Cibtech Journal of Bio-Protocols ISSN: 2319–3840 (Online)
An Online International Journal Available at http://www.cibtech.org/cjbp.htm
2013 Vol. 2 (1) January-April, pp.1-7/Ghanta and Mondal
Research Article

EFFECT OF SOME NUTRIENTS ON IN *VITRO* POLLEN GERMINATION OF *CRESCENTIA CUJETE* L

Ramanjan Ghanta and *Subrata Mondal
Department of Botany, Visva-Bharati, Santiniketan-731235 (India)
**Author for correspondence*

ABSTRACT
The titled research has been undertaken to study the effect of different nutrients like sucrose, boric acid at various concentrations separately and in combinations and salts of Calcium, Magnesium and Potassium on *in vitro* pollen germination of *Crescentia cujete* L., a medicinally important plant belonging to the family Bignoniaceae to know the pollen viability and optimum nutrient requirements for *in vitro* pollen germination. It flowers during July to November. Flowers generally open at17.30 hrs. - 20:30 hrs. But sporadic flower opening takes place through out the night after which anther dehiscence take place. Maximum 97% pollen germination along with 4173µm pollen tube development was observed in 20% sucrose solution supplemented with 100 ppm boric acid and among the salts; maximum 70% pollen germination along with 1456µm pollen tube was observed in 100 ppm Calcium nitrate solution. Pollen grains which were collected in the evening (18.30 hrs. - 19.30 hrs.) showed best results.

Key Words: *Pollen germination, Sucrose, Boric acid, Salts, Crescentia cujete L.*

INTRODUCTION
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 *Crescentia cujete* L. (Calabus tree) belonging to the family Bignoniaceae (Fig. 1) which a medicinally important plant (Chopra *et al.*, 1956; Singh *et al.*, 1996 and Udayan and Balachandran, 2009) and native to Maxico and Central America (Arango-Ulloa e*t al.*, 2009).

MATERIALS AND METHODS
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, 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 experiment it was found that 86% pollen germination along with 2340 µm long pollen tube development occurred in 20% sucrose solution (Table-1). Individually, 100ppm boric acid showed 66% germination along with 1014 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. Same thing happened in case of boric acid also. The highest germinating pollen (97%) along with 4173µm long pollen tube developed in 20% sucrose solution supplemented with 100 ppm boric acid (Table-3, Fig. 2).

Among the salts, maximum 70% pollen germination along with 1456µm pollen tube development in 100 ppm Calcium nitrate solution following 56% pollen germination along with 767µm pollen tube was observed in 200 ppm Potassium nitrate solution and 38% pollen germination along with 494µm long pollen tube in 300 ppm Magnesium sulphate solution (Table-4). Both Potassium and Calcium showed good results, however maximum pollen germination as well as tube development was observed in Potassium nitrate solution. Pollen germination and tube length decreased in lower as well as in higher concentrations (Table-4).

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

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)
5	7	260	8	280	10	390
10	15	360	24	790	28	1040
15	43	430	69	1300	76	1560
20	***56***	***520***	***78***	***1680***	***86***	***2340***
25	42	400	62	920	68	1300
30	26	390	28	880	32	1240

Table 2: Effect of boric acid on *in vitro* pollen germination of *Crescentia cujete* L

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	8	70	16	91	18	130
50	28	260	42	650	45	897
100	***36***	***390***	***62***	***910***	***66***	***1014***
200	32	320	45	780	54	845
300	10	78	25	234	28	260

Table 3: Effect of sucrose and boric acid on *in vitro* pollen germination of *Crescentia cujete* L

Conc. (Sucrose+ Boric acid)	After 1 hr.		After 2 hrs.		After 4 hrs.	
	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)
20%+50ppm	67	520	78	1700	86	3250
20%+100ppm	***72***	***560***	***82***	***1820***	***97***	***4173***
20%+200ppm	70	480	74	1680	82	2840
20%+300ppm	62	420	65	1300	74	1900

Table 4: Effect of $Ca(NO_3)_2$, KNO_3 and $MgSO_4$ on *in vitro* pollen germination of *Crescentia cujete* L

Salts	Conc. (ppm)	After 1 hr.		After 2 hrs.		After3 hrs.	
		Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)	Germination (%)	Mean tube length (μm)
$Ca(NO_3)_2$	50	28	320	42	870	48	910
	100	***36***	***520***	***60***	***1023***	***70***	***1456***
	200	32	480	54	930	62	1040
	300	20	240	29	518	40	650
	400	15	220	22	260	27	390
	500	9	70	12	110	16	130
KNO_3	50	19	230	28	324	32	369
	100	21	280	32	428	38	520
	200	***29***	***376***	***42***	***678***	***56***	***767***
	300	19	213	25	265	30	326
	400	16	198	20	230	26	260
	500	10	78	12	122	14	130
$MgSO_4$	50	10	73	13	119	15	130
	100	17	198	20	236	24	268
	200	20	210	26	327	32	360
	300	***24***	***234***	***30***	***368***	***38***	***494***
	400	20	125	25	148	29	168
	500	5	72	6	80	7	82

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.

Figure 1: *Crescentia cujete* L.

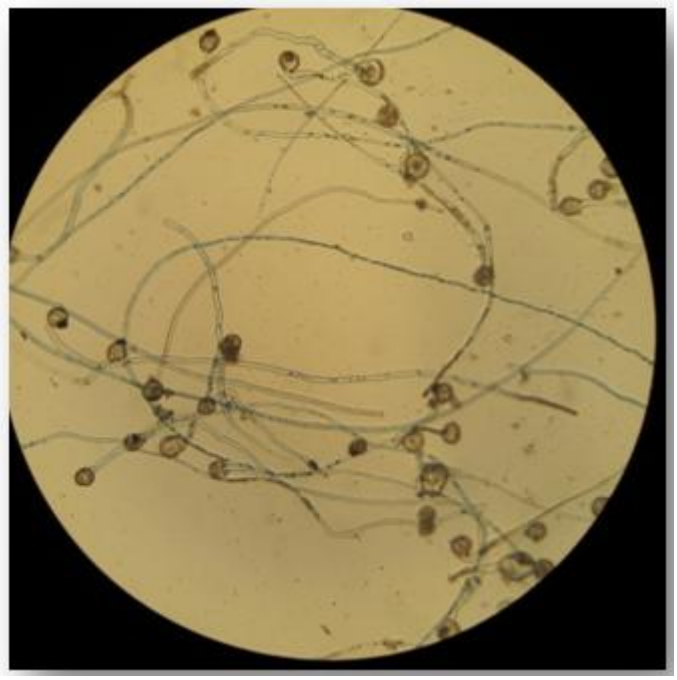

Figure 2: *In vitro* germinating pollen grains of *Crescentia cujete* L

The results indicate that Calcium ion was the most effective to influence the pollen germination. 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. 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^{+} both 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). According to Fan *et al.*, (2001) external supply of K^{+} ion enhanced the rate of pollen germination as well as pollen tube growth in *Arabidopsis*. Moore and Jung (1974) pointed out that NO_3^{-} and Mg^{++} enhanced the tube growth in the case of *in vitro* pollen germination of sugarcane. Prajapati and Jain (2010) indicated that Calcium, Magnesium and Nitrate play a key role in pollen tube growth of *Luffa aegyptica.*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), Choudhury *et al.*, (2012) and Choudhury *et al.*, (2013).

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