Advances in Bioresearch
Volume 3 [3] September 2012: 63 - 66
ISSN 0976 – 4585

ABR
www.soeagra.com/abr/abr.htm

ORIGINAL ARTICLE

Studies on *in Vitro* Pollen Germination of *Lawsonia inermis* Linn.

Subrata Mondal* and Ramanjan Ghanta
Department of Botany, Visva-Bharati, Santiniketan-731235
*E-mail:submondal@rediffmail.com

ABSTRACT

The present investigation reveals the effect of sucrose and boric acid on in vitro pollen germination of a medicinal as well as dye yielding important plant Lawsonia inermis Linn. belonging to the family Lythraceae. It flowers almost throughout the year with a peak during June to August. Flowers open in the early morning (04.00 hrs.-06.00 hrs.) after which anther dehiscence take place. The pollen grains are 3- colporate. The maximum 92% pollen germination along with 1144 µm long pollen tube developed in 15% sucrose solution supplemented with 100 ppm boric acid. Pollen grains which were collected in the morning (06.00 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 study the effect of sucrose and boric acid on *in vitro* pollen germination of *Lawsonia inermis*, a medicinally important plant [1,2,3] also having long traditional use as natural dye and cosmetic [4,5] belonging to the family Lythraceae. It is a native of Arabia and Persia and now being cultivated mainly in Hariana, Gujrat, Madhyapradesh, Rajasthan and naturalized all over India [1,3].

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 nutrients like 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 an 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 [6]. A pollen grain was considered as germinated if pollen tube length atleast becomes twice greater than the diameter of the pollen grains [7].

RESULTS AND DISCUSSION

Studies on *in vitro* pollen germination at different time intervals after anthesis indicated that 72% germinating pollen with a mean of 858 µm long pollen tube development was observed in 15% sucrose solution (Table-1). Individually, 100 ppm boric acid showed 84% germination along with

897 µm long pollen tube (Table-2). The maximum 92% pollen germination along with 1144 µm long pollen tube developed after 3 hours in 15% sucrose solution supplemented with 100 ppm boric acid (Table-3, Fig-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-ionised sugar molecules [8,9].

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

Conc. (%)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination(%)	Mean tube length (µm)	Germination(%)	Mean tube length (µm)	Germination(%)	Mean tube length (µm)
Distilled water	--	--	--	--	--	--
1	--	--	--	--	--	--
2	--	--	--	--	--	--
5	--	--	13	273	16	312
10	34	273	46	455	58	598
15	38	278	56	552	72	858
20	26	208	34	390	42	494
25	16	198	29	264	32	299

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

Conc.(ppm)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination(%)	Mean tube length (µm)	Germinatio(%)	Mean tube length (µm)	Germination(%)	Mean tube length (µm)
25	29	152	44	370	54	412
50	32	208	56	680	74	756
100	40	299	74	754	84	897
200	32	287	63	698	70	845
300	26	280	51	650	58	819
400	19	274	28	362	32	416

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

Conc.	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination(%)	Mean tube length (µm)	Germination(%)	Mean tube length (µm)	Germination(%)	Mean tube length (µm)
100ppm+5%	16	308	28	416	36	512
100ppm+10%	32	414	51	688	58	884
100ppm+15%	68	544	84	890	92	1144
100ppm+20%	54	480	78	800	86	1084
100ppm+25%	36	410	52	611	58	762

The pronounced effect of sucrose and boric acid on increasing trend of germinating pollen might be reflected with the views of Johri and Vasil [10] and Shivanna and Johri [11] 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 [9,12]. The studies of Stanley and Loewus [13] indicated that boron is directly involved in pectin synthesis and thus indirectly involved in development of pollen tube membrane.

Scott [14] 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 [15]. 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 [16]. Brewbaker and Kwack (1964) reported the induced role of Calcium and Boron on *in vitro* pollen germination [17]. Boron plays a role in flowering and fruiting process in pistachio [18] and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth [19]. 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; and it is well appreciated that elimination of boric acid from the culture medium often leads to tube bursting [20,21].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* [22]. Acar *et. al*. (2010) also reported the stimulatory effect of boron on *in vitro* pollen germination of *Pistacia vera* [21]. Thus, the present work gets supports from Vasil [23], Gupta *et. al*. [7], Pal *et. al.* [24], Mondal *et. al*. [25], Bhattacharya *et. al*. [26] and Bhattacharya and Mandal [27], Biswas *et. al*. [28] and Acar *et. al*. [21].

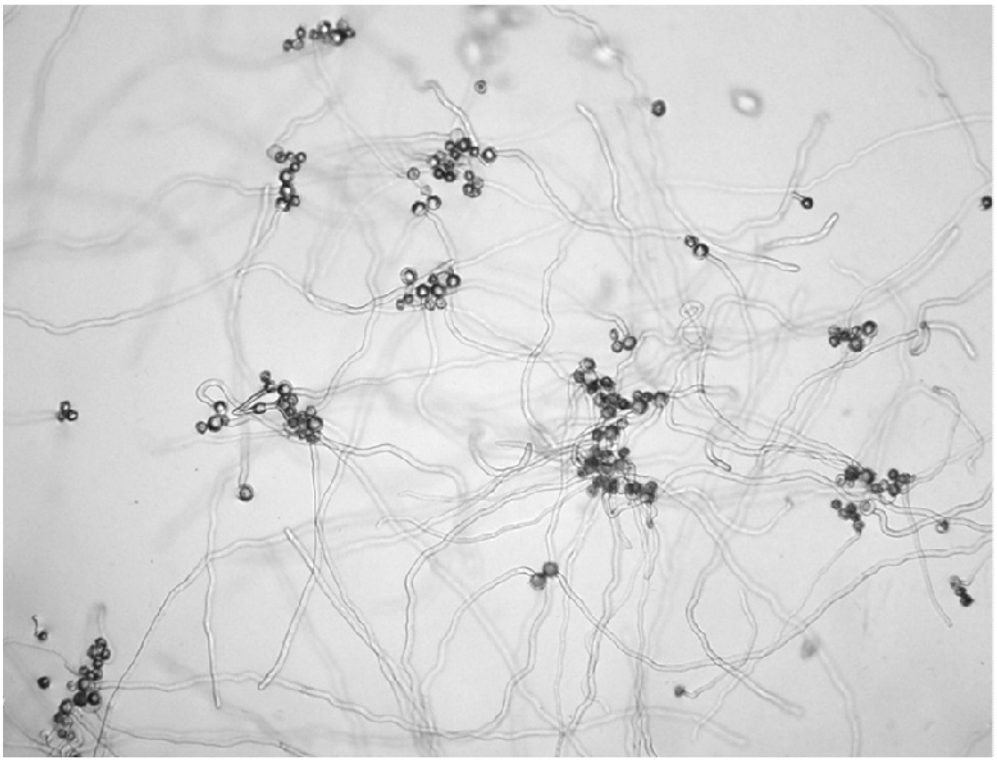

Fig.1. In vitro germinating pollen of *Lawsonia inermis*.

REFERENCES

1. Chopra, R. N., Nayar, S. L. & Chopra, I. C. (1956). Glossary of Indian Medicinal Plants, C.S.I.R., NewDelhi.
2. Muhammad, H. S. and Muhammad, S. (2005). The use of *Lawsonia inermis* Linn. (henna) in the management of burn wound infections. *Afr. J. Biotechnol.* 4 (9): 934-937.
3. Khare, C. P. (2007). Indian medicinal plants.Springer (India) Pvt. Ltd, New Delhi.
4. Zavada, Michael, S. (1993). The historical use of Henna (*Lawsonia inermis* L.). Thaiszia, Kosice, 3:97-100.
5. Kumar, S., Singh Y. V., & Singh, M. (2005). "Agro-History, Uses, Ecology and Distribution of Henna (*Lawsonia inermis* L. syn. Alba Lam)". *Henna: Cultivation, Improvement, and Trade.* Jodhpur.
6. Shivanna, K. R. and Rangaswamy, N. S. (1993). Pollen biology – A laboratory manual.- Narosa publ. House, New Delhi.
7. Gupta, S., Bhattacharya, K. N. and Chanda. S. (1989). *In vitro* pollen germination of *Solanum sisymbriifolium* Lamk, J. Palynol. 25 : 65 –72.
8. Gauch,H.G. and Dugger, W. M. Jr. (1953).The role of boron in the translocation of sucrose, Plant Physiol.28:457-466.

9. Sidhu, R. J. K. and Malik, C. P. (1986). Metabolic role of boron in germinating pollen and growing pollen tubes. –In : Biotechnology and Ecology of Pollen (eds. Mulcahy *et.al*.). 373-378, Springer, New York.
10. Johri, B. M. and Vasil, I. K. (1961). Physiology of Pollen, Bot. Rev. 27(3):318-381.
11. Shivanna, K.R. and Johri, B.M. (1985). The angiosperm pollen structure and function. Wiley Eastern Ltd., New Delhi.
12. Lewis, D. H. (1980). Boron lignification and the origin of vascular plants. A unified hypothesis, New Phytol. 84 : 209-229.
13. Stanley, R.G. and Loewus, F. A. (1964). Boron and myo-inositol in pollen pectin biosynthesis. In Pollen Physioilogy and Fertilisation. Edited by H. F. Linkens. North Holland. Publ. Co. Amsterdam. 128-139.
14. Scott, E. G. (1960). Effect of supra optimal boron levels on respiration and carbohydrate metabolism of *Helianthus annuus*. Plant Physiol., 35: 653
15. Richards, A.J. (1986). Plant Breeding Systems . George Allen Unwin, London ,England.
16. Brewbaker, J., Majumder, S.K. (1961). Cultural studies of pollen population effect and self –incompatibility inhibition. Am. j.Bot.48: 457
17. Brewbaker, J. L. and Kwack, B. H. (1964). The calcium ion and substance influencing pollen growth, Pollen Physiology and Fertilization. Edited by H. F. Linskens North-Holland publishing, Amsterdam. 143-151.
18. Brown, P. H., Ferguson, L. and Picchioni, G. (1994). Boron nutrition of pistachio:Third year report. California Pistachio Industry, Annual Report- Crop Year 1992-1993. 60-63.
19. Nyomora, A. M. S. and Brown, P. H. (1997). Fall foliar-applied boron increases tissue boron concentration and nut set of almond. *J. Am. Soc. Hort. Sci.* 122(3): 405-410.
20. Holdaway-Clarke, T. L. and Hepler, P. K. (2003). Control of pollen tube growth:role of ion gradients and fluxes. New Phytol. 159: 539-563.
21. Acar, I., Ak, B. E. and Sarpkaya, K. (2010). Effect of boron and gibberellic acid on *in vitro* pollen germination of Pistachio (*Pistacia vera* L.), *Afr. J. Biotechnol*., 9 (32): 5126-5130.
22. Wang, Q., Lu, L., Wu, X., Li, Y. and Lin, J. (2003). Boron influences pollen germination and pollen tube growth in *Picea meyeri*, Tree Physiol., 23 (5): 345-351.
23. Vasil, I. K. (1964): Effect of boron on pollen germination and pollen tube growth. In *Pollen Physiology and Fertilization*. Edited by H. F. Linskens., North-Holland Publ. Co. Amsterdam.. 107-119
24. Pal, J.K., Mandal S. and Bhattacharya, G.N. (1989). Studies on the *in vitro* pollen germination of the two varieties of *Butea monosperma* (Lam.)Taub. J. of Palynol. (25)113-120.
25. Mondal, S., Bhattachaya, K. N. and Mandal, S. (1991). Studies on *in vitro* pollen germination of *Holarrhena antidysenterica* Wall, Indian Biol. 23 : 33-35.
26. 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.
27. Bhattacharya, A. and Mandal, S. (2004). Pollination, pollen germination and stigma receptivity in *Moringa oleifera* Lamk, Grana 43 : 48 – 56.
28. 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.