Indian Journal of Plant Sciences
Vol. 41 No. 1 (January-April, 2020)

Received: 27th December 2019 Revised : 2nd February 2020 Accepted: 19th March 2020

Research Article

STUDIES ON IN VITRO POLLEN GERMINATION OF *HELICTERES ISORA* LINN

Subrata Mondal* and Ramanjan Ghanta
Department of Botany, Visva-Bharati, Santiniketan-731235
**Author for Correspondence*

ABSTRACT
The present investigation reveals the effect of sucrose and boric acid on *in vitro* pollen germination of a medicinally important plant *Helicteres isora* Linn. Belonging to the family Sterculiaceae. Flowers open in the early morning (5.00 hrs.-6.00 hrs.) after which anther dehiscence take place. The maximum 98% pollen germination along with 806 μm long pollen tube developed in 5% sucrose solution supplemented with 100 ppm boric acid. Pollen grains which were collected in the morning (6.00 hrs. - 7.00 hrs.) Showed best results.

Key Words: *Pollen Germination, Pollen Tube, Pollen Viability*

INTRODUCTION
Germination is the first critical morphogenetic event in the pollen towards fulfilling its ultimate function of discharge of male gametes in the embryo sac. It is therefore important to understand the physiology and biochemistry of pollen germination. The stigma provides a suitable site for pollen germination. However studies on *in vitro* are not easily feasible because of the complications involving in pistillate tissue. It is possible to germinate pollen grains of a number of taxa using rather a simple nutrient medium and to achieve a reasonable length of tube growth. Our knowledge on physiology and biochemistry of pollen germination and tube growth comes largely from *in vitro* studies. Pollen grains, being the sexual reproductive unit and the carrier of male genetic material in higher plants, play a vital role in breeding programme and assists successful fruit-set. High crop yield generally depends on viable pollen grains. Pollen fertility and viability have a paramount importance in hybridization programme. Pollen performance in terms of germinating ability may have the relative importance upon not only fruit-set but also the flower-flower and flower-pollinator interaction. The present work is aimed to estimate the effect of sucrose and boric acid on *in vitro* pollen germination of *Helicteres isora* Linn., a medicinally important plant (Chopra *et al.,* 1956; Khare, 2007) belonging to the family Sterculiaceae.

MATERIALS AND METHODS
For the study of *in vitro* pollen germination, newly opened flowers were collected in the morning (6.00 hrs.-8.00 hrs.) and transferred to polythene bag. *In vitro* pollen germination was studied to know the effect of sucrose and boric acid at different concentration individually as well as in combinations. The fresh pollen samples were sown on several grooved slides containing solution of sucrose and boric acid at different concentrations separately or in combinations. Slides were then kept in Petridishes lined with moist filter paper and examined under a Olympus microscope at low magnification (10x X 15x) at different time intervals to know the germination percentage and pollen tube length following the method of Shivanna and Rangaswamy (1993). A pollen grain was considered as germinated if pollen tube length atleast becomes twice greater than the diameter of the pollen grains (Gupta *et al.,* 1989).

RESULTS AND DISCUSSION
Studies on *in vitro* pollen germination at different time intervals after anthesis indicated that 72% germinating pollen along with a mean of 559 μm long pollen tube development was observed in 8% sucrose solution (Table 1). Individually, 100 ppm boric acid showed 85% germination along with 741 μm long pollen tube (Table 2). The maximum 98% pollen germination along with 806 μm long pollen tube developed after 4 hours in 5% sucrose solution supplemented with 100 ppm boric acid (Table 3, Figure 1). Though the

effect of either sucrose or boric acid individually showed good results, but sucrose in combination with boric acid promoted pollen germination as well as tube development, because boron makes a complex with sugar and this sugar-borate complex is known to be capable of better translocation than non-borate, non-ionized sugar molecules (Gauch and Dugger, 1953; Sidhu and Malik, 1986).

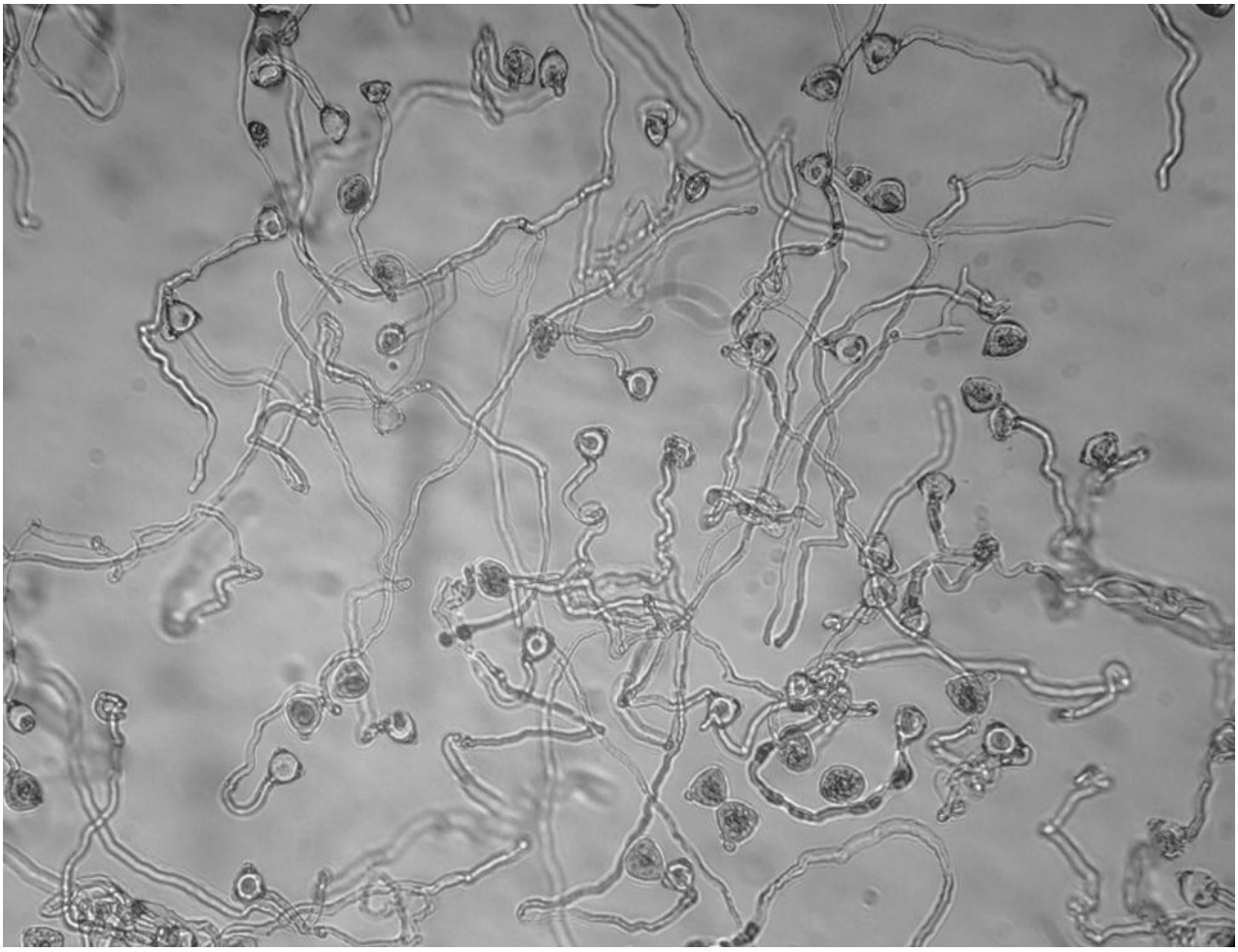

Figure 1: *In vitro* germinating pollen of *Helicteres isora*

The pronounced effect of sucrose and boric acid on increasing trend of germinating pollen might be reflected with the views of Johri and Vasil (1961) and Shivanna and Johri (1985) who stated that the externally supplied sucrose maintains the osmotic pressure and acts as a substrate for pollen metabolism. The role of boron has been confirmed in germinating pollen and growing pollen tubes in vascular plants (Lewis, 1980; Sidhu and Malik, 1986). The studies of Stanley and Loewus (1964) indicated that boron is directly involved in pectin synthesis and thus indirectly involved in development of pollen tube membrane. Scott (1960) suggested that boron could exert a protective effect in preventing excessive polymerization of sugars at sites of sugar metabolism. In nature water, sugar, amino acids are supplied by the style to nourish the growing pollen tube. Boron is also provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986). Boric acid is known to be crucial for pollen germination and tube growth and it is required at concentration of 100 ppm for most species (Brewbaker and Majumder, 1961).

Table 1: Effect of sucrose on *in vitro* pollen germination.

Conc. (%)	After 1 hr.		After 2 hrs.		After 4 hrs.	
	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)
Distilled water	--	--	--	--	--	--
1	13	130	26	195	30	260
2	36	234	44	260	52	325
5	42	247	56	429	64	460
8	48	286	67	494	72	559
10	25	221	35	260	48	312
15	16	78	18	156	25	195
20	3	78	8	104	10	117

Table 2: Effect of boric acid on *in vitro* pollen germination.

Conc. (ppm)	After 1 hr.		After 2 hrs.		After 4 hrs.	
	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)
25	17	364	27	546	30	624
50	55	481	65	507	74	650
100	60	481	78	585	85	741
200	45	208	71	351	75	390
300	40	182	54	273	60	325
400	27	117	30	143	36	208

Table 3: Effect of sucrose and boric acid on *in vitro* pollen germination.

Conc.	After 1 hr.		After 2 hrs.		After 4 hrs.	
	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)
100ppm +2%	43	234	52	364	60	442
100ppm+ 5%	65	390	89	650	98	806
100ppm +10%	66	364	92	416	95	533
100ppm +15%	37	234	42	377	50	468

Brewbaker and Kwack (1964) reported the induced role of Calcium and Boron on *in vitro* pollen germination. Boron plays a role in flowering and fruiting process in pistachio (Brown *et al.,* 1994) and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth (Nyomora and Brown, 1997). Boron takes part in pollen germination and style tube formation and therefore has a vital function in fertilization of flowering crops. Boron added in the form of boric acid, is also essential for the *in vitro* culturing of pollen from most species; for example, it is well appreciated that elimination of boric acid from the culture medium often leads to tube bursting (Holdaway-Clarke and Hepler, 2003 ; Acar *et al.,* 2010).Wang *et al.,* (2003) studied the effect of boron on the localization of pectins and callose in the wall of pollen tubes in *Picea meyeri*. Acar *et al.,* (2010) also reported the stimulatory effect of boron on *in vitro* pollen germination of *Pistacia vera*. Thus, the present work gets supports from Vasil (1964), Gupta *et al.,*

(1989), Pal *et al.,* (1989), Mondal *et al*., (1991), Bhattacharya *et al*., (1997) and Bhattacharya and Mandal (2004), Biswas *et al.,* (2008, 2009) and Acar *et al*., (2010).

REFERENCES

Acar I, Ak BE and Sarpkaya K (2010). Effect of boron and gibberellic acid on *in vitro* pollen germination of Pistachio (*Pistacia vera* L.), *African Journal Biotechnology* **9**(32) 5126-5130.

Bhattacharya A and Mandal S (2004). Pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lamk, Grana **43** 48–56.

Bhattacharya A, Mondal S and Mandal S (1997). *In vitro* pollen germination of *Delonix regia* (Boj.) *Raf. Sci. and Cult.* **63**(5-6) 143-144.

Biswas K, Mondal S and Mandal S (2008). Studies on *in vitro* pollen germination of *Solanum surattense* Burm.f. and *Solanum nigrum* L, *Sci. and Cult.* **74**(3-4) 149-152.

Biswas K, Mondal S and Mandal S (1961). Pollination Biology and Role of Carpenter bee (*Xylocopa sp.*) on fruit set of *Solanum torvum* Swartz. *Advances in Plant Biology*, edited by Mandal and Bhattacharya, 374-384.

Brewbaker J and Majumder SK (2009). Cultural studies of pollen population effect and self – incompatibility inhibition. *Am. Journal Botany* **48** 457.

Brewbaker JL and Kwack BH (1964). The calcium ion and substance influencing pollen growth. In: Pollen Physiology and Fertilization (Ed. Linskens HF) North-Holland publishing, Amsterdam 143-151.

Brown PH, Ferguson L and Picchioni G (1994). Boron nutrition of pistachio: Third year report. California Pistachio Industry, Annual Report- Crop Year 1992-1993 60-63.

Chopra RN, Nayar SL and Chopra IC. Glossary of Indian Medicinal Plants, C.S.I.R., NewDelhi. 1956.

Gauch HG and Dugger WMJr. The role of boron in the translocation of sucrose. *Plant Physiology* **28** 457-466.

Gupta S, Bhattacharya KN and Chanda S (1989). *In vitro* pollen germination of *Solanum sisymbriifolium* Lamk, Journal of Palynology **25** 65-72.

Holdaway-Clarke TL and Hepler PK (2003). Control of pollen tube growth:role of ion gradients and fluxes. *New Phytology* **159** 539-563.

Johri BM and Vasil IK (1961). Physiology of Pollen, *Botany Revisio* **27**(3) 318-381.

Lewis DH (1980). Boron lignification and the origin of vascular plants. *A Unified hypothesis, New Phytology* **84** 209-229.

Khare CP (2007). *Indian Medicinal Plants* .Springer (India) Pvt. Ltd, New Delhi.

Mondal S, Bhattachaya KN and Mandal S (1991). Studies on *in vitro* pollen germination of *Holarrhena antidysenterica* Wall, *Indian Biology* **23** 33-35**.**

Nyomora AMS and Brown PH (1997). Fall foliar-applied boron increases tissue boron concentration and nut set of almond. *Journal of Am. Sociology Horticulture Science* **122**(3) 405-410.

Pal JK, Mandal S and Bhattacharya GN (1989). Studies on the *in vitro* pollen germination of the two varieties of *Butea Monosperma* (Lam.) Taub. *Journal of Palynology* **25** 113-120.

Richards AJ (1986). Plant Breeding Systems. George Allen Unwin, London, England.

Scott EG (1860). Effect of supra optimal boron levels on respiration and carbohydrate metabolism of *Helianthus annuus. Plant Physiology* **35**(5) 653-661.

Sidhu RJK and Malik CP (1986). Metabolic role of boron in germinating pollen and growing pollen tubes. In : *Biotechnology and Ecology of Pollen*, edited by Mulcahy *et al.*, (Springer, New York) 373-378.

Shivanna KR and Johri BM (1985). The angiosperm pollen structure and function. Wiley Eastern Ltd., New Delhi.

Shivanna KR and Rangaswamy NS (1993). *Pollen Biology*: A laboratory Manual. - Narosa publ. House, New Delhi.

Stanley RG and Loewus FA (1964). Boron and myo-inositol in pollen pectin biosynthesis. In: Pollen Physiology and Fertilization, edited by Linskens HF, North-Holland publishing, Amsterdam: 128-139.

Research Article

Vasil IK (**1964).** Effect of boron on pollen germination and pollen tube growth. In: *Pollen Physiology and Fertilization*, edited by Linskens HF) North-Holland publishing (Amsterdam) 107-119.

Wang Q, Lu L, Wu X, Li Y and Lin J (2003). Boron influences pollen germination and pollen tube growth in *Picea Meyeri*, *Tree Physiology* **23**(5) 345-351.