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Research Article

ROLE OF SOME NUTRIENTS ON IN *VITRO* POLLEN GERMINATION OF *SOLANUM TORVUM* SWARTZ

Kakali Biswas, *Subrata Mondal and Sudhendu Mandal
Department of Botany, Visva-Bharati, Santiniketan India-731235
**Author for correspondence*

ABSTRACT
The present investigation has been undertaken to study the role of different nutrients like sucrose, boric acid at various concentrations separately and in combinations and salts like Calcium nitrate, Magnesium sulphate and Potassium nitrate on *in vitro* pollen germination of *Solanum torvum* Swartz., a medicinally important plant belonging to the family Solanaceae to know the pollen viability and optimum nutrient requirements for *in vitro* pollen germination. It flowers during January-April. Flowers generally open in early morning (04.00 – 06.00 hrs.), after which anther dehiscence take place by apical pores. Pollen grains are 3-colporate. Maximum 95% pollen germination along with 1014µm pollen tube development was observed in 20% sucrose solution supplemented with 100 ppm boric acid and among the salts; maximum 45% pollen germination along with 285µm pollen tube was observed in 500 ppm Magnesium sulphate solution. Pollen grains which were collected in the morning (05.30 hrs.- 07.30 hrs.) showed best results.

Key Words: *Pollen Germination, Sucrose, Boric Acid, Salts, Solanum Torvum Swartz*

INTRODUCTION
Pollen germination, pollen tube growth and pollen pistil interactions are key phenomena in plant sexual life cycle. 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. 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 vivo* 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 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 basic needs for the improvements of plants before going to the breeding programme are pollen fertility, viability and its longevity. The present work is aimed to study the effect of sucrose and boric acid at various concentrations separately and in combinations and salts of Calcium, Magnesium and Potassium on *in vitro* pollen germination of *Solanum torvum* Swartz belonging to the family Solanaceae (Fig. 1).
The Fruits of the plant are eaten as a vegetable and said to be good for enlargement of the spleen (Chopra *et. al.*, 1956; Singh *et. al.*, 1996; Khare, 2007 and Udayan and Balachandran, 2009). Fruits contain sterolin (sitosterol-d-glucoside) and 0.1% gluco-alkaloid Solasonine (Khare, 2007).

MATERIALS AND METHODS
Fresh flowers were collected in the early morning (05:30 hrs. - 07:30 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, Magnesium and Potassium. 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 (1993). A pollen grain was considered as germinated if pollen tube length atleast becomes twice greater than the diameter of the pollen (Gupta *et al.*, 1989).

RESULTS

In the present study, it was found that 48% pollen germination along with 395 μm long pollen tube development occurred in 15% sucrose solution (Table-1). Individually, 100ppm boric acid showed 55% germination along with 495 μm long pollen tube (Table-2). The pollen germination as well as tube development decreased in lower concentrations as well as in higher concentrations of sucrose and also in case of boric acid. The highest germinating pollen (95%) along with 1014μm long pollen tube developed in 20% sucrose solution supplemented with 100 ppm boric acid (Table-3, Fig. 2).

Among the salts, maximum 45% pollen germination along with 285μm long pollen tube was observed in 500 ppm Magnesium sulphate solution following 26% pollen germination along with 240μm pollen tube in 300 ppm Potassium nitrate solution and 23% pollen germination along with 224μm pollen tube development in 500 ppm Calcium nitrate solution (Table-4).

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

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)
1	--	--	--	--	3	24
5	7	52	10	85	15	95
10	12	75	22	110	30	125
15	***25***	***105***	***30***	***225***	***48***	***395***
20	20	90	22	115	35	285
25	15	60	18	75	20	115
30	5	35	7	55	12	80

Table 2: Effect of boric acid on *in vitro* pollen germination of *Solanum torvum* Swartz

Conc. (ppm)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)
50	15	95	25	115	30	220
100	***48***	***258***	***52***	***421***	***55***	***495***
200	35	128	45	218	50	362
300	28	89	31	126	40	203
500	10	60	15	90	22	150

Table 3: Effect of sucrose and boric acid on *in vitro* pollen germination of *Solanum torvum* Swartz

Conc. (Sucrose+ Boric acid)	After 1 hr.		After 2 hrs.		After 3 hrs.	
	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)
20%+50 ppm	30	176	38	426	55	615
20%+100 ppm	***75***	***720***	***80***	***975***	***95***	***1014***
20%+200 ppm	52	380	65	516	70	650
20%+300 ppm	25	119	30	235	42	428
20%+500 ppm	12	67	22	98	30	226

Table 4: Effect of $Ca(NO_3)_2$, KNO_3 and $MgSO_4$ on *in vitro* pollen germination of *Solanum torvum* Swartz (After 3hrs.)

Conc. (ppm)	$Ca(NO_3)_2$		KNO_3		$MgSO_4$	
	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)
100	--	--	--	--	--	--
200	--	--	18	125	12	135
300	9	106	**26**	**240**	30	234
500	**23**	**224**	14	132	**45**	**285**
600	15	134	7	98	24	242

DISCUSSION

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 and 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). 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 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 (Holdaway-Clarke and Hepler, 2003 and 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*. Though the effect of either sucrose or boric acid individually showed good results, however 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; Vasil, 1964 and Sidhu and Malik, 1986).

Salts of Calcium, Magnesium and Potassium like Calcium nitrate, Potassium nitrate and Magnesium sulphate were used to study the effect of Ca, K and Mg ions on *in vitro* pollen germination. The results indicate that Magnesium ion was the most effective to influence the pollen germination. According ot Moore and Jung (1974) NO_3^- and Mg^{++} enhanced the tube growth in the case of *in vitro* pollen germination of sugarcane. Fan *et al.* (2001) suggested that external supply of K^+ ion enhanced the rate of pollen germination as well as pollen tube growth in *Arabidopsis*. Pollen germination and pollen tube growth are significantly 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). It is also known that K^+ is required for both pollen germination and tube growth (Brewbaker and Kwack, 1963; Weisenseel and Jaffe, 1976 and Feijo *et al.,* 1995). Both Ca^{++} and K^+ are interdependent each other because the inward K^+ channels are greatly regulated by Ca^{++} as in case of *Arabidopsis* pollen (Fan *et al.,* 2001) as well as stomatal guard cells (Schroeder and Hagiwara, 1989; Blatt *et al.,* 1990; Fairley-

Grenof and Assmann, 1992a,b; Lemtri-Chlieh and MacRobbie, 1994; Kelly *et al.,* 1995 and Grabov and Blatt, 1997). Prajapati and Jain (2010) indicated that Calcium, Magnesium and Nitrate play a key role in pollen tube growth of *Luffa aegyptica.*

Figure 1: *Solanum torvum* Swartz

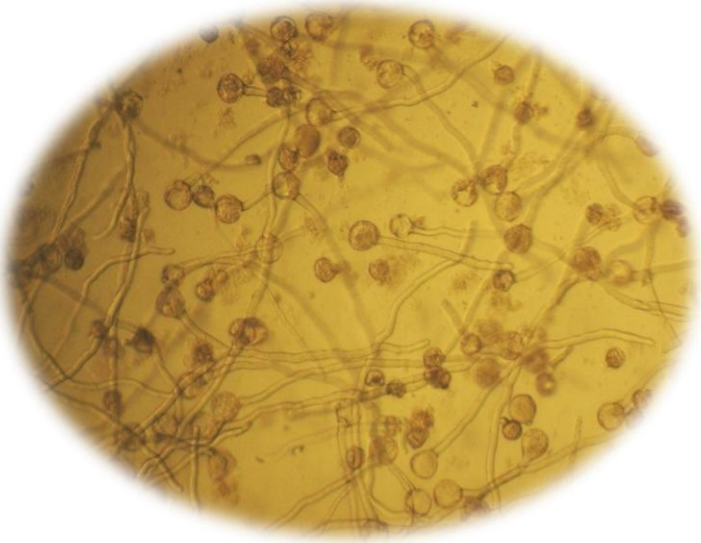

Figure 2: *In vitro* germinating pollen grains of *Solanum torvum* Swartz

Calcium is one of the most important cations involved in cell metabolism. It is also known to be important in maintaining membrane integrity and permeability (Jones and Lunt 1967 and Brewbaker and Kwack 1964). According to Kwack (1967) Calcium probably gives rigidity to the pollen tube wall by binding pectic carboxyl groups and also induced pollen germination. Picton and Steer (1983) and Miller *et al.,* (1992) demonstrated that Calcium concentration plays a critical role in maintaining the tube growth. Mondal *et al.,* (1997) and Choudhury *et al.,* (2013) studied the role of sucrose, boric acid and

different salts like Calcium nitrate, Potassium nitrate and Magnesium sulphate on *in vitro* pollen germination.

Thus, the present findings corroborate the findings of Vasil (1964), Brewbaker and Kwack (1964), Kwack (1967) , Jones and Lunt (1967), Gupta *et al*., (1989), Pal *et al.*, (1989), Mondal *et al*., (1991), Mondal *et al.*, (1997), Bhattacharya *et al*., (1997), Fan *et al.*, (2001), Holdaway-Clarke and Hepler (2003), Bhattacharya and Mandal (2004), Biswas *et al.*, (2008), Acar *et al*., (2010), Prajapati and Jain (2010), Mondal and Ghanta (2012) and Choudhury *et al.*, (2012, 2013).

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