In Vitro Pollen Germination and Tube Development Study of Pisum Sativum L.

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Abstract :

The present study revealed the effect of various nutrients such as sucrose and boric acid at different concentrations, individually and in combination, along with calcium, magnesium and potassium salts, on *in vitro* pollen germination of *Pisum sativum* L.., belonging to the family Fabaceae. Flowers bloom from December to March. Flowers usually open at 6pm. - 7:30 PM In 20% sucrose solution and saline supplemented with 100 ppm boric acid, up to 75% pollen germination and 656 *µm* pollen tube development were observed. After incubation in 50 ppm calcium nitrate solution for 6 h, maximum germination of 50% of pollen and pollen tubes of 452 *µm* were observed.

Key Words: Pollen germination, Sucrose, Boric acid, Salts, *Pisum sativum L.*.

Introduction :

Pollen germination and pollen tube growth are essential processes to ensure the productivity of flowering plants. Complex processes involve many signal events. This includes cell-environment interactions, cell-cell communication, and intracellular signaling (Taylor and Hepler, 1997; Franklin-Tong, 1999). The physiological and molecular mechanisms regulating pollen germination and pollen tube growth have been extensively studied (Heslop Harrison, 1989; Taylor and Heplar, 1997; Franklin-Tong, 1999).
Pollen tube formation in many species is a huge structure compared to the storage of material stored in the pollen grain, and that storage is often quickly consumed. The energy required for pollen grain germination and the formation of cell wall components and callose in angiosperms is provided by nutrients stored in the pollen grain. The stores of these nutrients are fats, starches, and sugars. Lipids and sugars are generally exists in pollen grains (Baker and Baker, 1979).
In vitro pollen germination is an effective technique for understanding the basic and applied aspects of pollen biology (Heslop Harrsion, 1989; Mascarenhas, 1993 and Herreo, 1991). Such studies have provided important insights into the physiology and biochemistry of pollen germination and pollen tube growth (Johri and Vasil, 1961; Shivanna and Johri, 1989; Heslop Harrison, 1989). Pollen germination studies include evaluation of several nutrients that stimulate pollen germination and pollen tube growth. Brewbaker and Kwak (1963). Imani *et al* (2011) suggested that in addition to carbohydrates, the growth medium should contain germination promoters such as boric acid, calcium nitrate, potassium nitrate, and magnesium sulfate. Therefore, an *in vitro* pollen germination test was conducted to investigate the effects of different nutrients such as sucrose, boric acid, calcium nitrate, magnesium sulfate, and potassium nitrate at different concentrations on the germination of *Pisum sativum* L. medicinally important plant which is used as a thirst reliever and laxative, roots are used for purgative, antibilious, vermifuge and antidiarrhoeal, leaves are used for emetic, purgative and treatment of alopecia. Fruits are generally used to improve appetite and cure of biliousness (Khare, 2007).

MATERIALS AND METHODS

To study pollen germination in vitro, newly opened flowers were collected in the evening (6:30–7:30 pm) and transferred to plastic bags. Fresh pollen samples were seeded onto several trough slides containing solutions of sucrose and boric acid at different concentrations, either individually or in combination. We also added salts of calcium Ca(NO3)2, magnesium (MgSO4), and potassium (KNO3). The slides were then examined at different time intervals to measure germination percentage and pollen tube length, following the method of Shivaanna and Rangaswamy (1993). A pollen grain was considered as germinated if the pollen tube length at least becomes twice greater than the diameter of the pollen (Gupta *et al.,* 1989).

RESULTS AND DISCUSSION

Studies on *in vitro* pollen germination at different time intervals after anthesis indicated that 60% germinating pollen along with a mean of 505 µm long pollen tube developments was observed in 20% sucrose solution (Table 1). Individually 200 ppm boric acid showed 50% germination along with 303 µm long pollen tube (Table- 2). The maximum 75% pollen germination along with 656 µm long pollen tube developed after 6 hours in 20% sucrose solution supplemented with 100 ppm boric acid (Table -3 Fig.-3).

Among the salts, maximum 50% pollen germination along with 452 µm pollen tube development in 50 ppm calcium nitrate solution following 45% pollen germination along with 302 µm long pollen tube in 100 ppm potassium nitrate solution and 35% pollen germination along with 292 µm pollen tube was observed in 100 ppm magnesium sulphate solution (Table-4). Both calcium and potassium showed good results, however maximum pollen germination as well as tube development was observed in calcium nitrate solution.

The combination with boric acid promotes pollen germination and tube development. This is because boron forms a complex with the sugar, and this sugar-borate complex is known to have a superior migration capacity than non-borate and non-ionized sugar molecules (Gauch and Dugger, 1953; Sidhu and Malik, 1986). 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), Shivanna and Johri (1989) 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 may have a protective effect by preventing excessive sugar polymerization at sites of sugar metabolism. In nature, water, sugar and amino acids are provided by the body to nourish growing pollen tubes. Boron is also provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube (Richards, 1986).

Salts like calcium, potassium and magnesium were used to study the effect of Ca, K and Mg ions on in vitro pollen germination. The results show that calcium ions most effectively affect pollen germination. Calcium is one of the most important cations involved in cellular metabolism. It is also known for its important role in maintaining membrane integrity and permeability (Jones and Lunt, 1967 and Brewbaker and Kwack, 1964). According to Kwak (1967), calcium probably strengthens the pollen tube wall by binding to pectin carboxyl groups and also induces pollen germination. Picton and Steer (1983) and Miller *et al*. (1992) demonstrated that calcium concentration plays an important role in maintaining oviduct growth. Pollen germination and pollen tube growth are primarily regulated by the transport of inorganic ions, such as Ca++ and K+, across the plasma membrane of pollen and/or pollen tubes (Feijo et al., 1995 and Taylor and Hepler, 1997). According to Huang et al. (2001), external supply of K + ions increased pollen germination rate and pollen tube growth in Arabidopsis. Moore and Young (1974) pointed out that NO3- and Mg++ promoted tube growth during sugarcane pollen germination in vitro. The stimulatory role of magnesium in pollen germination and tube development has been established in tobacco (Loguercio, 2002) and gossypium (Burke et al., 2004). Prajapati and Jain (2010) showed that calcium, magnesium, and nitrate play important roles in pollen tube growth of Aedes aegypti. According to Brewbaker and Kwak (1963), magnesium ions enhance the effect of calcium ions, which leads to vigorous growth of the pollen tube. Mondal et al. (1997); Chowdhury et al. (2013); Ganta and Mondal (2013) studied the role of sucrose, boric acid and various salts such as calcium nitrate, potassium nitrate and magnesium sulfate on pollen germination in vitro.

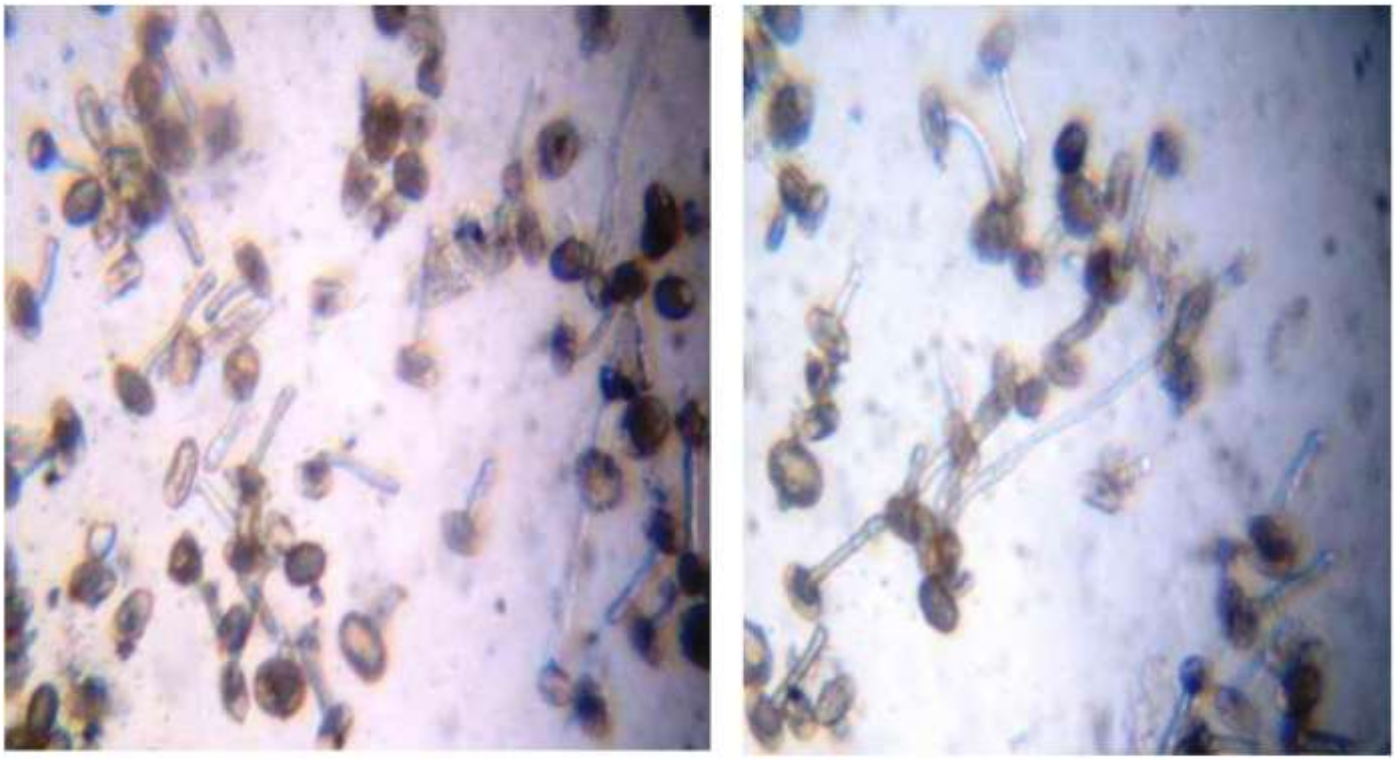


Fig.-3. Germinating Pollen Grains

Table-1. Effect of sucrose on *in vitro* pollen germination of *Pisum sativum L.*.

Con of Sucrose	After- 1hr.		After-3 hrs.		After- 6hrs.	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length (µm)
Distilled water	-	-	-	-	-	-
8%						
10%	5	30	10	78	30	251
15%	10	101	30	151	40	352
20%	15	151	40	252	60	505
25%	10	101	30	202	40	404
30%	5	50	10	89	25	201

Table -2. Effect of boric acid on *in vitro* pollen germination of *Pisum sativum L.*.

Concentration of Boric acid (ppm)	After 1hr.		After- 3 hrs.		After-6hrs.	
	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)	Germination (%)	Tube length (µm)
100	10	50	15	101	30	202
200	15	101	35	202	50	303
300	10	78	20	101	35	252
500						

Table-3.Effect of sucrose and boric acid (100 ppm) on *in vitro* pollen germination of *Pisum sativum* L.

Con of Sucrose + 100 ppm Boric acid	After 1hr.		After- 3 hrs.		After- 6hrs.	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)
8%						
10%	5	50	10	101	35	303
15%	10	101	35	222	50	404
20%	20	202	55	253	75	656
25%	15	101	40	252	55	504
30%	5	50	10	101	30	201

Table -4. Effect of $Ca(NO_3)_2$, KNO_3 and $MgSO_4$ on *in vitro* pollen germination of *Pisum sativum L.*.

Salts	Conc (ppm)	After 1 hr.		After-3 hrs.		After-6hrs.	
		Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)
$Ca(NO_3)$	25			10	50	28	101
	50	5	12	20	151	50	452
	100			9	40	21	80
	200						

KNO_3	25						
	50			15	50	35	80
	100	10	50	30	101	45	302
	200			10	40	25	60
	300						
$MgSO_4$	25						
	50			8	40	20	70
	100	10	40	20	101	35	292
	200			10	50	22	100
	300						

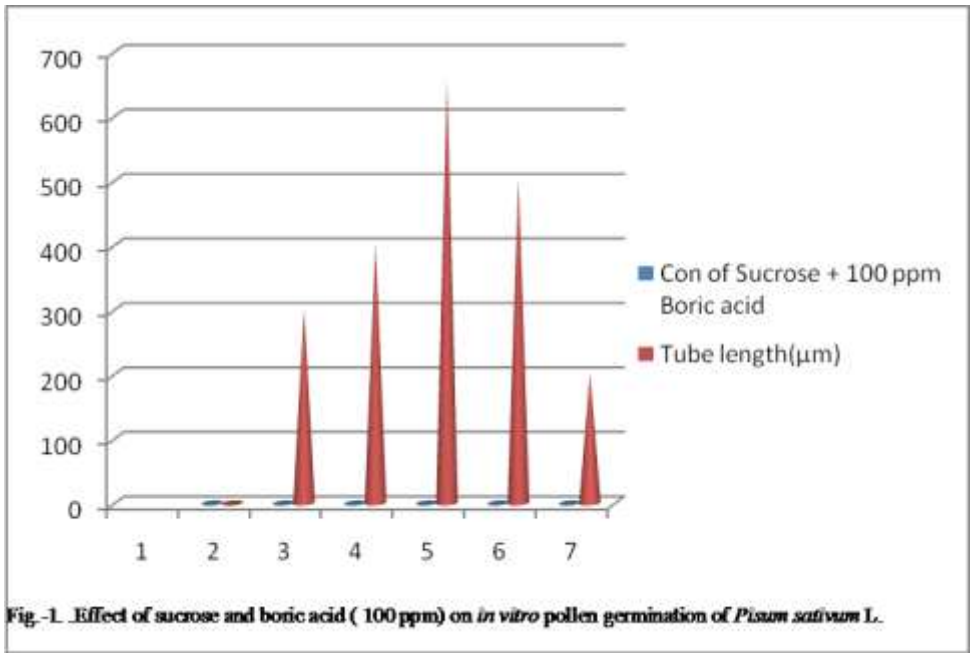


Fig.-1. Effect of sucrose and boric acid (100 ppm) on *in vitro* pollen germination of *Pisum sativum* L.

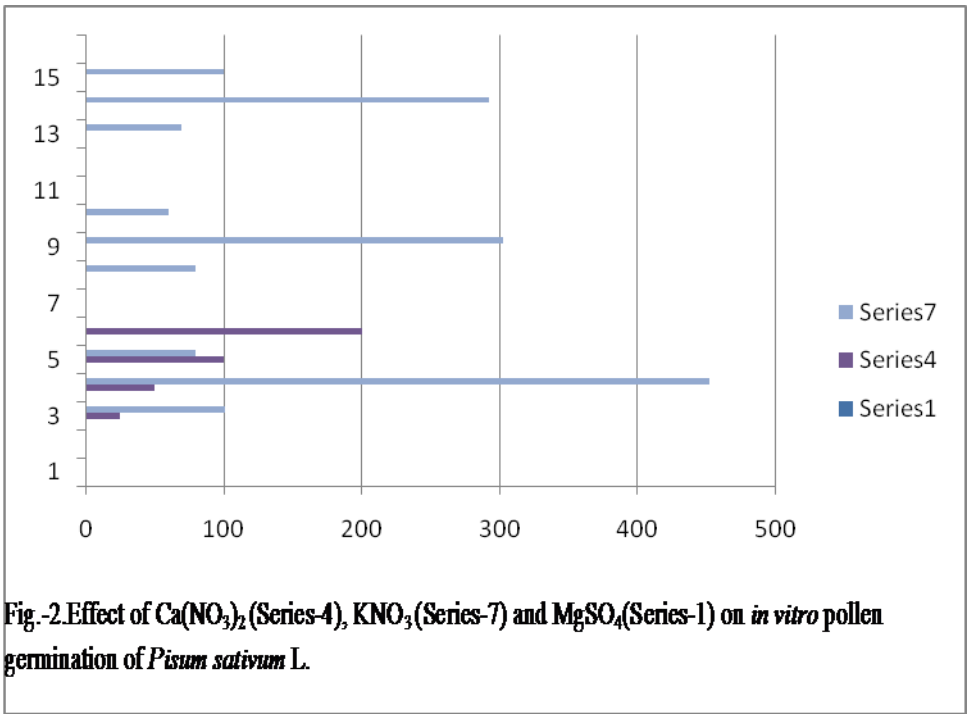


Fig.-2.Effect of $Ca(NO_3)_2$ (Series-4), KNO_3 (Series-7) and $MgSO_4$ (Series-1) on *in vitro* pollen germination of *Pisum sativum* L.

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