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Effect of sucrose on *in-vitro* pollen germination of *Gloriosa superba* L. - a medicinal plant from the Garhwal Himalaya, India.

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Abstract

Pollen germination and pollen tube growth are prerequisites for fertilization in seed bearing plants. The present study deals with the morphology, production, pollen-ovule ratio, and effect of sucrose on *in-vitro* germination of pollen grains of *Gloriosa superba* L., family Colchicaceae. The *G. superba* L. pollen were radially symmetrical, prolate shape, 2-3 zonicolpate with striate surface. A single anther produced an average of 350,000 pollen grains. Pollen-ovule ratio was 58333.33/1. The maximum pollen germination (98.86%±0.641) along with 676.2µm pollen tube growth was observed in 5% sucrose solution, while in 20% sucrose concentration the germination percent decreased by 90% and the pollen grains' cytoplasm also shrinked. The pollen viability was found 96.22% and 91.79% in IKI and acetocarmine stain respectively. The study is useful in determining the pollen production and potential pollination capacity of *Gloriosa superba* L., as it is found that the species is xenogamous (self-compatible). The findings will be useful in assessing the pollen vigour by monitoring the rate of germination over a period of time on the length of pollen tubes.

***Key Words*: *Gloriosa superba* L., Pollen morphology, Pollen/ovule ratio, Pollen fertility, Sucrose effect, Garhwal Himalaya.**

Introduction

Gloriosa superba L. belongs to family Colchicaceae and popularly known as glory lily, creeping lily or flame lily in English, while Kalihari in Hindi and locally as Lakya and Langlya. It is a native of tropical Asia and Africa and found growing throughout tropical India upto an altitude of 2500m, including the foot hills of Himalaya (Chopra *et al.*, 1956). It is one of the endangered species among the medicinal plants (Badola, 2002). The genus *Gloriosa* comprises of 10 to 15 known species, out of which *G. superba* L. and *G. rothschildiana* L. are found in India (Tarar and Vishwakarma, 1995). It is a perennial tuberous climbing herb with solitary axillary flower, and is characterized by its low seed set in nature (Padamapriya *et al.*, 2015). The plant has fleshy cylindrical tubers which grow well in sandy loam soil. The flower has analgesic, anti-inflammatory, antimicrobial, antitumor and enzyme inhibition potential and is also used in treatment of snake bite, skin diseases and respiratory disorders (Abhishek *et al.*, 2011; Rehana *et al.*, 2011). In the Indian system of medicine, the tubers are used as tonic, antiperiodic, antihelmentic and also against snake bite (Gupta *et al.*, 2005). The tuber and seeds of *G. superba* contain valuable alkaloids *viz.*, colchicine and colchicoside which are exclusively used to treat cancer related diseases, almost every part of the plant contain these. Glory lily is mainly cultivated for its seeds due to medicinal property but in nature seed set of these plants is low. Pollen germination and pollen tube growth are prerequisites for fertilization and seed development. *In-vitro* germination determines the actual germination ability of pollen under suitable conditions (Tuinstra and Wedel, 2000), and it is the most widely used method of testing pollen viability in breeding programs (Marcellán and Camadro, 1996). The pollen is considered mature when the pollen tube length is longer than the diameter of the pollen grain (Wang *et al.*, 2004). Studies have suggested that *in-vitro* appropriate concentrations of sucrose can promote the germination and growth of the pollen tube of pollen grains (Pan *et al.*, 2009; Zhang and Huang, 2009). Pollen tubes are an excellent system for the study of polarized tip growth, cell movement, cell to cell communication, cell to cell recognition and signalling in plants (Megha and Subrata, 2014). In recent years, pollen

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germination along with pollen tube growth is used as research material to determine the importance of cytoskeleton in cell growth and differentiation (Ma *et al.* 2000; Mautinho*et al.*, 2001). P/O ratios are widely used in breeding system (Cruden, 1977). The P/O ratios reflect the likelihood of sufficient pollen grains reaching each stigma to result in maximum seed set (Cruden, 1977). The aim of present study is to find out the suitable nutrient media for *in-vitro* pollen germination and to evaluate the maximum pollen tube length for further biological studies.

Materials and Method

Study Area

The study was carried out in a semi-urban locality (Chauras campus) on the right flank of river Alaknanda in Garhwal Himalaya, India, at 30.22°N 78.78°E., with an elevation of 560 m asl. (Plate 1. Fig. A-B).

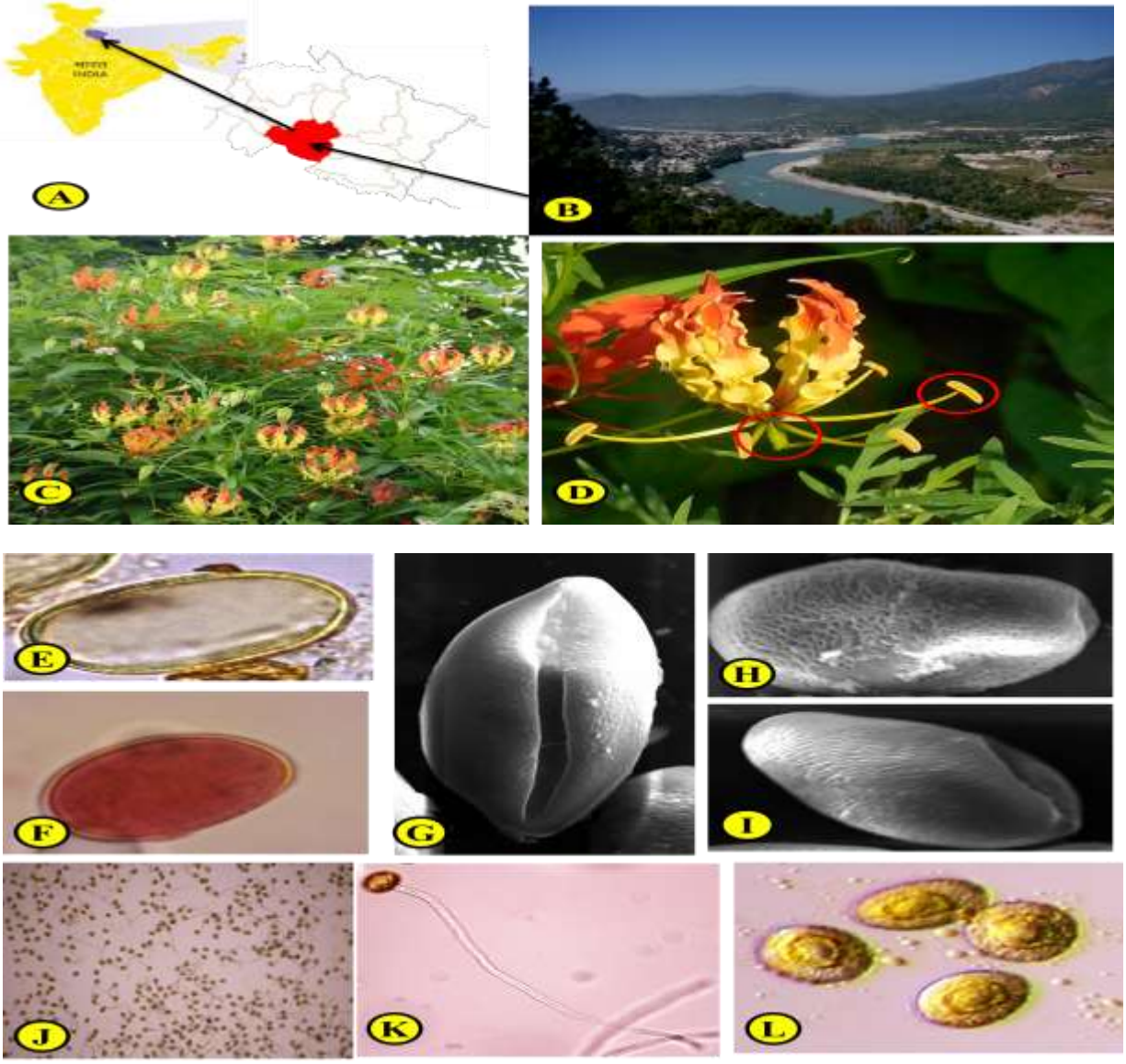

Plate 1. Photograph of study site, plant (*Gloriosa superba*, L.) and pollen grain

Fig A. Study area Fig B. Study site Fig C Plant *G. superba* L. Fig D. Flower Fig E. Light microscopy of pollen grain in equatorial view (1000X), Fig F. SEM of pollen aperture, Fig G. Pollen exine surface. Fig H , I. Pollen germination and pollen tube growth Fig J, K. Pollen germination and pollen tube growth, Fig l. Pollen cytoplasm shrink in 20% sucrose solution.

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The plant was identified following Gaur (1999) (Plate 1. Fig. C-D). The anther sample of the plant was collected in the morning after the anthesis and immediately transferred to the laboratory for further analysis. Pollen morphology was done following Erdtman (1952). Pollen production per flower and pollen/ovule ratio was calculated as per Cruden (1977). Fertility of pollen was tested by following Brewbaker and Kwack (1963).

Results and Discussion

The flowers of *G. superba* L. showed a typical structure such as solitary flowers, with 6 perianth lobes bent backward, six radiating anthers and the style almost 90^0 at the point of attachment to the ovary, these characters do not make them suitable for pollination by small insects (Gupta and Raina, 2001; Anandhi *et al.*, 2013). The anther dehiscence in *Gloriosa* starts from 7:30 a.m. goes upto 9:30 a.m. as also reported earlier by Mamatha, (1989); Rajamani *et al.*, (2009) and Anandhi *et al.*, (2013). The pollen observed were oval, prolate shape, 64.4±5.57µm in size, 2-3 zonicolpate with striate exine surface (Plate 2, Fig. E-I, Table 1).

Table 1. Pollen characters of *G. superba* L.

Character	Dimensions
Anthesis	7:30-9:30 a.m.
Anther dehiscence	Longitudinal
Pollen size	64.4±5.57(µm)
Pollen production	350,000 pollen/ anther
Pollen/ovule ratio	58,333.33/1
Pollen viability	96.22% (IKI) 91.77% (acetocaramine)
Pollen morphology	amb ellipsoidal, monad, radially symmetrical, shape prolate, size 64.4µm, 2-3 zonicolporate, exine 1.33µm thick, striate surface.

Pollen production was estimated 350,000/ anther, and the pollen/ovule ratio was observed 58,333.33/1 (Table 1). According to Cruden, (1977), the P/O ratios of above 19523, the plant shows xenogamous flowers. In the present study, the flower type of *G. superba* L. was xenogamous i.e, the species is self-compatible, as the the p/o ratio 58,333.33/1 was observed (Table 1).

Pollen VIABILITY showed 96.22% and 91.22 % in IKI and acetocaramine respectively (Table 1) and pollen germination(%) ranged from 9.37 (20% sucrose solution) to 98.86 (5% sucrose solution) (Fig 1), with the pollen tube length 106.25µm to 676.21µm respectively, (Table 2, Plate 2. Fig. J - L). The high pollen viability 96.22% and 91.22 % in IKI and acetocaramine respectively were observed. The similar results on high pollen viability were also observed by Anandhi *et al.*, (2013), (98.33%) on the day of anther dehiscence. We also found that *Gloriosa* pollen were viable on the third day also, with a mean of 88.25%, which is also in conformity with Anandhi *et al.*, (2013), as they found that *G. rotschilidiana* pollen showed 91.17% pollen viability till the third day. The duration of pollen viability depends on the internal, morphological factors of pollen and on the environmental conditions (Dafni and Firmage, 2000). Now a days, the pollen tubes are used as a model for studying the cell biology of plant cells (Mautinho *et al.*, 2001), because the pollen grains, are single celled structures which provide a unique system for *in vitro* studies (Katara, 2013). Sucrose is an important carbohydrate source for *in vitro* pollen germination, as it maintains osmotic pressure of the medium and act as a substrate for pollen metabolism (Shivanna and Johri, 1985). The optimum uptake of sucrose solution varied from species to species (Bhattacharya and Mandal, 2000). It is evident from the Table 2, that the highest pollen germination 98.86%, with a mean of 676.21±86.94µm pollen tube length was found in 5% sucrose solution after the 3 hours of experiment setup. Although the pollen germination in 10% sucrose solution is almost nearer to the maximum range 97.82% with a mean of 595.48µm pollen tube length, the minimum pollen germination 9.37% was found in the 20% sucrose solution with a mean of 106.25µm pollen tube length and on these concentration the pollen cytoplasm found to be

gradually shrinked. The findings of present study, (98.86%) pollen germination in 5% sucrose solution are in line with the earlier reported value 98.61% in 10% sucrose solution by Mamatha *et al.*, (1992) on *Gloriosa superba*. While Anandhi *et al*., (2013), observed 98.08% pollen germination with a mean of 47.72µm pollen tube length in *Gloriosa rothschildiana* L. In the present study pollen tube length of *Gloriosa superba* is observed 676.21µm, which is quite high then the previous one.

Table 2. Pollen viability (mean±SD) of *G. superba* L.

Time in hour		Different sucrose concentration			
		0%	**5%**	**10%**	**20%**
After 1 hour	A	30.36±7.08	70.41±11.98	46.36±9.32	1.62±1.06
	B	57.946±9.66	70.873±5.56	62.803±4.85	54.73±5.58
After 2 hour	A	50.1±5.76	88.81±2.6	86.38±4.19	5.19±0.704
	B	131.92±24.171	163.19±15.84	157.26±19.5	77.233±9.65
After 3 hour	A	75.36±7.08	98.86±0.641	97.82±1.02	9.37±3.92
	B	218.96±20.12	676.21±86.94	595.486±24.27	106.25±25.56

Where, A - Pollen germination (%); B - Pollen tube length (µm)

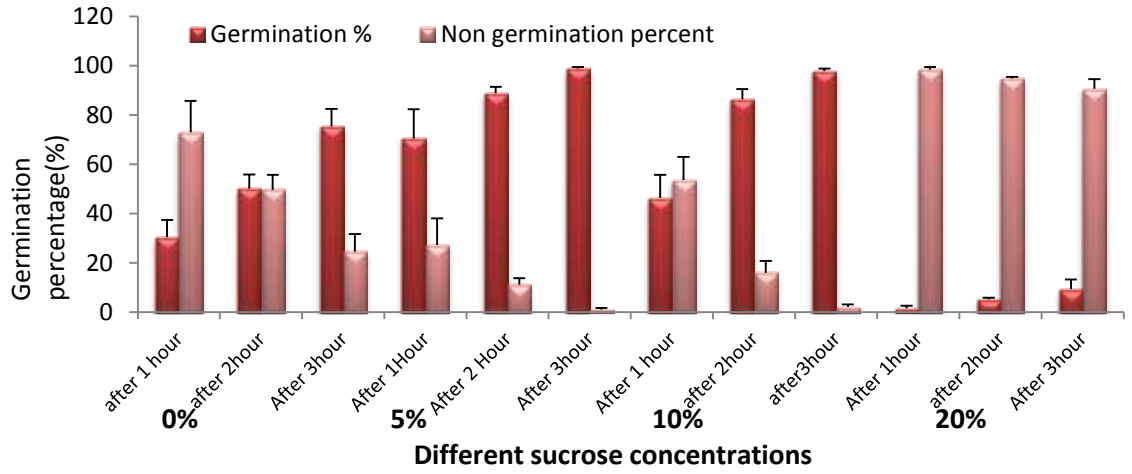

Figure 1. Effect of sucrose concentration on pollen germination(%)

Conclusion

Present study revealed that *Gloriosa superba* L. pollens have good fertility (98.86%) and are best for breeding and cell biology studies in plant cells with a 5% sucrose solution for *in-vitro* germination. High pollen germination of these plants need a low level of sucrose, if sucrose concentration is high the pollen cytoplasm shrinked, and not respond to germination. Although the plant is xenogamous and produces good amount of pollen grains but, due to it's typical flower structure which is not suitable for natural self and cross pollination, results into less number of seed set. Further studies need to be attempted on pollen tube growth and its respective inner constituents.

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