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Original Research Paper

Pollen germination studies in Giant Himalayan Lily (*Cardiocrinum giganteum* Wall.) a high value of ornamental plant in Western Himalayan region

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ABSTRACT

Giant Himalayan lily (*Cardiocrinum giganteum* Wall.) is a perennial bulbiferous herb of Liliaceae and an endemic species in the eastern Himalayan region, which has become one of the new flower crops because of its high ornamental value. The present investigationwas conducted to study pollen morphologyi.e., fresh pollen grains size was measured in different media, *viz.*, water, acetocarmine, glycerol and without any medium (dry). The pollen grains of Giant Himalayan lily exhibited the average size (length x width) of (22.64 x 19.72µ) in water suspension. The result revealed that in glycerine and dry condition shape of pollen grains was perprolate. However, in water and acetocarmine it looked was round in shape. The result also shown that highest pollen germination (54.70%, 63.69%) and pollen tube growth (89.24µ, 175.85µ) in 10% sucrose solution for 12 hours and 24 hours respectively. In control light treatments, red light was found to be best in pollen germination and pollen tube growth.

Keywords : Pollen morphology, pollen tube, growth regulators, pollen germination.

INTRODUCTION

The Giant Himalayan lily (*Cardiocrinum giganteum* Wall.) is the largest species of the lily plants, belong to the family Liliaceae. The genus *Cardiocrinum* consists of three species distributed geographically in the Himalayas (*C giganteum),* China (*C. cathayanum)* and Japan (*C. cardatum)* among that *C. giganteum* (Wallich.) Makino [syn. Lilium giganteum (Patterson and Givnish 2002)], commonly known as giant Himalayan lily. It has two varieties: *C. giganteum* var. *Giganteum* and *C. giganteum* var. *yunnanense*. *C. Giganteum* var. giganteum is native to the Himalayan region of tropical Asia (Bhutan, India, Nepal and Mayanmar) and *C. Giganteum* var. *Yunnanense* to temperate Asia (Southeast China) (USDA- ARS 2009). In India, *C. giganteum* var. *giganteum* grows in dense deciduous moist temperate forest woodlands at altitudes of about 1,500-3,000 m in the Himalayas (Genders 1994 and Guar 1999).The plants produce bulblets at the base of the taxon (Fox 1985 and Huxley 1992). It is monocarpic in nature and produce flowers in monsoon (July- August) and seed setting occur in the autumn (October- November). *Cardiocarinum giganteum* is grown as an ornamental plant in temperate region of the Northern Hemisphere including the United States (Phartyal *et al*., 2012). Oldfield (1991) confirmed its continuation in international trade in horticultural bulbs of wild origin from India. In addition to being an introduced ornamental, the species is hermaphroditic and has been reported to be an invasive species in its introduced habitat. Phartyal *et al.,*(2012) stated that Giant Himalayan Lily has are suitable for screening purpose, grown in shaded and Bog Garden, border making and bedding purpose. The flowers can be used as a cut flower formatting bouquet, Flower arrangement and decorations. Beside ornamental value; plants known to contain bioactive compounds, such as isopimarane type diterpenoids (Liu,1984) locally used for medicinal purpose. The starchy bulbs of *C. giganteum* are the staple food of local people in Guangxi and Yunnan (Li, 1997). The great economic value of *Cardiocrinum* species has brought about over exploitation and habitat fragmentation/ isolation of their natural populations (Li *et al.,* 2012) which might decrease not only population size but also genetic diversity. Present studies on by keeping the above points in considered to the study of pollen germination and pollen tube growth for further biological studies.

MATERIALS AND METHODS

A field experiment was conducted at College of Horticulture, VCSG, UUHF, Bharsar, Pauri Garhwal, Uttarakhand, India during June 2020. The experimental site located at an altitude of 1900 m asl at a Longitude of 78.99^0 E and Latitude of 30.056^0 N. The climate of Bharsar is typically temperate type with mild summer, higher precipitation during rainy season and severe cold prolonged winter with occasional snowfall (Bisht and Sharma 2014).

The size of pollen grains was measured with the help of ocular micro indexed against stage micrometre (Aneja, 2003). The pollen morphology size measurement was done in different media, *viz.,* water, acetocarmine, glycerol and no medium (dry). The experiment used replicated five times in complete randomized block design (Gomez and Gomez 1984). In second experiment effect of different chemicals *viz.,* sucrose, IAA, IBA and GA_3 (1, 5, and 10 ppm each) on pollen germination was done. The treatment was replicated three times in complete randomized block design. In third experiment effect of different light colours *viz.,* red, blue, green violet and pink in pollen were also tested and replicated five times in complete randomized block design. Pollen was collected from mature anthers. Bulk of the pollen was distributed into germination media in cavity slides and placed at room temperature (15±22 ºC) for 12 and 24 hours. Germination was quantified as the percentage of germinated pollen grains per 100 evaluated. Pollen grain was considered germinated when the pollen tube length was greater than the diameter of the pollen grain (Tuinstra and Wedel, 2000).

RESULTS AND DISCUSSION

Pollen morphology (Size and shape)

The pollen grains of Giant Himalayan lily (Table 1 and Fig. 1) exhibited the maximum size (length x width) of 22.64 x19.72µ in T_1 (water suspension).The minimum size of pollen grains in length acetocarmine staining 19.02µ and width dry conditions 11.37µ. The shape of pollen grains in glycerine and dry condition were found perprolate whereas, in acetocarmine and water it looked round. The result of present investigation was accordance with the finding of Selvarasu *et al.,* (2019) found the average fresh pollen size was 65.24 µm in *G. rothschildiana*. In dry conditions and glycerol shape of pollen was perprolate which was actual shape of pollen grains, while in other media *i.e.,* acetocarmine and under water conditions it looked like round. The perprolate shape in glycerine and dry conditions might be due to inner gradient matter which in not equally proportion to the exine material of pollen grains. Prativa *et al.* (2012) the differences in the pollen diameter among the varieties might be due to their genetic makeup in rose. Hemanta *et al.,* 2017.The comparison of pollen diameter between the small bud and large bud showed that the large bud had bigger pollen diameter than the small bud. The reason for bigger pollen diameter of large bud might be due to the bigger size of bud and maturity.

Pollen germination and pollen tube length

The data of pollen germination and pollen tube length in Giant Himalayan Lily were taken at 12 hours intervals. Presented in Table 2 and Fig. 1. The data revealed that the maximum pollen germination

Table 1 : Variation in pollen shape and size in different media

Treatment	Size of pollen grain (µ) ± SE (m)	
	Length	Width
T_1 - Water (control)	22.64 ± 0.56	19.72 ± 0.40
T_2 - Dry conditions	21.80 ± 0.18	11.97* ± 0.18
T_3 – Acetocarmine	19.02*± 0.89	17.81* ± 0.66
T_4 – Glycerol	20.42*± 0.39	13.05 *± 0.24
SE(d)	0.810	0.590
$CD_{(0.05)}$	1.732	1.260
C.V	6.107	5.960

µ = micron (unit of pollen length and width)
*Significant at 5 % level of significance as compared with (control)

Table 2 : Pollen germination percentages and pollen tube elongation at different incubation time

Treatment	Pollen germination (%) ± SE (m) (12 hours)	Pollen germination (%) ± SE (m) (24 hours)	Pollen tube length(µ) ± SE (m) (12 hours)	Pollen tube length(µ) ± SE (m) (24 hours)
T_1 Water	20.37±0.74	32.36±1.05	16.63±0.96	32.25±3.86
T_2 1 % Sucrose	40.73*±1.29	55.86*±5.73	38.65±12.30	55.57*±9.15
T_3 5 % Sucrose	49.46*±4.62	56.74*±1.63	61.18*±4.74	119.06*±11.16
T_4 10 % Sucrose	54.70*±2.32	63.69*±3.56	89.24*±39.73	175.85*±8.50
T_5 20 % Sucrose	36.26*±3.15	40.45*±2.09	21.86±1.46	44.41±3.14
T_6 1 ppm GA_3	37.73*±0.82	48.50*±1.88	22.24±1.38	36.60±1.01
T_7 5 ppm GA_3	32.43*±0.36	38.02±1.12	23.41±2.80	32.50±1.81
T_8 10 ppm GA_3	27.68*±0.34	35.81±1.23	24.83±2.69	32.48±1.33
T_9 1 ppm IBA	25.12±0.97	38.08±0.78	21.04±0.87	73.46*±2.09
T_{10} 5 ppm IBA	29.19*±4.92	35.28±0.84	47.09±6.14	73.21*±9.97
T_{11} 10 ppm IBA	25.87±2.99	34.08±1.03	22.93±2.42	39.35±3.26
T_{12} 1 ppm IAA	26.50±0.57	39.34*±0.54	20.96±1.70	40.78±2.75
T_{13} 5 ppm IAA	25.20±2.03	37.21±0.54	29.45±1.84	51.91*±12.94
T_{14} 10 ppm IAA	23.46±0.56	35.53±1.26	28.41±5.55	40.65±1.58
SE (d)	3.355	3.032	16.276	9.277
$CD_{(0.05)}$	6.907	6.243	33.511	19.100
C.V	12.649	8.798	59.635	18.754

µ= micron (unit of the pollen tube length and width)
*Significant at 5% level of significance as compared with control

percentage was recorded with T_4 (54.70%) 10% sucrose solution. The minimum pollen germination percentage was recorded with T_1 (20.37%) water. All the treatments were found to be significant as compared to control except T_9 (IBA 1ppm), T_{11} (IBA 10ppm), T_{12} (IAA 1ppm), T_{13} (IAA 5ppm) and T_{14} (IAA 10ppm). The maximum pollen tube length was recorded in T_4 (10% sucrose) 89.24 µ and found statically at par with T_3 (5% sucrose) 61.18µ. Minimum pollen tube length (16.63 µ) was recorded in T_1 water (control).

The pollen germination and pollen tube length in Giant Himalayan lily at 24 hours interval has been depicted in Table 2 and Fig.3. It was recorded that the maximum pollen germination percentage was recorded in T_4 (63.69%) 10% sucrose solution. The minimum pollen germination percentage (32.36%) was in T_1water (control) and found statically at par with treatmentsT_7 ($GA_3$5ppm), T_8 (GA_3 10ppm),T_9 (IBA 1ppm), T_{10} (IBA 5ppm), T_{11}(IBA 10ppm),T_{13}(IAA 5ppm) andT_{14}(IAA 10ppm). The maximum pollen tube length was recorded in T_4 (175.85 µ). 10% sucrose solution. The minimum pollen tube length (32.25 µ) was recorded in T_1 water (control) and found statically at par with treatments T_5(Sucrose 20%), T_6(GA3 1ppm), T_7(GA3 5ppm), T_8(GA3 10ppm), T_{11}(IBA 10ppm), T_{12}(IAA 1ppm) and T_{14}(IAA 10ppm). Hemanta *et al.* (2017) who reported highest pollen germination with 15 % sucrose and 15% sucrose + 60 ppm boric acid in Tuberose. Chaudhary (1991) and Jisha (1999) who reported the best pollen germination of gladiolus under 15 % sucrose + 75 ppm boric acid in germination medium. Yuxin *et al.* (2005) also found that sucrose and boron have great effects on the germination of lily pollen. Assessment of pollen viability has direct relevance in hybridization as pollen of male parent takes part in the fertilization process. Therefore, pollen germination study is an important activity in order to determine the potentiality of male parent for fertilization and seed setting after crossing

(Shivanna *et al.,* 1991). Mascarenhas and Mermeistein (1981) also emphasized the need for newly synthesized protein for tube growth. An array of plant growth regulators and other chemicals have been empirically added to the culture medium to promote pollen germination and tube growth and the positive effects of some of these substances have led to speculation about their biochemical functions. Further, pollen germination and tube elongation are independent processes governed by separate sets of conditions (Malik, 1985).

Effect of light color on pollen germination and tube elongation

The pollen germination and pollen tube length in Giant Himalayan lily plants at 12- and 24-hours interval is depicted in Table 3 and Fig. 2. It was observed that pink light (no germination) and violet light (20.85%) inhibited pollen germination, and also pollen tube growth. The maximum pollen germination and pollen tube elongation were observed in red light recording 35.99% and 46.23µ respectively. Finding is accordance with report by Nautiyal *et al,* (2009) in *Aconitum balfourii* (Benth) Muk. and *Aconitum heterophyllum* Wall. found highest pollen germination and pollen tube growth in red and green light respectively. Maximum pollen germination and tube elongation in red color suggest the involvement of phytochromes, as red synthesizes phytochrome protein and its biological manifestation (Sharma and Malik, 1978; Korner 1999).

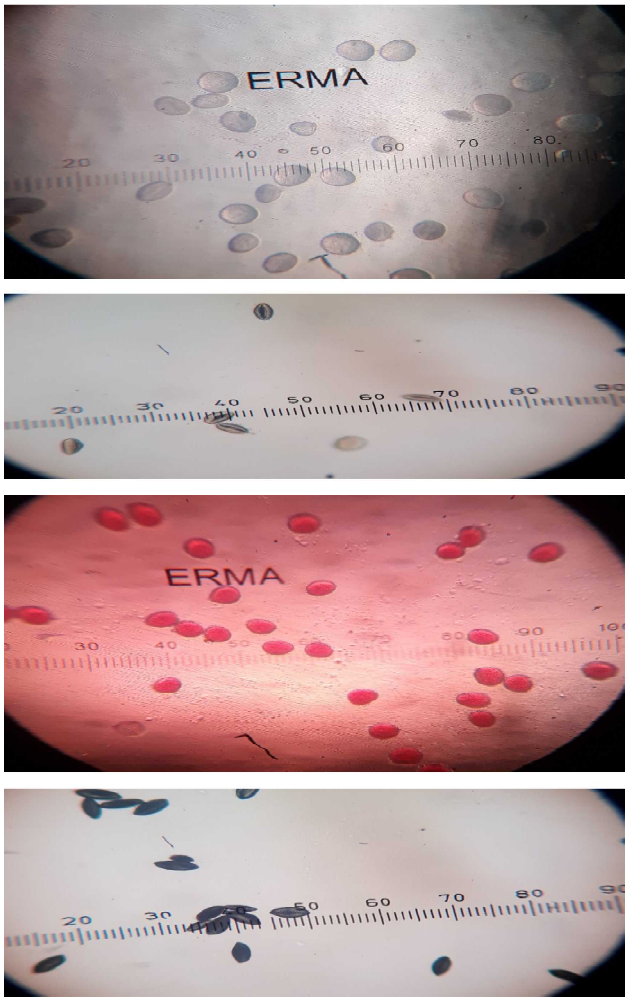

Fig. 1 : Pollen morphology Giant Himalayan Lilly a. water, b. acetocarmine, c. glycerine and d. dry (fresh condition)

Table 3 : Effect of light on pollen germination and pollen tube elongation at different incubation time

Light colour	Pollen germination (%) ± SE(m) 12 hours	Pollen germination (%) ± SE(m) 24 hours	Pollen tube length (µ) ± SE(m) 12 hours	Pollen tube length (µ) ± SE(m) 24 hours
T_1 Red	29.62±2.12	35.99±3.07	29.33±1.63	46.23±2.86
T_2 Blue	17.29*±0.98	27.38*±1.77	22.88*±1.36	31.94*±2.36
T_3 Green	23.50*±1.81	29.05*±2.21	27.49±3.80	36.10*±2.39
T_4 Violet	16.50*±1.33	20.85*±1.78	17.66*±0.80	30.87*±1.12
T_5 Pink	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
SE(d) 2.054	2.037	2.805	2.885	
$CD_{(0.05)}$ 4.315	6.052	5.891	6.060	
C.V. 18.683	20.105	22.770	15.711	

µ= micron (unit of pollen tube length and width)
*Significant at 5% level of significance as compared with T_1

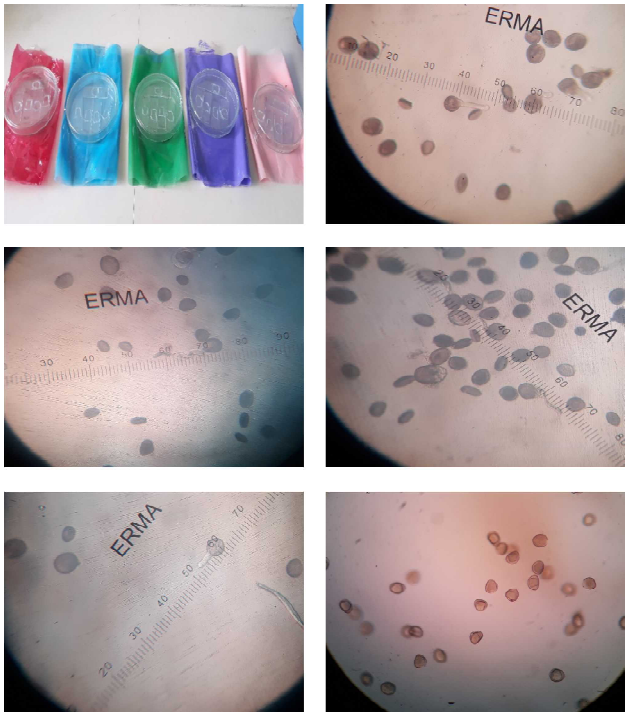

Fig. 2 : Effect of light on pollen germination and pollen tube elongation: a. different colours of light, b. red, c. blue, d. green, e. violet and f. pink.

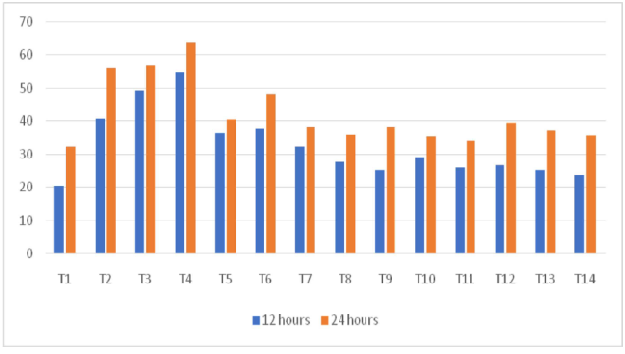

Fig. 3 : Percentage of pollen germination in Giant Himalayan Lilly

CONCLUSION

The pollen germination studies of *Cardiocrinum giganteum* Wall. Showed that maximum pollen size was recorded in T_1(water). The perprolate shape of pollen grain were obtained in glycerine and dry conditions whenever in water and acetocarmine it was found round shape. The maximum pollen germination and pollen tube growth were record in treatment 10% sucrose at 12- and 24-hours intervals. The result also showed among different light used red light is effective in improving pollen germination and pollen tube elongation.

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