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Original Research Paper

Efficient plant regeneration in arrowroot *(Maranta arundinacae* L.) for mass multiplication and *in vitro* germplasm conservation

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ABSTRACT

Arrowroot is a tuber crop gaining global importance as a potential source of high-quality natural starch, cultivated throughout the tropical and subtropical regions of the world. The present study aimed to standardize an efficient micropropagation protocol for rapid mass multiplication and *in vitro* germplasm conservation of arrowroot. Among the genotypes evaluated, the number of shoots per explant was highest (2.40) in the genotype M7 and was on par with M4 (2.21). Maximum regeneration was observed on the MS medium supplemented with 4.0 mg/l BAP and 0.5 mg/l NAA. The average number of shoots per explant (4.17±1.40), shoot length (3.78±0.37 cm) and number of leaves (6.92±2.23) were recorded maximum on the same medium. Whereas, the addition of TDZ at varying concentrations and combinations to the MS medium was found to be inhibitory to *in vitro* regeneration. Rooting of regenerated shoots was achieved on MS basal medium devoid of plant growth regulators. The regenerants were successfully acclimatized on a sterile mixture of soil, sand and farmyard manure at a 1:1:1 ratio before transferring to the main field.

Keywords: Arrowroot, germplasm conservation, mass multiplication, plant regeneration

INTRODUCTION

Arrowroot (*Maranta arundinacea*) is a monocotyledonous, herbaceous, perennial crop that belongs to the family Marantaceae. It produces several fusiform to cylindrical fleshy tubers and is cultivated as an annual crop. Its cultivation has spread throughout the tropical and subtropical regions of the globe. In India, arrowroot is mainly cultivated in Uttar Pradesh, Bihar, Orissa, West Bengal, Kerala, Karnataka and the North Eastern States of India (Shubhangi et al., 2022).

Arrowroot is an underexploited tropical tuber crop that has tremendous potential in the food and pharmaceutical industries. Its rhizome contains 10% to 25% extractable starch and is believed to be a good quality natural starch on earth (Spennemann, 1994). Consequently, its starch commands a high price in the international market due to its quality as a food ingredient. Arrowroot starch is utilized as an easily digestible food for babies, children, and individuals with dietary restrictions. It is also used in the preparation of various products such as biscuits, cakes, jellies, puddings, hot sauces, and more. Recent findings suggest that arrowroot flour is a potential source of prebiotics (Harmayani et al., 2011) and possesses immunostimulatory effects (Kumalasari et al., 2012). Consequently, it is preferred for the preparation of fine confectionery and is used in the pharmaceutical industries, competing with commercially modified starches. However, the shortage or unavailability of arrowroot starch in the market leads to a price increase and potential adulteration with commercial cornstarch or cassava starch (Jyothi et al., 2010; Guilherme et al., 2019).

The crop is commercially propagated vegetatively by planting entire rhizomes or rhizome bits and thus, requires large quantities (5.0-6.0 t/ha) of planting material. However, the spread and popularity of the crop is confined due to factors like slow multiplication rate (1: 4-7) and lack of good planting materials. In addition, vegetative propagation and the risks of disease transfer through planting materials are the major problems in the conservation and the exchange of germplasm. Thus, it was essential to standardise an efficient *in vitro* regeneration system for germplasm conservation and commercial production of arrowroot planting material through tissue culture. Very limited

work has been done on the micro-propagation of arrowroot (Daquinta et al., 2009; Sousa et al., 2019a; Souza et al., 2019b).

Considering the necessity for the development of high-frequency plant regeneration system and *in vitro* germplasm conservation, the present study on *in vitro* clonal propagation of arrowroot was carried out.

MATERIALS AND METHODS

The promising germplasm lines of arrowroot, M2, M4, M5 and M7 maintained by ICAR-Central Tuber Crops Research Institute were selected for *in vitro* propagation studies. Freshly harvested, healthy rhizomes were cleaned by washing with running tap water and incubated for sprouting at room temperature. Sprouts measuring 0.8 to 1.2 cm, which grew from these rhizomes, were excised (Fig. 2) and soaked for 4-5 minutes in distilled water with 2-3 drops of Tween-20 (Himedia®), followed by thorough washing with distilled water. Subsequently, the washed explants were surface sterilised with 1.5 per cent silver nitrate ($AgNO_3$) for 20 minutes under a laminar-airflow cabinet and finally rinsed 4 to 5 times with sterile double distilled water to take out the traces of $AgNO_3$ (Ines et al., 2013).

Fig. 2 : Mother plants (a) and sprouts raised from arrowroot rhizomes, (b) used to collect explants for *in vitro* regeneration experiments

Explants measuring 0.5 to 0.8 cm (without scales) were inoculated on MS medium containing 0.8% agar, 3.0% sucrose supplemented with varying concentrations and combinations of growth regulators to study the regeneration response of arrowroot (Table 1). The cultures were incubated at 25±2°C under 16 h light and 8 h dark cycles provided by white LED tube light. The growth of the cultures was examined at regular intervals and observations such as the number of shoots per explant, shoot length (cm), number of leaves and roots were recorded after 45 days of culture. The well-elongated individual shoots obtained from different treatments were separated and transferred to MS basal medium devoid of any plant growth regulators for root induction. After 45 days of culture, *in vitro* regenerants were carefully taken out from the culture tube without damaging their root systems and soaked in distilled water for five minutes. Then the regenerants were washed properly under running tap water to remove the traces of medium sticking to the roots. After washing, regenerated plantlets were planted in pots containing the mixture of garden soil, coarse sand and farmyard manure in a 1:1:1 ratio and placed under 50-60% shade. The plants were irrigated periodically to ensure soil moisture and relative humidity.

The experiment was conducted with 19 treatment combinations (Table 1) following a completely randomized design with three replications. To evaluate the data obtained from the different parameters, data were subjected to a two-factor analysis of variance (ANOVA) followed by Tukey's HSD test. All the analysis and preparation of graphs were carried out using the open-source statistical package 'R'.

Table 1 : Regeneration medium tested for the arrowroot multiplication

Treatment	MS medium supplemented with various concentrations and combinations of plant growth regulators (mg/l)		
	BAP	TDZ	NAA
T1			
T2	2.0		0.5
T3	2.0		1.0
T4	2.0		1.5
T5	3.0		0.5
T6	3.0		1.0
T7	3.0		1.5
T8	4.0		0.5
T9	4.0		1.0
T10	4.0		1.5
T11		0.5	0.5
T12		0.5	1.0
T13		0.5	1.5
T14		1.0	0.5
T15		1.0	1.0
T16		1.0	1.5
T17		1.5	0.5
T18		1.5	1.0
T19		1.5	1.5

Table 2 : ANOVA to investigate the effect of various treatments on *in vitro* regeneration of arrowroot genotypes

Source	Degree of freedom	No. of shoots/explants	Shoot length (cm)	No. of leaves	No. of roots/shoots
Treatments	18	12.43***	4.00***	27.95***	204.20***
Variety	3	2.77**	0.57***	3.57*	4.61
Treatments x Variety	54	0.25	0.02	0.63	0.40
Residuals/Error	152	0.55	0.08	1.33	3.70

*** significant at p< 0.001, ** significant at p< 0.01, *significant at p< 0.05

Table 3 : Response of arrowroot genotypes to *in vitro* regeneration

Varieties	No. of shoots/explants	Shoot length (cm)	No. of leaves	No. of roots/ shoot
M2	1.89±1.06^{b}	2.85±0.63^{ab}	4.25±1.70^{ab}	6.25±4.13^{a}
M4	2.21±1.29^{ab}	2.90±0.57^{a}	4.16±1.81^{ab}	6.75±4.67^{a}
M5	2.04±1.10^{b}	2.72±0.61^{b}	3.98±1.85^{b}	6.30±4.15^{a}
M7	2.40±1.31^{a}	2.95±0.66^{a}	4.58±1.90^{a}	6.77±4.46^{a}

RESULTS AND DISCUSSION

Healthy, actively growing arrowroot sprouts from axillary buds (Fig. 2) measuring 0.5 to 0.8 cm were inoculated vertically on regeneration medium to find out the optimum concentration of plant growth regulators for *in vitro* clonal propagation of arrowroot.

The ANOVA indicated the effect of treatments on *in vitro* regeneration of arrowroot genotypes as well as the interactions between treatments and genotypes. It revealed significant effects of treatments for parameters like number of shoots per explant, shoot length, number of leaves and roots. The result also showed a significant difference between the genotypes studied for these parameters except for the number of roots. However, there was no significant difference between genotype and treatment interactions for any of these parameters (Table 2).

The *in vitro* regeneration response of four arrowroot genotypes used in the study is presented in Table 3. Among the genotypes evaluated, the mean numbers of shoots per explants were highest (2.40) in M7 and were on par with M4 (2.21), while, lowest was observed in M2 (1.89) and M5 (2.04). The regeneration response of cultured explants was observed early in M7 and M4, hence the maximum shoot length was 2.95 cm and 2.90 cm, respectively after 45 days of culture, while, shoot length was recorded minimum in M5 (2.72 cm). Similarly, the number of leaves recorded highest in M7 (4.58), followed by M2 (4.25) and the lowest was recorded in M5 (3.98).

Regeneration and rooting were observed simultaneously on the regeneration medium. The number of roots per explant was not significant between the genotypes tested. Genotype M7 had a better performance in most of the traits evaluated.

The mean effect of the treatments on the regeneration of arrowroot genotypes differs significantly among themselves by Tukey's HSD test at $p \leq 0.05$ (Table 4). Induction of multiple shoots from the cultured explants was observed within 15 days of culture (Fig. 3a) on MS medium fortified with 6-Benzylaminopurine (BAP). *In vitro* regenerated arrowroot plants with swollen roots (Fig. 3b) were observed in the medium supplemented with varying concentrations of NAA irrespective of the addition of other growth regulators. Whereas, low regeneration efficiency was observed on the medium with TDZ. Similarly, turmeric responded well to regeneration on a medium supplemented with 4.0 mg/l BAP and 1.0 mg/l NAA (Nasirujjaman et al., 2005). Daquinta et al. (2009) found BAP was the potent cytokinin for arrowroot shoot proliferation and cultured arrowroot buds did not respond when cultured on a medium supplemented with TDZ. Cattarin et al. (2022) reported optimum shoot proliferation in

turmeric on MS medium containing 3 mg/l BAP. A maximum regeneration of ginger was achieved on MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l Kinetin (Genene et al., 2023). Relatively higher concentrations of NAA alone as well as in combination with other cytokinins promoted a higher percentage of callus induction in potato (Rahul et al., 2023).

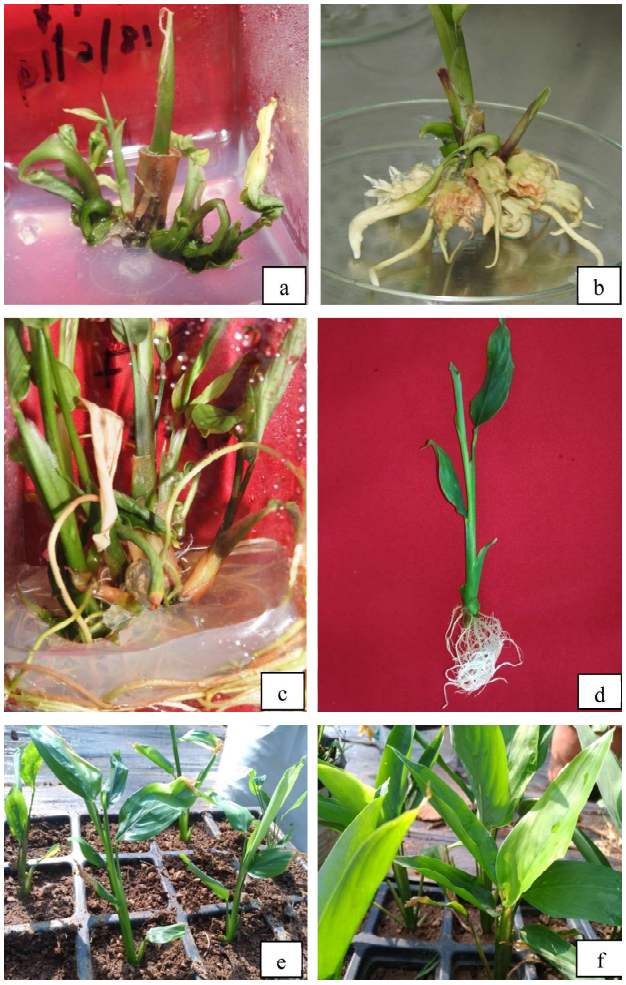

Fig. 3 : Regeneration response of cultured explants of arrowroot; a) *in vitro* proliferation; b) *in vitro* regenerated plants with swollen roots on MS medium supplemented with 3.0 mg/l BAP with 1.5 mg/l NAA; c) induction of multiple shoots on MS medium supplemented with 4.0 mg/l BAP with 0.5 mg/l NAA; d) *in vitro* regenerated arrowroot plantlet with a proper shoot and root system; e & f) acclimatization of regenerated plantlets

The average number of shoots per explant was maximum (4.17±1.40) in the medium T8 and the number of shoots per explant ranged from 2 to 6 (Fig. 1a and Fig. 3c). Similar effects were observed in the medium T9 (3.83±0.72), T5 (3.33±0.89) and T10 (3.25±0.87). The addition of thidiazuron (TDZ) to the MS medium was found inhibitory on the production of multiple shoots. The lowest number of shoots per explant, 1.08±0.29 was observed in the medium T16, T15 and T13 and followed by 1.17±0.39 in T19 (Table 4 & Fig.1a). Sayan et al. (2020) reported the highest number of shoots in wild turmeric on MS medium with 2.0 mg/l BAP and 0.75 mg/l IAA. Increased regeneration of cultured *Curcuma angustifolia* was observed when the BAP concentrations in the regeneration medium were increased from 2.9 to 13.3µM (Sudipta et al., 2018). MS medium with BAP at 13.31 µM was found most effective in inducing the maximum number of shoots of turmeric genotypes. Induction of a decreased number of shoots was also observed with the increased concentration of TDZ and KIN (Bhanuprakash et al., 2021).

Among all the media combinations, the highest shoot length (cm) was observed in T8 (3.78±0.37) and T1 (3.70±0.50) followed by T5 (3.52±0.11) and T2 (3.46±0.23). The effect of medium T8, T1, T5 and T2 did not differ significantly among themselves for shoot length. The shoot length on these media ranged from 3.0 cm to 4.4 cm (Fig.1b). Shortest shoots (1.87±0.17) were recorded in T19 (Table 4 & Fig.1b). Higher concentrations of NAA were found to be inhibitory to the shoot elongation irrespective of the BAP and TDZ concentrations used in the medium. The addition of TDZ was also found detrimental to shoot elongation.

The maximum number of leaves (6.92±2.23) recorded in the plants regenerated on the medium T8 (Table 4) and was on par with the plants regenerated on the medium T4 (5.58±1.31), T5 (6.42±1.38), T6 (5.83±1.27), T9 (6.33±1.15) and T10 (5.58±1.08). The number of leaves on these media ranged from 3 to 10 (Fig.1c) and the least number of leaves were noticed on the medium T16 (2.50±0.52) and T19 (2.50±0.67) followed by T13 (2.67±0.65), T15 (2.83±0.58), T17 (2.83±0.58) and T18 (2.67±0.65).

In the multiplication medium, rooting from the regenerated explants was also observed simultaneously. Comparable with media combinations, the average root numbers were maximum in the medium T1 (14.33±3.14); was followed by T2 (13.00±2.41) and T5 (12.75±2.49). The least number of roots were recorded in the medium containing TDZ (Table 4 & Fig. 1d).

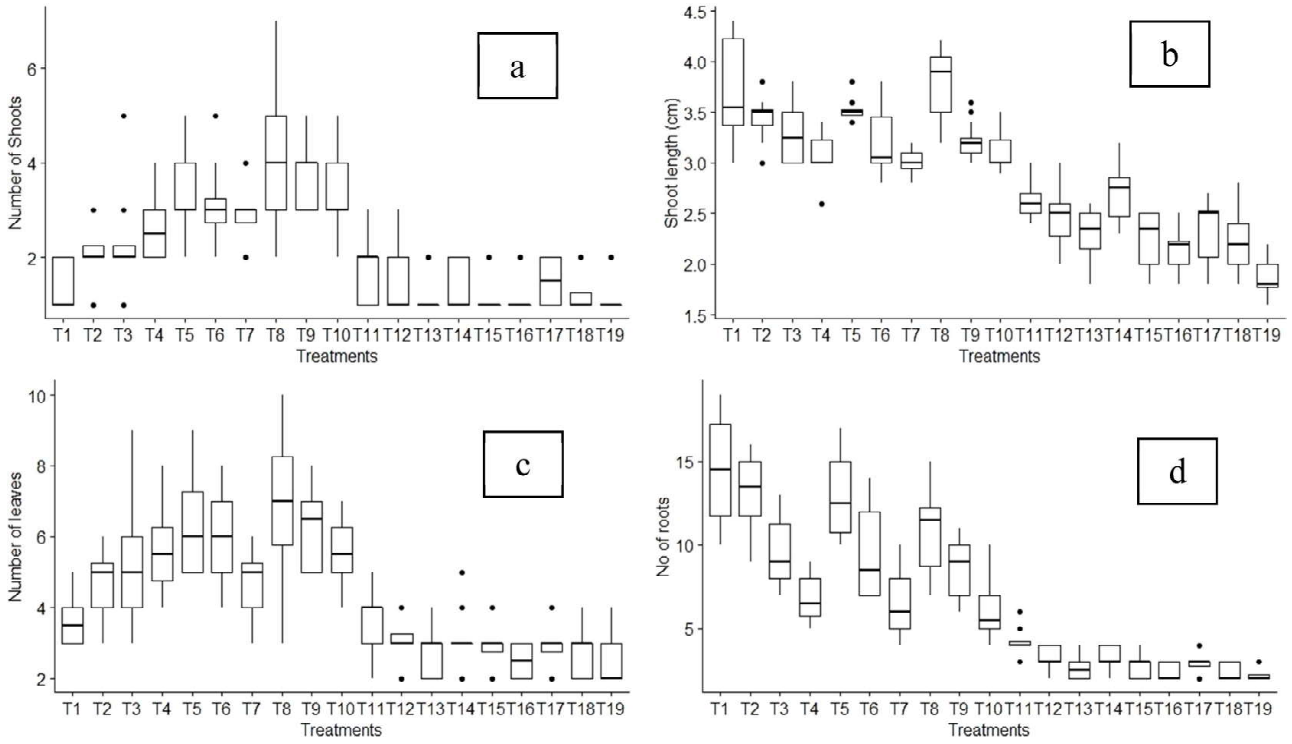

Fig.1: *In vitro* regeneration of arrowroot, a. Effects of treatments on the production of shoots, b. Effects of treatments on shoot length, c. Effects of treatments on number of leaves, d. Effects of treatments on number of roots

Table 4 : Effects of varying concentrations and combinations of growth regulators on *in vitro* regeneration of arrowroot

Treatment	No. of shoots/ explants	Shoot length (cm)	No. of leaves	No. of roots/ shoot
T1	1.42±0.51^{gh}	3.70±0.50^{a}	3.67±0.78^{cde}	14.33±3.14^{a}
T2	2.08±0.67^{efgh}	3.46±0.23^{abc}	4.75±1.06^{bcd}	13.00±2.41^{ab}
T3	2.25±1.06^{defg}	3.27±0.27^{bcd}	5.00±1.65^{bc}	9.50±2.07^{c}
T4	2.67±0.78^{cdef}	3.08±0.22^{cde}	5.58±1.31^{ab}	6.67±1.37^{def}
T5	3.33±0.89^{abc}	3.52±0.11^{ab}	6.42±1.38^{ab}	12.75±2.49^{ab}
T6	3.08±0.90^{bcde}	3.21±0.31^{bcd}	5.83±1.27^{ab}	9.33±2.50^{cd}
T7	2.83±0.58^{bcde}	3.00±0.14^{def}	4.75±0.97^{bcd}	6.50±1.83^{ef}
T8	4.17±1.40^{a}	3.78±0.37^{a}	6.92±2.23^{a}	10.92±2.64^{bc}
T9	3.83±0.72^{ab}	3.23±0.19^{bcd}	6.33±1.15^{ab}	8.83±1.70^{cde}
T10	3.25±0.87^{abcd}	3.11±0.18^{bcd}	5.58±1.08^{ab}	6.17±1.80^{ef}
T11	1.75±0.62^{fgh}	2.62±0.17^{fgh}	3.58±1.00^{cde}	4.17±0.83^{fg}
T12	1.42±0.67^{gh}	2.43±0.29^{ghi}	3.08±0.67^{de}	3.25±0.75^{g}
T13	1.08±0.29^{h}	2.30±0.27^{ghi}	2.67±0.65^{e}	2.58±0.67^{g}
T14	1.33±0.49^{gh}	2.69±0.29^{efg}	3.08±0.79^{de}	3.25±0.62^{g}
T15	1.08±0.29^{h}	2.26±0.25^{hij}	2.83±0.58^{e}	2.75±0.62^{g}
T16	1.08±0.29^{h}	2.15±0.20^{ij}	2.50±0.52^{e}	2.42±0.51^{g}
T17	1.50±0.52^{gh}	2.33±0.30^{ghi}	2.83±0.58^{e}	2.83±0.58^{g}
T18	1.25±0.45^{gh}	2.23±0.31^{hij}	2.67±0.65^{e}	2.33±0.49^{g}
T19	1.17±0.39^{h}	1.87±0.17^{j}	2.50±0.67^{e}	2.25±0.45^{g}

*values represent the means ±SD of three replications. Means followed by the same letter do not differ significantly among themselves by Tukey's HSD test at ($p \leq 0.05$)

Arrowroot plantlets with the proper shoot and root system were obtained after subculture (Fig. 3d) on an MS basal medium devoid of plant growth regulators. Similarly in sweet potato, the root number per plant and root length were reported to be highest in MS basal media (Arathi et al., 2019). The *in vitro* regenerants, after 45 days of culture were carefully taken out from the culture tube without damaging their root systems and soaked in distilled water for five minutes. Then the regenerants were washed properly under running tap water to remove the traces of medium sticking to the roots. The plantlets were transferred to pots containing a sterile mixture of soil, sand and farmyard manure at a 1:1:1 ratio and kept under shade for hardening. About 100% of regenerants survived (Fig. 3e & f) and were transferred to the main field after 30 days of acclimatization and observed normal growth. Similarly, survival of about 95% of *Curcuma angustifolia* regenerants was reported during acclimatisation on the potting medium containing 1:1:1 ratio of soil, sand and cow dung mixture (Sudipta et al., 2018).

CONCLUSION

An efficient micropropagation protocol has been standardised in arrowroot. Maximum regeneration efficiency was observed on the MS medium supplemented with 4.0 mg/l BAP and 0.5 mg/l NAA. Whereas, the lowest regeneration efficiency was observed on the medium with TDZ as the least number of shoots, shoot length, number of leaves and root numbers were recorded. Rooting was achieved on MS basal medium devoid of plant growth regulators. Plantlets were acclimatized under shade on a sterile mixture of soil, sand and farmyard manure at 1:1:1 ratio. Observed uniform normal growth when transferred to the main field and plants were phenotypically similar to their mother plant. This arrowroot regeneration procedure could be helpful for rapid mass multiplication, *in vitro* germplasm conservation and genetic improvement of arrowroot by transferring novel candidate genes.

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