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Studies on *In vitro* Pollen Germination and Tube Development of *Lens culinaris* Medik

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Abstract

The present investigation reveals the effect of different nutrients like sucrose and boric acid at various concentrations separately and in combinations and salts of calcium, magnesium and potassium on *in vitro* pollen germination of a medicinally and as well important crop plant *Lens culinaris Medik* belonging to the family Fabaceae. It flowers during June - September. Flowers generally open at 18:00 hrs. - 19:30 hrs. Maximum 80% pollen germination along with 1180 µm pollen tube development was observed in 1% sucrose solution supplemented with 100 ppm boric acid and among the salts; maximum 43% pollen germination along with 707µm pollen tube was observed in 100 ppm calcium nitrate solution after 4 hours incubation.

***Key words*:** Pollen germination, Sucrose, Boric acid, Salts, *Lens culinaris Medik*

Pollen germination as well as pollen tube growth is vital processes that make sure the productivity of flowering plants. The complex processes involve a number of signaling events; including cell-environment interaction, intercellular communication and intracellular signaling [1-2]. Physiological and molecular mechanisms of regulation of pollen germination and pollen tube growth have been extensively studied in the past [1-3]. The pollen tube formed in many species is a massive structure relative to the reserve materials stored in the pollen grain, and the reserves often are quickly consumed. The requirement of energy for the germination of pollen grains, formation of cell wall components and callose in angiosperms is provided from the nutriment reserves stored in pollen grains. These nutriment reserves are lipids, starches and sugars. Lipids and sugars are generally existing in pollen grains [4].

In vitro pollen germination is an effective technique for understanding the basic and applied aspects of pollen biology [3-6]. Such studies have provided considerable information on the physiology and biochemistry of pollen germination and pollen tube growth [7-8]. Pollen germination studies involve assessment of several nutrients which are stimulants for pollen germination and pollen tube growth, it is suggested that, the culture medium should contain in addition to carbohydrates germination-stimulating substances such as boric acid, calcium nitrate, potassium nitrate, magnesium sulphate [9-10]. So, *in vitro* pollen germination assay was conducted to determine the effect of different nutrients like sucrose, boric acid, calcium nitrate, magnesium sulphate and potassium nitrate at various concentrations on pollen germination of *Lens culinaris Medik* an medicinally important plant which is effective in bilious fever as a thirst reliever and laxative, root-purgative and tonic, antibilious, vermifuge, antidiarrhoeal leaves-emetic and purgative, treatment of alopecia. Fruits are generally used to improve appetite, cure biliousness [11].

MATERIALS AND METHODS

For the study of *in vitro* pollen germination newly opened flowers were collected in the evening (18.30 hrs. - 19.30 hrs.) and transferred to polythene bags for the *in vitro* pollen germination study. The fresh pollen samples were sown on several grooved slides containing solution of sucrose and boric acid at various concentrations separately and in combinations and salts of calcium ($Ca(NO_3)_2$), magnesium ($MgSO_4$) and potassium (KNO_3). Slides were then examining at different time of intervals to know the germination percentage and pollen tube length following the method of [12]. A pollen grain was considered as germinated if pollen tube length at least becomes twice greater than the diameter of the pollen [13].

RESULTS AND DISCUSSION

Studies of in vitro pollen germination at various time intervals after flowering reveal 65% pollen germination in 1% sucrose solution (Figure 1) and an average pollen tube development of 808 µm (Table 1, Fig 2), indicating 100 ppm boron. The germination rate was 43% for 606 µm pollen tubes (Table 2, Fig 3). After 4 hours in a 1% sucrose solution supplemented with 100 ppm boric acid, pollen tubes with a length of 1180 µm were developed and up to 80% pollen germination was observed (Table 3, Fig 4). In salt, a maximum pollen germination rate of 43% and pollen tube development of 707 µm was observed for the 100-ppm calcium nitrate solution, followed by a 200-ppm calcium nitrate solution with a pollen germination rate of 40% and pollen tube development of 707 µm. Magnesium solution. 404 µm was observed. In the 200-ppm potassium nitrate solution, the pollen germination rate was 26% and the pollen tube length was 252 µm (Table 4, Fig 5). Good results were obtained with both calcium and potassium,

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but maximum pollen germination and tube development were observed with calcium nitrate solution.

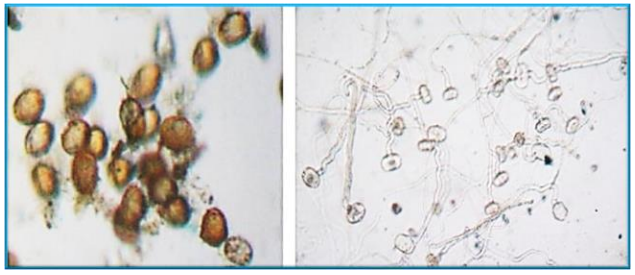
The action of sucrose or boric acid alone showed good results, but combining sucrose and boric acid promoted pollen germination and tube growth. This is because boron forms complexes with sugars, and this sugar-boric acid complex is known to have better translocation capabilities than non-punched sugar molecules. and non-ionized [14-15]. The pronounced effect of sucrose and boric acid on increasing pollen germination propensity is reflected, with sucrose maintaining osmotic pressure at surface conditions and showing that it acts as a substrate for pollen metabolism [7-8]. A role for boron in pollen germination and pollen tube development in vascular plants has been confirmed [15-16].

Table 1 Effect of sucrose on *in vitro* pollen germination of *Lens culinaris Medik*

Conc. of sucrose (%)	After 1 hour		After 2 hours		After 4 hours	
	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
Distilled water	-	-	-	-	-	-
0.5	6	101	18	202	38	323
1	17	202	38	404	65	808
2	10	110	25	330	45	606
5	7	65	17	202	31	445
8	5	50	8	99	21	234
10	4	40	7	82	14	126

Table 2 Effect of boric acid on *in vitro* pollen germination of *Lens culinaris Medik*

Concentrated of boric acid (ppm)	After 1 hour		After 2 hours		After 4 hours	
	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
50	7	81	12	172	15	222
100	20	202	35	303	43	606
200	9	101	19	245	41	450
300	5	50	10	91	22	224



Pollen grains Germinating pollen grains

Fig 1 Pollen and germinating pollen

Since boron is directly involved in pectin synthesis, it is indirectly involved in pollen tube membrane development [17], and boron exerts a protective effect by preventing excessive sugar polymerization at sites of sugar metabolism [18]. Natural water is supplied with sugars and amino acids to nourish the growing pollen tubes. Boron is also supplied by the stigma and style, facilitates sugar uptake, and plays a role in pectin production within the pollen tube [19].

External supply of K+ ions improved pollen germination rate and pollen tube development in Arabidopsis [20]. NO_3- and Mg++ promote tube growth in germinated sugarcane pollen in vitro [21]. A stimulating role for magnesium in pollen germination and tube development has been demonstrated in tobacco [22] and Gossypium [23]. Calcium, magnesium, and nitrates play important roles in pollen tube development in *Aedes loofah* [24].

Magnesium ions enhance the effect of calcium ions, resulting in vigorous pollen tube growth [9]. Role of sucrose, boric acid and various salts such as calcium nitrate, potassium nitrate and magnesium sulfate in pollen germination *in vitro* [25-29].

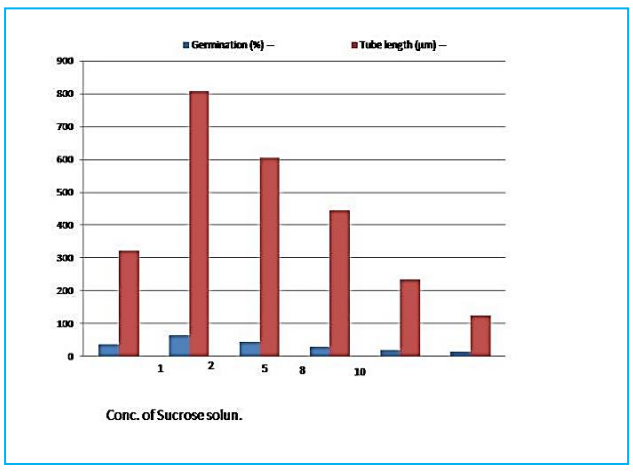


Fig 2 Pollen germination in sucrose solution

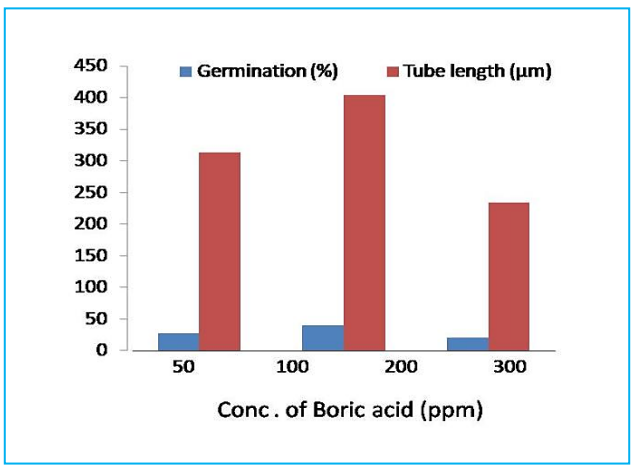


Fig 3 Pollen germination in boric acid solution

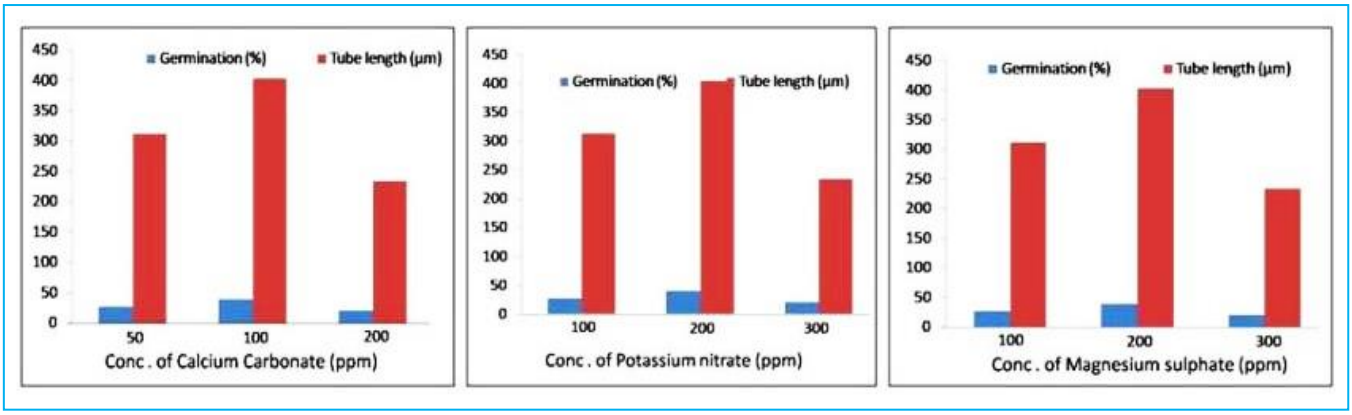


Fig 5 Pollen germination in salt of $Ca(NO_3)_2$, KNO_3 and $MgSO_4$ solution

Table 3 Effect of sucrose and boric acid (100 ppm) on *in vitro* pollen germination of *Lens culinaris Medik*

Conc. of sucrose (%) + Boric acid (ppm)	After 1 hour		After 2 hours		After 4 hours	
	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
0.5+ 100	14	151	42	363	55	838
1 + 100	23	404	51	808	80	1180
2 + 100	17	202	28	414	60	850
5 + 100	11	114	21	212	45	620
8 + 100	8	91	12	112	32	431
10 +100	5	54	9	89	20	291

Table 4 Effect of $Ca(NO_3)_2$, KNO_3 and $MgSO_4$ on *in vitro* pollen germination of *Lens culinaris Medik*

Salts	Conc. (ppm)	After 1 hour		After 2 hours		After 4 hours	
		Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
$Ca(NO_3)_2$	50	8	121	22	303	49	606
	100	12	220	31	404	43	707
	200	6	78	13	212	23	403
KNO_3	100	4	20	10	45	15	121
	200	8	56	16	144	26	252
	300	5	23	11	50	16	131
$MgSO_4$	100	7	67	18	187	27	313
	200	9	89	19	215	40	404
	300	5	52	12	154	21	234

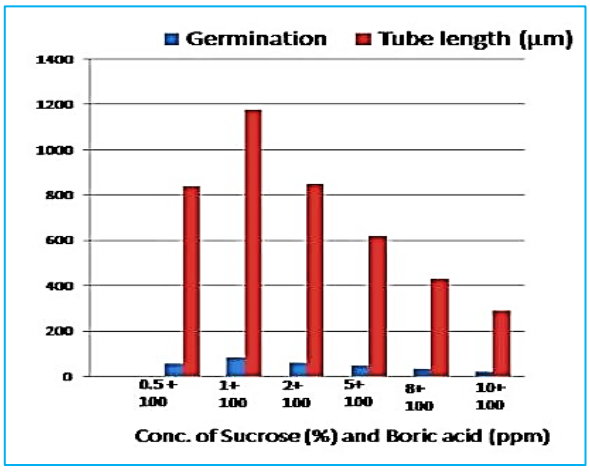


Fig 4 Pollen germination in sucrose solution with boric acid

CONCLUSION

The study you mentioned focuses on assessing pollen viability through in vitro pollen germination, which is a common and quantitative method used for this purpose. In this experiment, the researchers studied the effects of various concentrations of sucrose, boric acid, and certain salt solutions on pollen germination and tube growth. In vitro pollen germination is the most common and quantitative test for assessing pollen viability. Therefore, pollen germination and tube growth were studied in vitro using various concentrations of sucrose, boric acid and some salt solutions. Externally supplied sucrose maintains osmotic pressure and serves as a substrate for pollen metabolism. The effects of sucrose or boric acid individually showed good results, but combining sucrose and boric acid promoted pollen germination and tube development. This is because it is known that boron forms a complex with sugars and this sugar-boric acid complex can achieve better translocation than non-boronated, non-ionized sugar molecules. Overall, the combination of sucrose and boric acid in the growth medium creates an optimal environment for pollen germination and tube development in vitro, making it a preferred method for assessing pollen viability.

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LITERATURE CITED

1. Taylor LP, Hepler PK. 1997. Pollen germination and tube growth. *Annual Review of Plant Physiology and Plant Molecular Biology* 48: 461-491.
2. Franklin –Tong VE. 1999. Signalling and the modulation of pollen tube growth. *The Plant Cell* 11: 727-738.
3. Heslop Harrsion J. 1989. Pollen germination and pollen tube growth. *International Review of Cytology* 107: 1-78.
4. Baker HB, Baker I. 1979. Starch in angiosperm pollen grains and; its evolutionary significance. *American Journal of Botany* 66(5): 591-600.
5. Mascarenhas JP. 1993. Molecular mechanism of pollen tube and differentiation. *The Plant Cell* 5: 1303-1314.
6. Herreo M. 1991. Pollen tube development in *Petunia hybrida* following compatible and incompatible intraspecific mattings. *Journal of Cell Science* 47: 365-383.
7. Johri BM, Vasil IK. 1961. Physiology of pollen. *Botanical Review* 27(3): 318-381.
8. Shivanna KR, Johri BM. 1989. *The angiosperm Pollen Structure and Function*. Wiley Eastern Ltd., New Delhi.
9. Brewbaker JL, Kwack BH. 1963. The essential role of calcium ion in pollen germination and pollen tube growth. *American Journal of Botany* 50: 859-865.
10. Imani A, Kazem B, Saeed P, Seiyed HM. 2011. Storage of apple pollen and *in vitro* germination. *African Journal of Agricultural Research* 6: 624-629.
11. Khare CP. 2007. *Indian Medicinal Plants: An Illustrated Dictionary*. Springer.
12. Shivanna KR, Rangaswamy NS. 1993. *Pollen biology - A laboratory manual*. Narosa Publication House, New Delhi.
13. Gupta S, Bhattacharya KN, Chanda S. 1989. *In vitro* pollen germination of *Solanum sisymbriifolium* Lamk. *Journal of Palynology* 25: 65-72.
14. Gauch HG, Dugger WM. 1953. The role of boron in the translocation of sucrose. *Plant Physiology* 28: 457-466.
15. Sidhu RJK, Malik CP. 1986. Metabolic role of boron in germinating pollen and growing pollen tubes. *In*: Biotechnology and Ecology of Pollen. (Eds) Mulcahy *et al.* Springer New York. pp 373-378.
16. Lewis DH. 1980. Boron lignification and the origin of vascular plants: A unified hypothesis. *New Phytologist* 84: 209-229.
17. Stanley RG, Loewus FA. 1964. Boron and myo-inositol in pollen pectin biosynthesis. *In*: (Eds) Pollen Physiology and Fertilization. Linkens HF. North-Holland Publishing Company, Amsterdam. pp 128-139.
18. Scott EG. 1960. Effect of supra optimal boron levels on respiration and carbohydrate metabolism of *Helianthus annuus*. *Plant Physiology* 35(5): 53-661.
19. Richards AJ. 1986. *Plant Breeding Systems*. George Allen Unwin, London, England.
20. Fan L, Wang Y, Wang H, Wu W. 2001. *In vitro Arabbidopsis* pollen germination a characterization of inward potassium currents in *Arabidopsis* pollen grain protoplasts. *Journal of Experimental Botany* 52(361): 1603-1614.
21. Moore PN, Jung WL. 1974. Studies in sugarcane pollen. *In vitro* germination of pollen. *Phyton, International Journal of Experimental Botany* 32(2): 147-158.
22. Loguercio L. 2002. Pollen treatment in high osmotic potential: A simple tool for *in vitro* preservation and manipulation of viability in gametophytic populations. *Braz. Jr. Plant Physiology* 14: 1-9.
23. Burke JJ, Velten J, Oliver MJ. 2004. *In vitro* Analysis of cotton pollen germination. *American Society of Agronomy* 96: 359-368.
24. Prajapati PP, Jain BK. 2010. Effect of sucrose, boron, calcium, magnesium and nitrate during *in vitro* pollen germination in *Luffa aegyptiaca* Mill. *Prajna* 18: 5-8.
25. Vasil IK 1964. Effect of boron on pollen germination and pollen tube growth. *In*: (Eds) Pollen Physiology and Fertilization. Linkens HF. North-Holland Publishing Company, Amsterdam. pp 107-119.
26. Bhattacharya A, Mondal S, Mandal S. 1997. *In vitro* pollen germination of *Delonix regia* (Boj.) *Raf. Science and Culture* 63(5/6): 143-144.
27. Mondal S, Ghanta R. 2012. Effect of sucrose and boric acid on *in vitro* pollen germination of *Solanum macranthum* Dunal. *Indian Journal of Fundamental and Applied Life Sciences* 2(2): 202-206.
28. Saha H, Mondal S, Mandal S. 2016. Studies on *in vitro* pollen germination and tube development of *Borassus flabellifer* L. *International Journal of Recent Scientific Research* 7(6): 11985-11989.
29. Yao W, Li C, Lin S, Wang J, Fan T, Zhao W. 2022. The structures of floral organs and reproductive characteristics of ornamental bamboo species, *Pleioblastus pygmaeus*. *Horticultural Plant Journal* 9(3): 589-601.