Social Sciences (Agricultural Economics), College of Forestry, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India. The Cobb-Douglas production function was used for studying the relationship between output of vegetables and the various inputs of each vegetable (Cobb and Douglas, 1928; Lokapur *et al.,* 2014).

$$Y = aX_1^{b_1}X_2^{b_2}X_3^{b_3}\ldots X_n^{b_n}e_u$$

or

$$\text{Log}Y = \text{Log}\, a + b_1\text{Log}X_1 + b_2\text{Log}X_2 + b_3\text{Log}X_3 \ldots\ldots\ldots + b_n\text{Log}X_n + U$$

Where, Y = Gross return (quintal); X_1 = Expenditure on human labour (manday); X_2 = Expenditure on FYM (quintal); X_3 = Expenditure on Plant protection (kg); X_4 = Expenditure on fertilizers (kg); X_5 = Expenditure on seed (kg); a = intercept and b_1 to b_5 are the elasticity coefficients and u = error term.

Adjusted R^2 is the modified version of R that has been adjusted for the number of predictors in the model. Adjusted R is the statistic based on the number of 30 independent variables in the model which is the desired property of a goodness of fit statistic. The adjusted value of R^2 is calculated as follow:

$$\bar{R}^2 = 1-(1- R^2)\ \frac{n-1}{n-k}$$

Where, R^2 = Coefficient of multiple determination; n = Number of sample observation; k = number of parameters estimated and R^2 = Adjusted R^2

'F' test was used to test the overall significance of explanatory variables to check if they affected the dependent variable or not. The expression for the test is as under:

$$F\ (k\text{-}1,\ n\text{–}k)\ df = \frac{R^2}{1-R^2}\frac{n-k-1}{k}$$

Where, k= Number of parameters; n = Number of observations in the sample and R^2 = Coefficient of multiple determination.

Estimation of resource use efficiency

The marginal value product of a particular resource represents the expected addition to the gross returns caused by an additional unit of a resource, while other inputs are kept constant (Kireeti and Guleria, 2015). The marginal value product (MVP) of the resources employed in vegetable production was calculated by multiplying the marginal physical product (MPP) by the unit price of the output(P_y), as given below:

$$MVP_{xi} = MPP_{xi}.P_y$$

Where, MVP_{xi} = Marginal value product of i^{th} input; MPP_{xi} = Marginal physical product of the i^{th} input and P_y = Price of unit output.

The estimation of MVP-Factor Cost Ratio was done using the formula given below:

$$r = MVP_{xi}/MFC$$

Where, r = Efficiency ratio; MVP_{xi} = Marginal value product; MFC = Marginal factor cost; If r = 1 resource is efficiently used; r > 1 resource is under-utilized and r\ < 1 resource is over utilized

The elasticity of production was calculated as:

$$e_p = MPP_{xi}/\ APP_{xi}$$

where , e_p= elasticity of production; MPP_{xi} = Marginal physical product and APP_{xi} = Average physical product

RESULTS AND DISCUSSION

One of the main objectives of a production unit is to co-ordinate and utilize resources or factors of production in such a manner that together they yield the maximum net returns. The cost and return analysis does not put sufficient light on the efficiency of resource allocation. It just depicts the general idea about the different factors of production or inputs used in the cultivation and production. In order to explain the contribution of individual input in the total output, production function analysis is helpful to evaluate the efficiency of various inputs used by the farmers. The elasticity of inputs used in the production of vegetables has been worked out by fitting Cobb-Douglas production function (Goni *et al.,* 2013). The analysis was carried out at overall basis as there was no significant difference was observed among various categories of farm.

Cobb - Douglas production function in tomato, cauliflower and peas

The estimated Cobb-Douglas production function for tomato is presented in Table 1. The production function analysis shows that in case of tomato, 88 % of variation in output was explained by the variables under study. The sum of elasticity coefficients in case of tomato was greater than unity (Σb_i = 1.22) which was statistically significant and showed increasing returns to scale which meant that the output increased in a greater proportion than the increase in input. The plant protection and labour were found statistically significant at 1 and 5 % respectively.

Cobb-Douglas production function for cauliflower is represented in Table 1. The sum of elasticity coefficients in case of cauliflower was greater than unity (Σb_i = 1.56) which was statistically significant and showed increasing returns to scale which meant that the output increased in a greater proportion than the increase in input. Seed, FYM, fertilizer and labour were found to be statistically significant at 1 % level of significance.

In case of peas (Table 1), the sum of elasticity coefficients in case of pea was greater than unity (Σb_i = 1.31) , which was statistically significant and showed increasing returns to scale which meant that the output increased in a greater proportion than the increase in input. FYM and labour were found to be

Table 1 : Estimated Cob-Douglas production function in tomato, cauliflower and pea

Function	Tomato			Cauliflower			Pea		
	Coefficient	Standard Error	P-value	Coefficient	Standard Error	P-value	Coefficient	Standard Error	P-value
Intercept	54.95	-0.49	0.00	120.00	-0.07	0.25	4.27	-1.42	0.66
Seed	0.03	-0.06	0.61	0.11*	-0.04	0.01	-0.07	-0.13	0.61
FYM	-0.04	-0.1	0.67	0.10*	-0.04	0.01	0.02*	-0.01	0.00
Labour	0.29**	-0.12	0.02	1.11*	-0.05	0.00	0.90*	-0.36	0.01
Fertilizer	-0.02	-0.11	0.00	0.24*	-0.07	0.00	-0.07	-0.13	0.61
Plant Protection	0.93*	-0.10	0.87	0.03	-0.03	0.26	0.39**	-0.18	0.04
Σb_i	1.22**	R^2	0.88	1.56*	R^2	0.99	1.31*	R^2	0.81
F	80.62	Adjusted R^2	0.87	1332.99*	Adjusted R^2	0.99	11.46*	Adjusted R^2	0.77

* and ** significant at 1 and 5 % level respectively

Table 2 : Estimated resource use efficiency and elasticity of production in tomato, cauliflower and pea

Crop	Function	Coefficient	APP	MPP	Py	MVP	MFC	r
Tomato	Seed	0.03	1.70	0.06	1000.00	55.72	275.00	0.2
	FYM	-0.04	1.61	-0.07	1000.00	-71.63	150.00	-0.48
	Labour	0.29	1.26	0.37	1000.00	368.22	350.00	1.05
	Plant Protection	0.93	2.26	2.10	1000.00	2095.52	250.00	8.38
	Fertilizer	-0.02	1.88	-0.03	1000.00	-30.83	525.15	-0.06
Cauliflower	Seed	0.11	1.68	0.18	1200.00	215.20	200.00	1.08
	FYM	0.10	1.65	0.16	1200.00	190.85	150.00	1.27
	Labour	1.11	1.56	1.73	1200.00	2072.81	350.00	5.92
	Fertilizer	0.24	1.82	0.44	1200.00	528.17	525.15	1.01
	Plant Protection	0.03	1.96	0.06	1200.00	75.01	250.00	0.30
Pea	Seed	-0.07	1.5	-0.10	4885.26	-488.54	200.00	-2.44
	FYM	0.02	1.53	0.03	4885.26	129.89	150.00	0.87
	Labour	0.90	1.19	1.07	4885.26	5245.21	350.00	14.99
	Fertilizer	-0.07	1.75	-0.12	4885.26	-572.25	525.15	-1.09
	Plant Protection	0.39	2.10	0.82	4885.26	4017.05	250.00	16.07

significant at 1% level whereas, plant protection was found to be significant at 5 % level.

Resource use efficiency and elasticity of production in tomato, cauliflower and peas

Resource use efficiency indicates whether a particular input is used efficiently or not as dictated by its economically optimum level. If a particular input is used up to that level where its marginal factor cost equal to the value of associated marginal products, then the resource use is said to be efficient. If the efficiency ratio is less than one it indicates that the resource is being over utilized and if ratio is more than one, the resource is being under-utilized.

For tomato, it was observed that the ratio of MVP to MFC represented by value of r in case of plant protection and labour wasgreater than unity which meant these were under-utilized and an increase in their usage would increase the production. Values of fertilizer, seed and FYM were less than unity, which meant these were over utilized and a reduction in their usage would lead to the maximization of profits in the sampled households.

In case of cauliflower, the ratio of MVP to MFC represented by value of r in case of seed, FYM, fertilizers and labour was greater than unity which showed under-utilization of these inputs and increasing their use would increase the production. Value of plant protection on the other hand was less than unity which showed its over-utilization and a reduction in their use would lead to maximization of profits.

In case of pea, the ratio of MVP to MFC represented by value of r in case of plant protection chemicals and labour was greater than unity which means these were under-utilized and an increase in the use of these would increase the production. Values for fertilizer, seed and FYM were less than unity, which meant these were over utilized and a reduction in their use would lead to the maximization of profits in the sampled households.

CONCLUSION

The Cobb-Douglas production function analysis indicated that the labour and plant protection had significant impact on output of tomato, whereas seed, labour, FYM and fertilizer significantly contributed towards cauliflower production. In case of pea, role of FYM, human labour and plant protection played a significant role in increasing the production. The efficiency ratios for the significant variables indicated that the farmers were not using the resources judiciously. The reason for this may be lack of awareness and knowledge. Therefore, it is suggested that the farmers should be trained for judicious use of resources.

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Short Communication

Over expression of anti-apoptotic gene in banana cv Rasthali enhances resistance against *Fusarium oxysporum* f. sp. *cubense* Race 1

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ABSTRACT

The most popular banana cv Rasthali was transformed with anti-apoptotic gene, *AtBAG4* regulated with two different promoters *viz*., ZmBgl and ubiquitin to enhance the tolerance levels to *Fusarium oxysporum* f. sp. *cubense* Race 1 (FOC1). The differences in gene expression driven by two promoters revealed that stronger expression of *AtBAG4* gene under the ubiquitin promoter suppressed the infection and spreading processes of FOC1 in transgenic banana under standard bioassay systems. Analysis using the real time PCR showed the varying levels of *AtBAG4* gene expression under two promoters. It was evident that ZmBgl driven *AtBAG4* lead to lower gene expression in leaves which correlated with lesser levels of resistance to FOC1. Constitutive expression of *AtBAG4* under the control of ubiquitin promoter showed increased transgene transcripts which directly correlated with the enhanced tolerance against FOC1 from seedlings stage to active vegetative phases. This study reveals the importance of constitutive expression of anti-apoptotic gene showing enhanced tolerance against the most dreaded FOC1 in highly susceptible variety Rasthali.

Keywords : Anti-apoptotic gene, banana, constitutive expression, fusarium wilt, rasthali

Fusarium wilt disease caused by fungal pathogen *Fusarium oxysporum* f. sp. *cubense* (FOC) is one of the major threats to the banana cultivation (Ploetz and Pegg, 2000). The fungal mycelium clogs the xylem vessels and hinders the supply of water and nutrients, showing the symptoms of discoloration and drooping of leaves, splitting of stem, eventually leading to the collapse of the plant (Li *et al.,* 2011). Based on virulence and host specificity FOC has been differentiated to four physiological races *viz*., races 1-4 (Moore *et al.,* 1993). Banana cv. Rasthali is highly susceptible to FOC1, due to which the area of cultivation has declined from 146 to 20 hectares (Singhal, 1999), continued cultivation in same condition worsen the situation which threatens total extinction (Thangavelu *et al*., 2001). Currently available methods to control the disease are ineffective, hence deploying resistance in susceptible variety by genetic transformation served as an alternative strategy (Ploetz, 2015). *F. oxysporum* species known to exhibit short biotrophic phase followed by complete necrotrophy in host plant (Thaler *et al.,* 2004). In view of this, employing genes which negatively regulate the cell death pathway in host were used to enhance resistance against necrotrophic fungi (Dickman and de Figueiredo, 2013). BAG genes encoding multifunctional group of proteins function by interacting with the signaling molecules and molecular chaperones, such as heat shock proteins resulting in inhibition of PCD (Sondermann *et al.,* 2001; Doukhanina *et al.,* 2006; Jacobs and Marnett 2009; Ge *et al.,* 2016). Most of the time, exogenous genes have mostly been expressed by ubiquitin promoter that drives high-level expression of transgenes in monocot plants (Jiang *et al.,* 2018). Beta-glucosidase promoter isolated from *Zea mays* (ZmBgl) shows much stronger activity in root parts (vigorous cell division zones associated with vascular tissues) compared to mature seeds and edible part of the crop (Gu *et al.,* 2006). Over expression of programmed cell death (PCD) gene, *AtBAG4* under two different promoters namely ZmBgl promoter and ubiquitin promoter was studied to confer FOC1 resistance in banana cv. Rasthali.

The embryogenic cell suspension (ECS) initiated from male inflorescence of banana cv. Rasthali was maintained in controlled condition as previously

described (Sunisha *et al.,* 2020a). ECS was heat shocked at 45ºC for 5 min and transformed with *AtBag4* gene constructs obtained from Queensland University Technology, Australia using *Agrobacterium* strain AGL as described by (Khanna *et al.,* 2004). The putative transformants developed with *AtBAG4* genes driven by ZmBgl promoter as well as ubiquitin promoter were subjected to PCR analysis. Total RNA was extracted from leaf tissue of untransformed and transformed plants using plant RNA isolation kit (Sigma, Aldrich). Briefly, 4 µg of RNA was subjected for DNase treatment and first strand cDNA synthesized using 2µg of RNA, Oligo (dT) $_{12\text{-}18}$ primer (Sigma), RevertAid™ M-MuLV Reverse Transcriptase (Thermo Scientific). Five transgenic lines from each construct were individually subjected to qPCR in three replications with a total volume of 20 µL reaction mixture containing 10 µL of PCR mix from SYBR Green kit (Takara), 1 µL of gene primer set and 5 µL of cDNA template in a 7500 Real-Time PCR system (Applied Biosystems). Banana Actin gene was used as an endogenous gene control for qRT-PCR. Amplification conditions were, initially 95 °C for 5 min followed by 40 cycles of 95 °C for 1 min, 60 °C for 45 sec, and 72 °C for 30 sec, each. Final PCR melt curve of 95°C for 1min and 60°C for 15 s was employed and $2^{-\Delta\Delta CT}$ method was used to analyze quantitative variation (Sunisha *et al.,* 2020b). Five transgenic lines from each construct along with the untransformed susceptible cv. Rasthali and resistant cv. Grand Naine controls were inoculated with FOC1 obtained from Plant pathology laboratory, ICAR-Indian Institute of Horticulture Research, Bangalore (India), as described previously by Smith *et al*. (2008) with slight modification in the potting mixture. External symptoms were recorded four weeks post inoculation by scoring each plant for yellowing and wilting symptoms (Paul *et al.,* 2011) using a 1–5 point scoring, where 1 represented -healthy, no symptoms; 2—slight symptoms (yellowing of the leaves); 3—advance symptoms (dropping of the leaves); 4—extensive symptoms (whole foliage got dried); 5—entire plant affected (complete dead plant). The data was analyzed by one-way analysis of variance (ANOVA) using GraphPad prism® software (USA).

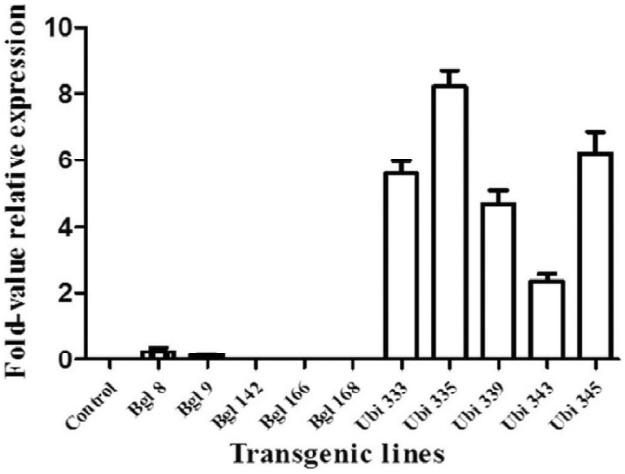

Fig. 1 Comparative *AtBAG4* gene expression analysis in leaves of transgenic plants

Lines 8, 9, 142, 166 and 168 represent transgenic banana cv Rasthali expressing AtBAG4 under ZmBgl promoter. Lines 333, 335, 339, 343 and 345 represent transgenic banana cv Rasthali expressing AtBAG4 under ubiquitin promoter

Table 1. Bioassay of *AtBAG4* transgenic lines of banana cv. Rasthali plants inoculated with FOC1 for symptom expression

Sl. No.	Transgenic Lines		External symtoms			
	ZmBgl: AtBAG4	Uboquitin: AtBAG4	ZmBgl:AtBAG4		Ubiquitin:AtBAG4	
			Yellowing	Wilting	Yellowing	Wilting
1	168	333	3.3	1.3	2.3	1.6
2	166	335	3.0	2.0	2.6	1.3
3	142	339	3.3	2.3	2.6	1.6
4	8	343	2.6	1.6	2.3	2.0
5	9	345	2.3	2.6	1.6	2.0
6	RS CONTOL		4.3	4.6	5.0	4.3
	C.D.		0.9	1.2	0.8	1.1

Transgenic lines expressing AtBAG4 gene; RS—control are untransformed cv Rasthali. Results are presented as score—yellowing and wilting: 1–5 scale, stem splitting: 1–3 scale. Five to seven leaf stage plants were subjected for the FOC root-challenge bioassay. The treatments were significantly dissimilar from susceptible RS control lines as $P < 0.05$ based on LSD post hoc test

Agrobacterium mediated transformation of ECS with binary vector containing *AtBAG4* gene, driven by ZmBgl and ubiquitin promoter resulted in total of 23 PCR confirmed putative transformants free from somaclonal variation. The well rooted plantlets were acclimatized in net house for further development. The efficient overexpression and transcript levels of the *AtBAG4* gene were examined using cDNAs derived from five selected transgenic lines of each construct and also in un-transformed control. The qRT-PCR data showed that the expression of *AtBAG4* gene driven by ubiquitin promoter ranged from 2 to 8.5-fold whereas the transgenic lines expressing *AtBAG4* gene under ZmBgl promoter showed less than one-fold expression in leaves compared to untransformed plants (Fig 1). The differential expression in the transgenic and non-transformed plants were statistically significant ($F = 131.7$; $P = < 0.0001$). Transformants expressing *AtBAG4* driven by ubiquitin and ZmBgl promoter showed reduced external symptoms compared to untransformed control plants four weeks post inoculation.

The ubiquitin promoter resulting transgenic events exhibited higher gene expression level in leaves compared to transgenic events developed with ZmBgl promoter. Also, transgenic lines over expressing anti-apoptotic *AtBAG4* gene driven by ubiquitin promoter exhibited reduction in external symptoms (< 3 rating for yellowing and wilting) and enhanced tolerance to Fusarium wilt caused by FOC 1 under pot condition. Similarly, the ubiquitin promoter used in transgenic plants for over expressing MusaBAG1 showed enhanced resistance to FOC infection (Ghag *et al.,* 2014). It is evident from the present study that anti-apoptotic *AtBAG4* gene driven by root specific ZmBgl promoter was having lower levels of expression in leaves. Similarly, the root specific ZmRCP-1 promoter isolated from maize failed to deliver gusA expression in leaves of transgenic plantains (Onyango *et al.,* 2016). However, the gene expression in roots and vascular discoloration index post FOC inoculation are to be further studied to know the efficacy of root specific ZmBgl promoter over constitutive ubiquitin promoter. Further, the ZmBgl promoter expressing in vascular tissues is developmentally and spatially regulated promoter and such promoters are under the influence of several endogenous elements (Schmitz *et al.,* 2022) which could be the reason for lower levels of transgene expression in leaves. In contrast the constitutively over expressing *AtBAG4* gene are expressed in all the tissues leading to higher oxygen scavenging activity and preventing tissue damage restricting the faster multiplication of FOC1 (Paul *et al.,* 2011).

Owing to the multiple reports confirming host PCD manipulation induced resistance to various FOC races (Magambo *et al.,* 2012; Dale *et al.,* 2017), the modification of genes regulating PCD pathways in plants is emerging as a promising strategy to provide broad-spectrum resistance to both biotic and abiotic stresses (Lincoln *et al.,* 2002; Li and Dickman, 2004;). Hence, our research mainly focused on improving resistance to Fusarium wilt by manipulating PCD pathways.

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Short Communication

Spectrum of chlorophyll mutations and morphological variations in *Abelmoschus esculentus* L. induced through gamma radiation

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ABSTRACT

Okra [*Abelmoschus esculentus* (L.) Moench], is an economically exploited important traditional vegetable crop of the world. The present investigation examined the variability in induced chlorophyll mutants and other morphological variations in okra. Seeds of two open pollinated popular varieties of okra namely Arka Anamika and Arka Abhay were irradiated with gamma doses of 30, 50 and 80 kR. The treatment 50 kR enhanced plant height, number of fruits per plant, fruit length, single fruit weight and total fruit yield per plant. Spectrum of several chlorophyll mutants were observed in the M_1 generation. Other macro-mutants such as early and late flowering types, dwarf statured plants, leaf and flower mutants were also noticed at different doses of gamma radiation. The total number of visible mutation followed a trend of increasing frequency with the increase in dose of radiation.

Keywords : Chlorophyll mutants, gamma radiation, mutation, okra, variability

INTROCUTION

Okra (*Abelmoschus esculentus* (L.) Moench), family Malvaceae, is one of the most cultivated multipurpose traditional crops of the world grown for its fresh tender fruits in the warm, sub-tropical and tropical areas. The genus *Abelmoschus* consists of thirty-eight species, among which *A. esculentus* is the widely cultivated. Okra fruits are rich in micro nutrients, vitamins, minerals and iodine and are also known for its medicinal values such as anti-inflammatory properties. The inadequacy of resistant genotypes for mitigating the climate change, biotic and abiotic stresses have limiting impacts on the total production and productivity of the crop. The inability to transfer available resistance genes from wild relatives to the cultivated lines as a result of chromosomal variations and faulty meiotic divisions also limits the hybridization programs in okra (Kumar *et al.,* 2019). Under these circumstances, mutation breeding has proven to be a useful technique in crops like okra, for inducing novel genetic variations such as development of mutant okra varieties *i.e.*, Punjab-8, and Parbhani Tillu. The role of mutation breeding in increasing the genetic variability for desired traits in various crop plants has been proved (Kozgar *et al.*, 2012). Chlorophyll mutations can be used as dependable indices for analyzing the efficiency of various physical and chemical mutagens in inducing genetic variability for the crop improvement in several crops (Gupta and Sood, 2019).

The present investigation was carried out to determine the response of okra to the varying doses of gamma radiation in terms of chlorophyll mutations and agro-morphological characters at the College of Agriculture, Keladi Shivappa Nayaka University of Agricultural and Horticultural Sciences, Shivamogga during 2019-2020. The 1000 seeds each of two open-pollinated varieties (Arka Anamika and Arka Abhay) were irradiated with three doses of gamma rays *i.e.*, 30 kR, 50 kR and 80 kR based on the LD_{50} value obtained from other research outcomes. Gamma irradiated seeds of both the varieties were sown with a spacing of 60 × 45 cm in a four meter row in an augmented block design during August 2019 to raise M_1 generation. In every block, untreated Arka Anamika, Arka Abhay, Parbhani Kranti (YVM tolerant) and Pusa Sawani (YVM susceptible) were sown as checks for respective treatments. The recommended package of practices

was ensured during the crop period. The observations on twelve quantitative characters were recorded from 40 randomly selected plants in each treatment and averaged. The chlorophyll mutations and other morphological variations were scored and recorded using DSLR camera. Chlorophyll mutants were scored on daily basis from 10th to 31st day after sowing and classified according to Gustafsson (1940) and the identification and description of morphological mutants were followed as proposed by Blixt (1961). The mean values recorded for the characters for each mutant line in all the blocks were subjected to statistical analysis in type 2 modified augmented block design (You *et al*., 2016) using R programme. The significance of difference among treatments in the M_1 generation of both the varieties were tested using Duncan's multiple range test (DMRT) method (Duncan, 1955).

Table 1 : Analysis of variance for growth and yield parameters in mutant families (M_1) of Arka Anamika variety

Source	Df	DFF	PH	NB	NF	FL	FW	INT	SFW	NS	SWT	TWT	YLD
Blocks	5	26.57 **	351.14 **	7.31 **	18.7 **	101.72 **	1.18 **	55.22 **	174.49 **	478.29 **	3.82 **	10.49 **	15710.90 **
Treatments	123	1.34 **	55.6 **	2.65 **	6.8 **	7.18 *	3.07 **	8.78 **	19.88 **	217.04 **	2.09 **	2.83 **	1340.08 **
Checks	3	17.15 **	42.52 **	5.59 **	7.88 **	14.98 **	0.01	0.13 *	2.78	2.76 *	0.64 **	5.9 **	1173.82 **
Mutants	119	1.86 **	70.92 **	2.81 **	6.55 **	11.19 **	0.51 **	11.39 **	27.27 **	243.15 **	2.3 **	2.57 **	1976.44 **
Mutants vs Check	1	23.87 **	25.03 **	10.73 **	125.75 **	0.69	21.92 **	0.26 *	54.18 **	138.08 **	0.5 **	20.85 **	3730.09 **
Residuals	15	0.28	0.83	0.05	0.03	2.73	0.05	0.04	0.9	0.77	0.03	0.33	55.39
CV		1.06	1.92	4.94	2.74	11.65	4.27	2.19	7.70	1.72	5.02	7.88	10.12
CD (5%) (Mutant v/s Control)		1.35	2.34	0.57	0.42	4.26	0.56	0.5	2.44	2.26	0.47	1.48	19.16
CD (5%) Two Mutants (Different Blocks)		1.77	3.07	0.75	0.55	5.57	0.73	0.65	3.2	2.96	0.62	1.93	25.08

Table 2 : Analysis of variance for growth and yield parameters in mutant families (M_1) of Arka Abhay variety

Source	Df	DFF	PH	NB	NF	FL	FW	INT	SFW	NS	SWT	TWT	YLD
Block	5	38.11 **	497.56 **	0.52 **	11.3 **	69.84 **	1.21 **	16.86 **	117.13 **	697.16 **	6.53 **	11.54 **	6680.28 **
Treatment	123	2.35 **	57.22 **	1.5 **	3.48 **	8.02 **	3.13 **	8.49 **	15.53 **	165.97 **	2.03 **	3.75 **	616.99 **
Check	3	17.15 **	42.52 **	5.59 **	7.88 **	14.76 **	0.02	0.13 *	2.78	2.76 *	0.64 **	5.9 **	946.96 **
Mutants	119	2.43 **	76.57 **	1.43 **	3.83 **	10.73 **	0.63 **	9.14 **	20.62 **	200.23 **	2.35 **	4.08 **	831.68 **
Mutants vs Check	1	137.9 **	284.07 **	0.01	4.82 **	0.39	18.64 **	40.83 **	23.62 **	57.97 **	0.53 **	8.87 **	1272.68 **
Residuals	15	0.28	0.83	0.05	0.03	2.60	0.05	0.04	0.9	0.77	0.03	0.33	45.88
CV		1.04	2.02	5.76	3.82	11.58	11.72	1.91	8.00	1.75	4.88	7.57	14.45
CD (5%) (Mutant v/s Control)		1.29	2.35	0.56	0.42	4.15	0.55	0.51	2.47	2.26	0.46	1.50	17.43
CD (5%) Two Mutants (Different Blocks)		1.75	3.10	0.75	0.55	5.44	0.72	0.65	3.2	3.04	0.71	1.95	22.83

** at 1% level of significance, * at 5 % level of significance

Analysis of variance for twelve parameters in the M_1 generation of Arka Anamika and Arka Abhay were performed and presented in Table 1 and Table 2, respectively. The ANOVA for both the varieties showed that mean sum of squares within blocks (eliminating treatments), within the treatments (eliminating blocks) and checks vs mutants were significant for all the 12 traits.

Significant differences in the mean values of most of the traits were observed for mutant lines of both okra varieties Arka Anamika and Arka Abhay, in response to varying doses of gamma radiation (Table 3). A lower dose of 30 kR affects most of the traits such as plant height, single fruit weight, number of seeds per fruit, test weight and total fruit yield in mutant lines compared to control in both the varieties.

The treatment 50 kR enhanced the economic yield of okra fruits per plant drastically compared to the other treatments and the control (Fig. 1). Traits such as plant height, number of fruits per plant, fruit length and single fruit weight were observed to be significantly higher in 50 kR treatment in mutants of Arka Anamika and Arka Abhay. Other traits such as number of seeds per plant, seed weight per fruit and test weight were also higher.

In general, a higher dose (80 kR) had detrimental effects on most of the traits such as plant height, number of fruits per plant, fruit length and width, single fruit weight, number of seeds per plant, seed weight per fruit, test weight and total fruit yield. Remarkable increase in the number of days to first flowering was also observed at a higher dose in both the varieties as also reported in number of fruits per plant (Warghat *et al.*, 2011), plant height, fruit length, average fruit weight and total fruit yield (Jadhav *et al.*, 2012) and number of seeds per plant, seed weight per fruit and test weight (Hegazi and Hamideldin, 2010). Mutant lines of 80 kR group recorded more number of branches per plant, increased number of nodes and shorter internodes on the main stem, making the plants looks dwarf and short stature.

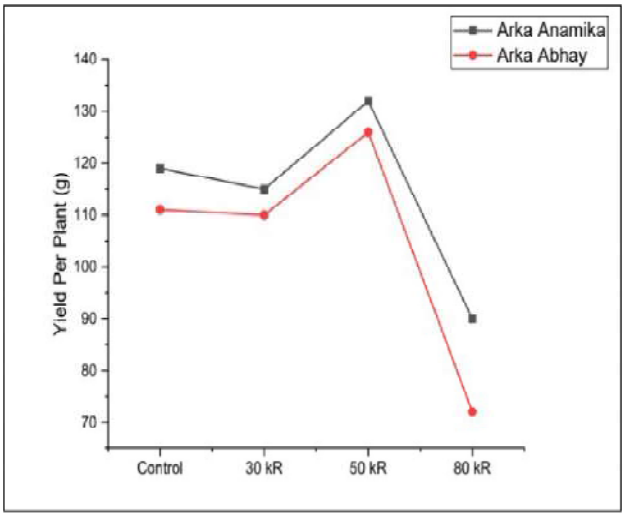


Fig. 1 : Effect of gamma radiation on yield per plant in two okra varieties

Table 3 : DMRT results for different treatments and characters among Arka Anamika and Arka Abhay mutant lines

Variety	Treatment	DFF	PH	NB	NF	FL	INT	SFW	NS	SWT	TWT	YLD
Arka Anamika	30 kR	48.25^{C}	47.17^{AB}	3.82^{BC}	7.32^{C}	13.89^{BC}	7.05^{B}	10.90^{B}	52.67^{A}	3.68^{A}	7.04^{A}	114.27^{AB}
	50 kR	49.35^{B}	51.15^{A}	4.92^{AB}	14.47^{A}	17.05^{A}	9.12^{AB}	14.39^{A}	54.10^{A}	4.00^{A}	7.35^{A}	131.80^{A}
	80 Kr	51.32^{A}	43.10^{B}	5.05^{A}	4.27^{D}	11.76^{C}	10.27^{A}	7.55^{C}	45.40^{B}	3.24^{A}	7.05^{A}	89.95^{B}
	Control	48.76^{BC}	47.01^{AB}	3.62^{C}	10.20^{B}	15.32^{AB}	8.75^{AB}	11.66^{B}	53.23^{A}	3.64^{A}	7.40^{A}	119.48^{AB}
	Mean	49.59	47.13	4.55	9.02	14.28	8.81	12.88	51.24	3.64	7.09	145.34
	CD (5%)	1.35	2.34	0.57	1.50	6.72	0.50	2.26	2.44	0.97	1.48	19.66
Arka Abhay	30 kR	49.27^{c}	46.40^{b}	3.70^{b}	8.57^{ab}	13.25^{bc}	9.12^{ab}	13.00^{a}	53.02^{ab}	4.00^{a}	7.44^{a}	100.72^{ab}
	50 kR	51.37^{b}	50.47^{a}	4.35^{b}	11.90^{a}	16.6^{a}	9.97^{a}	12.40^{a}	55.80^{a}	4.17^{a}	7.74^{a}	126.27^{a}
	80 Kr	56.50^{a}	38.62^{c}	6.05^{a}	3.39^{b}	12.16^{c}	8.98^{b}	8.82^{c}	44.90^{b}	3.16^{a}	7.43^{a}	72.70^{b}
	Control	49.41^{c}	47.01^{b}	3.62^{b}	7.37^{ab}	15.32^{ab}	8.75^{b}	10.00^{ab}	48.23^{ab}	3.64^{a}	7.40^{a}	110.70^{ab}
	Mean	51.01	44.61	4.64	8.62	14.04	10.28	12.29	51.09	3.77	7.53	114.89
	CD (5%)	1.29	2.35	0.56	1.42	6.80	0.51	2.47	2.26	0.46	1.50	17.43

Mean with same superscript within a column do not differ at 5% level of significance

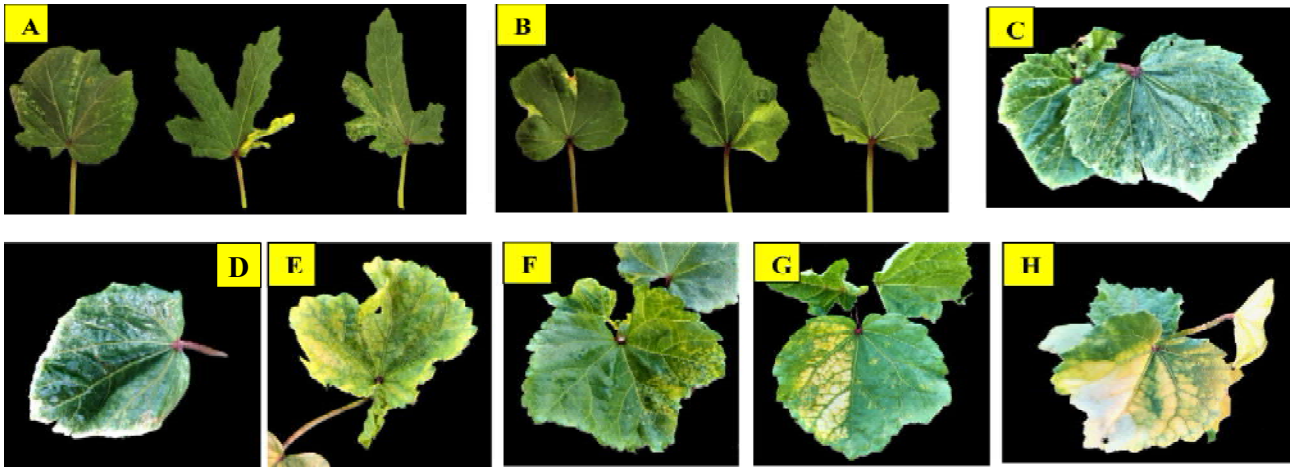
DFF-days to first flowering, PH-plant height, NB-number of branches, NF-number of fruits, FL-fruit length, INT-number of internodes, NS-number of seeds, SWT-seed weight, TWT-test weight, SFW-single fruit weight, YLD-yield per plant

Point mutations in the gene sequences of active photosynthetic parts of plants are the major source of chlorophyll mutations. Spectrum of various chlorophyll mutants in the M_1 generation of Arka Anamika and Arka Abhay are summarized in Table 4. Various chlorophyll deficient mutants such as chlorina, xantha, albina green, albino and viridis types were observed among the mutant plants, following the irradiation with gamma radiation (Fig. 2). Similar mutants were also documented by Mohite *et al.* (2019) in okra. The number of chlorina type of mutants was higher followed by xantha and viridis type. The appearance of chlorina, xantha and viridis types in a larger frequency implies the involvement of polygenic nature of chlorophyll formation (Gaul, 1964). It was also noticed that the frequency and spectrum of different chlorophyll mutations were higher at lower dose (30 kR) and was least at highest dose (80 kR). Kolar *et al*. (2011) opined that strong mutagens such as gamma radiations reach their saturation point even at lower or moderate doses and further increase in the dose may not yield higher frequency of mutants. An increase in the dose beyond a threshold level might generate more toxic effects with the strong mutagens which in fact exactly substantiate this research outcome.

The number of various visible mutants in the M_2 generation were recorded and presented in Table 5. Plants were significantly shorter in the highest dose (80 kR) which may be attributed to the sensitivity of plants to the extreme radiation dose. Number of dwarf plants were considerably high at extreme dose (10 and 8 for Arka Anamika and Arka Abhay, respectively), whereas, taller mutant plants was a peculiar observation in 50 kR treatment. The plants in 30 kR were almost synchronized with control plants for the onset of flowering, whereas, plants in 50 kR were early to flower in both the varieties. However, the extreme dose (80 kR) delayed flowering.

Table 4 : Spectrum of induced chlorophyll mutants by different doses of gamma radiation in M_1 generation

Type of mutation	Arka Anamika					Arka Abhay				
Chlorophyll mutants	Control	30 kR	50 kR	80 kR	Total	Control	30 kR	50 kR	80 kR	Total
Chlorina	-	4	2	1	7	-	3	3	1	7
Xantha	-	3	2	1	6		3	2	0	5
Viridis	-	3	0	0	3	-	3	1	0	4
Albina green	-	3	0	0	3	-	2	0	0	2
Albino	-	1	0	0	1	-	0	0	0	0



A and B: Chlorina, C and D: Albina green, E and F: Viridis, G and H: Xantha

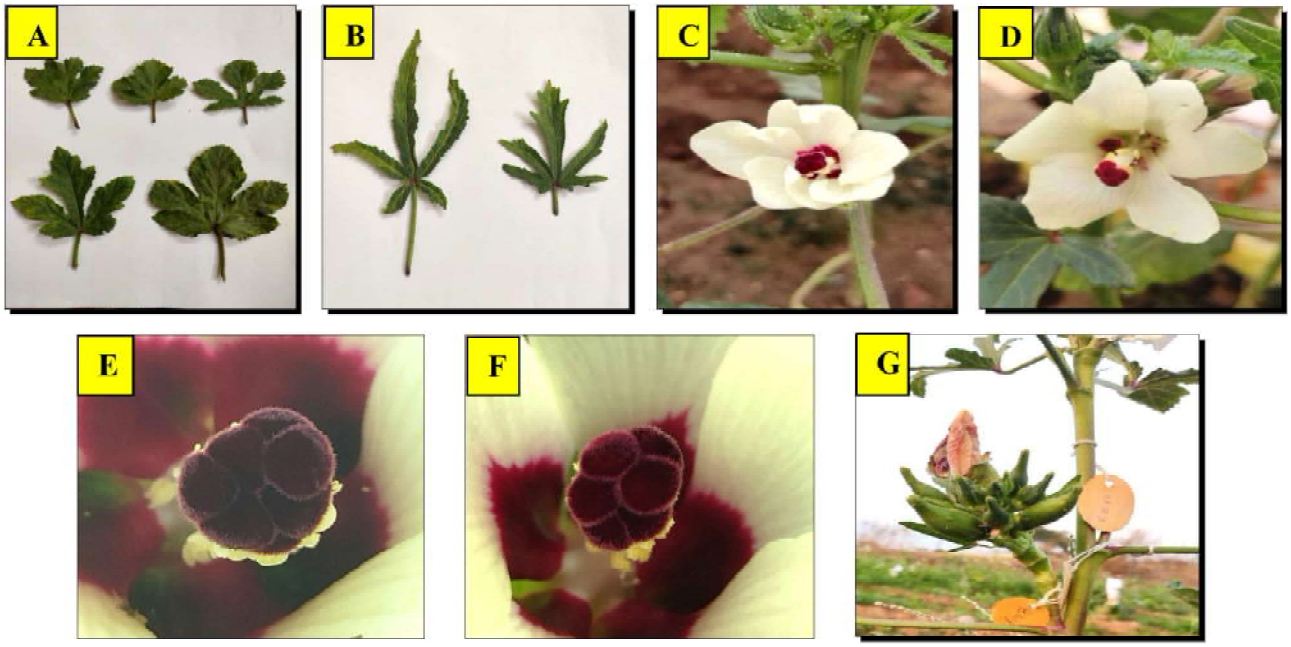
Fig. 2 : Spectrum of chlorophyll mutations induced by gamma radiation

Table 5 : Spectrum of induced visible mutations by different doses of gamma radiation in M_2 generation

Type of mutation	Arka Anamika					Arka Abhay				
	Control	30 kR	50 kR	80 kR	Total	Control	30 kR	50 kR	80 kR	Total
Dwarf	-	1	1	8	10	-	0	0	8	8
Tall	-	1	3	0	4	-	0	4	0	4
Early flowering	-	0	3	0	3	-	1	5	0	6
Late flowering	-	1	1	5	7	-	1	2	6	9
Leaf mutants	-	3	2	4	9	-	2	3	6	11
Flower mutants	-	0	0	2	2	-	0	0	1	1
Pod mutants (Increased ridge number)	-	4	6	15	25	-	6	7	12	25

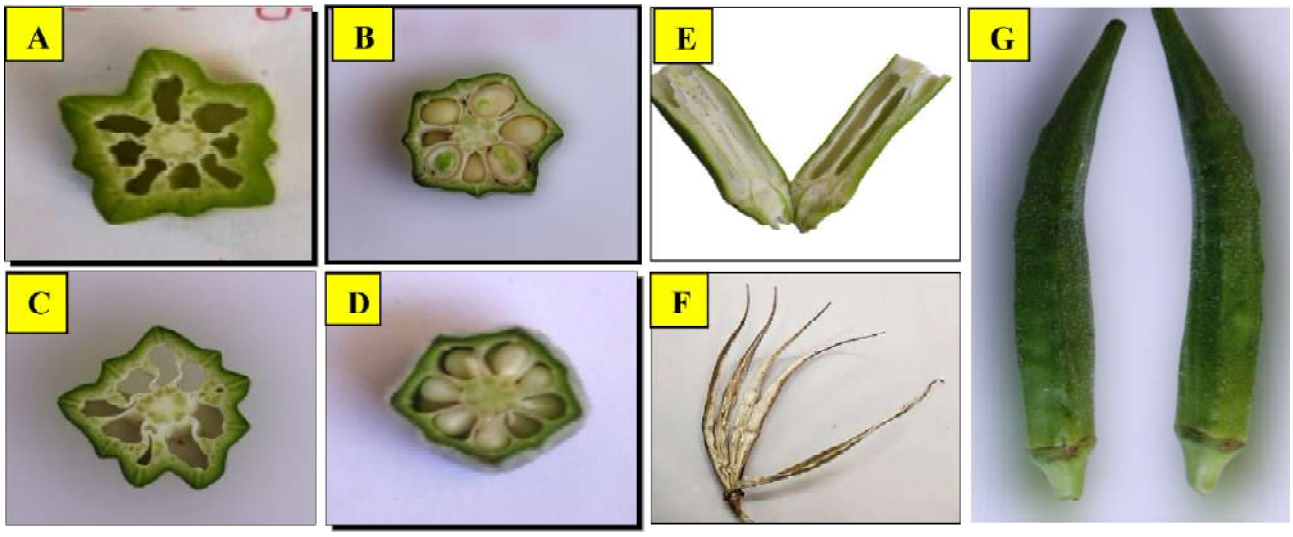
Different leaf shape mutants such as rosette, round and lobed types were also observed (Fig. 3). Leaf mutants were scored based on the sizes, shapes and colours. Although the leaf mutants were identified in all treatments, comparatively a higher number was recorded in 80 kR. Leaves were smallest in 80 kR treatment and also varied for the shapes such as rosette. Similar types of leaf mutants were also observed in okra (Surendran and Udayan, 2017; Gupta and Sood, 2019). The abnormalities observed in leaves may be attributed to various causes such as mutations in phytochromes, disturbances in chromatin material, mineral deficiencies, disturbance in DNA synthesis or mitotic division, enlargement of palisade, spongy and mesophyll cells (Gupta and Sood, 2019).

Flower mutants with six and more number of petals and altered aestivation were noticed at higher doses of gamma radiation. Considerable amount of sterility was also noticed. Record of different flower mutants following the gamma irradiation in okra is available (Surendran and Udayan, 2017). A mutant line with a bunch of fruits in a single node (Fig. 3; G) was isolated. Extremely short as well as long fruited mutants were recovered among different family. The number of ridges and locules per fruits increased from



A: round leaves, B: rosette leaves, C and D: increased number of petals, E and F: increased number of stigmata, G: multiple flower buds in a bunch

Fig. 3 : Morphological mutations for leaves and flowers induced by gamma radiation



A, B C and D: increased number of ridges and locules, E and F: sterile/ absence of seeds, G: hard pubescence

Fig. 4 : Morphological mutations for fruits, ridges and locules induced by gamma radiation

five up to eight, altering the normal ridge shape (Fig. 4). Pods with sterile seeds or miniature, shrunken seeds were also identified. The total number of visible mutations followed a trend of increasing frequency with the increase of the dose of the mutagen. A maximum number of viable and morphological mutations were obtained at a moderate dose in the study. Mohite *et al.* (2019) reported that the number of total lethal mutations increases corresponding to the increase in doses in mesta plant.

CONCLUSION

The present study, using gamma radiation confirmed the creation of genetic variability by exhibiting wide range of macro and micro mutations. The frequency and spectrum of different chlorophyll mutations such as chlorina, xantha, viridis and albino green were higher at lower dose (30 kR) and kept decreasing as the dose increased. Prominent changes in leaf morphology, including notable alterations in shape, size, increased surface roughness, and enhanced trichome sharpness and visibility were observed in higher dosage. On the other hand, the total number of visible agro-morphological mutations followed a trend of increasing frequency with the increase dose of gamma radiations. Economic traits such as total yield per plant, average fruit weight, number per plant, fruit length and seed yield per fruit were found highest at 50 kR followed by control set. Dose 50 kR can be suggested to improve agronomic characters in okra and superior mutant lines can be identified and stabilized during next generation of M_2.

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Short Communication

Morphological characterization of standard chrysanthemum (*Chrysanthemum morifolium* Ramat.)

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ABSTRACT

Ten diverse chrysanthemum varieties were evaluated for their suitability as cut flower, flower arrangement and pot plant. The maximum plant height at bud appearance (71.82 cm) and at anthesis (77.23 cm) was recorded in Snow Ball, while, it was recorded minimum at bud appearance (44.08 cm) and flower opening stage (48.10 cm) in Purnima. The longest duration of flowering (33.73 days) was recorded in Thai Chen Queen, whereas, the least flowering duration (23.63 days) was recorded in Swan Dance. The variety Pusa Centenary exhibited the longest vase life (22.00 days), however, the least vase life (16.00 days) was recorded in Valliant. Depending upon the compactness, medium size and vase life, Thai Chen Queen, Purnima, Pusa Centenary, Otam Blaze and Denise Oatridge were found suitable for pot culture, cut flower and flower arrangements, whereas, the varieties with big flower such as Snow Ball, Kikobiory, Sonar Bangla, Valliant and Swan Dance were identified for pot culture and exhibition purpose.

Keywords : Chrysanthemum, evaluation, floral characters, vase life

Chrysanthemum belongs to the family Asteraceae, native of Asia and Europe (Asha *et al.,* 2016), is commercially cultivated in for the exquisite flowers. It is a leading flower in the global market and commonly grown for cut flower, loose flower, pot plant and garden decoration throughout the world. In India, is being commercially grown in 31.40 thousand hectare area with 482.54 thousand metric tons loose flower and 28.73 thousand metric tons of cut flower production (Anon., 2022). Chrysanthemum flowers have high potential and price because of its variable flower shape, size, forms and distinctiveness for flower hues and shades (Kaushal and Bala, 2019). There is demand for superior varieties over the existing ones, thus, there is need to evaluate and categorize chrysanthemum varieties on the basis of their commercial significance (Bala, 2015). The objective of this study was to evaluate diverse standard varieties of chrysanthemum having potential for pot culture, exhibition, and cut flower with commercial significance.

The experiment was conducted with ten standard varieties of chrysanthemum *i.e.*, Snow Ball, Pusa Centenary, Sonar Bangla, Thai Chen Queen, Purnima, Kikobiory, Swan Dance, Otam Blaze, Valliant, Denise Oatridge, in 8 inch pots, replicated thrice in completely randomized block design (CRD) at Research Farm, Department of Floriculture and Landscaping, Punjab Agricultural University, Ludhiana, during 2018-19. Substrate media of soil : leaf mold : sand (2:1:1) was used for pot filling. Disbudding was done in September and October to maintain a healthy terminal flower on the single stem. The observations on various growth and flowering parameters such as plant height at bud appearance and at anthesis, number of leaves per plant, days to bud initiation, days to flower opening stage, maturity group (early, medium, late), flower diameter, duration of flowering, flower colour, vase life, flower form and commercial use were recorded. Data were subjected to statistical analysis by using CPCS-1 software and comparisons were made at 5% level of significance.

All the varieties differed significantly with each other with regard to various vegetative and floral parameters (Table 1). The maximum plant height at bud appearance (71.82 cm) and anthesis (77.23 cm) was recorded in variety Snow Ball which was significantly higher than at bud appearance (68.30 cm) and at anthesis (73.47 cm) in Kikobiory, however, the minimum plant height (44.08 cm) at bud appearance and at anthesis (48.10 cm) was observed in Purnima. The height of plants should be proportionate *i.e.*,

Table 1 : Evaluation of chrysanthemum varieties for vegetative and floral characters

Variety	Plant height (cm)		Number of leaves/ plant	Flower diameter (cm)	Duration of floweri-ng (days)	Floret colour code (RHS colour chart)
	at bud appearance	at anthesis				
Denise Oatridge	61.27	65.07	13.65	14.33	32.97	Purple Violet group (N 80 D)
Kikobiory	68.30	73.47	10.80	15.82	26.87	Yellow group (6 A)
Otam Blaze	57.59	60.73	13.20	14.50	26.70	Orange Red group (31 A)
Purnima	44.08	48.10	11.37	13.30	30.60	White group (15 N)
Pusa Centenary	61.33	65.80	18.50	14.41	29.57	Yellow group (6 C)
Snow Ball	71.82	77.23	13.37	17.69	31.07	White group (155 A)
Sonar Bangla	58.84	62.70	13.03	16.85	25.17	Yellow White group (158 C)
Swan Dance	66.94	70.80	14.87	19.50	23.63	White group (155 B)
Thai Chen Queen	54.10	58.70	14.67	15.80	33.73	Yellow Orange group (22 C)
Valliant	60.13	64.27	13.07	16.73	27.90	Red Purple group (62 D)
SEm±	0.47	0.23	0.32	-	-	-
LSD (0.05)	1.40	0.68	0.94	0.77	1.24	-

2-2.5 times to the size of pot for its effective display. The seasonal variation pertaining to environmental conditions such as light and temperature also affect the plant architecture (Suvija *et al.,* 2016). The variation in plant height could be due to genetic and environmental factors (Baskaran *et al.,* 2010). The highest number of leaves per plant (18.50) was recorded in the variety Pusa Centenary, whereas, the lowest leaf count per plant (10.80) was observed in Kikobiory. The diverse genetic makeup of different genotypes along with variable response to prevailing environmental conditions likely resulted in variation in leaf number (Suvija *et al.,* 2016).

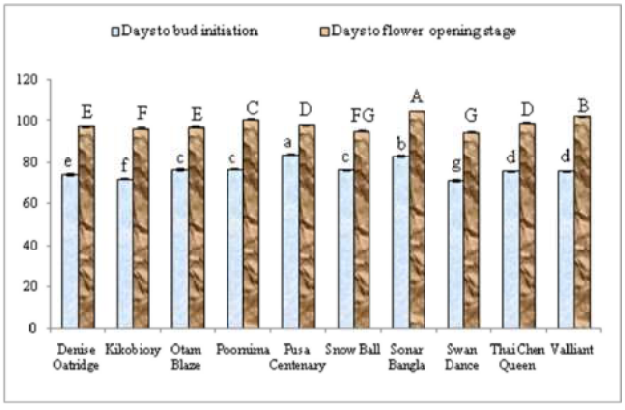

Fig. 1 : Days to bud apperance and flower opening stage

The number of days to bud appearance and flower opening differed significantly among the varieties (Fig. 1). The variety Swan Dance (71.30 days) recorded early bud initiation and it was statistically at par with Kikobiory (72.20 days). The variety Pusa Centenary registered the highest number of days to bud initiation (84.03 days) and was found statistically at par with Sonar Bangla (83.33 days) to initiate the flower buds. The days to bud initiation to first flower bud appearance is an important parameter reflecting earliness as well as late flowering habit of a variety, and holds a significance pertaining to the availability of flowers in the market (Behera *et al.,* 2002). The highest number of days to flower opening (104.87 days) was observed in the variety Sonar Bangla. The variety Swan Dance bloomed earliest (94.87 days) to anthesis, which was statistically at par with Snowball and Kikobiory. The cultivar which bloom early likely to reach or capture the market relatively earlier and could be a decisive factor for the farmer to cultivate

colourful varieties taking into consideration their varying response groups and their optimum stage for marketing (Laxmi *et al.*, 2008).

The flower diameter is an important floral parameter that determines the likely weight of a flower which can be used as loose flower or for exhibition purpose. The large sized chrysanthemum inflorescence is desired for exhibition purpose and sometimes raised by the growers owing to consumer demand (Kireeti *et al.,* 2017). The maximum diameter of flower was observed in Swan Dance (19.50 cm) followed by 'Snow Ball' (17.69 cm), while, minimum flower diameter (13.30 cm) was observed in Purnima. Similar variations in diameter of flower have been reported by Kumar and Polara (2017).

Considerable variations were recorded for the duration of flowering in different chrysanthemum varieties (Table 1). The variety Thai Chen Queen exhibited longest flowering duration (33.73 days), whereas, the shortest duration (23.63 days) was recorded in Swan Dance. These variations in flower diameter are requisite for the commercial flower market which provides an opportunity to select the varieties with profuse flowering with long blooming period. Similar variation in flowering among chrysanthemum varieties have also been reported (Srilatha *et al.*, 2015).

Flower colour of different varieties was observed and the colour codes were designated as per the standard Royal Horticultural Society Colour Charts (RHSCC), London. Variations in flower colour were observed among the ten varieties and are categorized into white, yellow, yellow white, orange red, red purple and purple violet group. The variation in flower colour among chrysanthemum varieties may also be due to the distinct genetic makeup and different proportion of pigments present in a particular genotype. The longest vase life (22 days) was observed in 'Pusa Centenary' and the shortest vase life (16.00 days) was observed in variety Valliant (Fig. 2). The variation in vase life may be due to genetic makeup of cultivars (Singh *et al.,* 2017).

The varietal differentiation according to the maturity group is important for consumer preference. The variation among maturity and flowering duration is determining factors, especially for pot cultivation of chrysanthemum. The observations revealed that all the ten varieties assessed matured between 8 to 12 weeks, thus have been categorized under the medium maturity group (Table 2). Wide range of variation with respect to flower form *viz*., regular incurve, decorative, irregular incurve and spider etc. was observed. The trait such as flower shape and flower form is totally accredited to the genetic factor (Behera *et al.,* 2002). All the evaluated varieties can be used for pot culture and exhibition purposes depending upon consumer preferences.

Table 2 : Characterization of chrysanthemum varieties for different maturity groups and commercial utilization

Variety	Flowering season	Maturity group	Flower form	Pot culture/ Exhibition	Cut flower
Denise Oatridge	November-December	Medium	Irregular Incurve	√	√
Kikobiory	November	Medium	Regular incurve	√	×
Otam Blaze	November	Medium	Decorative	√	√
Purnima	November	Medium	Decorative	√	√
Pusa Centenary	November	Medium	Decorative	√	√
Snow Ball	November	Medium	Regular incurve	√	×
Sonar Bangla	November	Medium	Regular incurve	√	×
Swan Dance	October-November	Early to medium	Spider	√	×
Thai Chen Queen	November	Medium	Decorative	√	√
Valliant	November	Medium	Spider	√	×

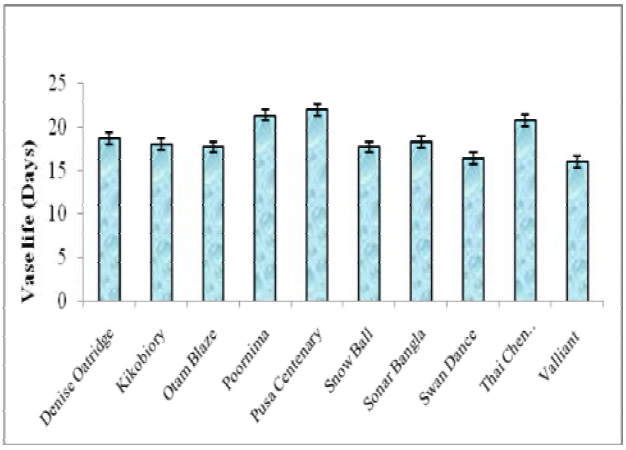

Fig. 2 : Vase life (days) of standard chrysanthemum

Therefore, the varieties Thai Chen Queen, Purnima, Pusa Centenary, Otam Blaze and Denise Oatridge with medium sized flowers and better keeping quality were found to be most suitable for pot culture, cut flower and flower arrangement, whereas, the varieties Snow Ball, Kikobiory, Sonar Bangla, Valliant and Swan Dance with bigger sized flowers were found suitable for pot culture and exhibition purpose.

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