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Research Article

INFLUENCE OF SOME NUTRIENTS ON *IN VITRO* POLLEN GERMINATION OF *RICINUS COMMUNIS* L.

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ABSTRACT
Present paper deals with 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 *Ricinus communis* L. an economically important plant belonging to the family Euphorbiaceae. Pollen grains showed different rates of viability and germ inability in relation to nutrient requirements. Flowers start to open in the morning at 07:00 – 09:30 hrs., after which anther dehiscence take place. Maximum 90% pollen germination along with 260µm pollen tube development was observed in 12% sucrose solution supplemented with 100 ppm boric acid. Among the salts, maximum 40% pollen germination along with 156 µm pollen tube development was observed in 300ppm Calcium nitrate solution. Pollen grains which were collected in the morning (09:30-11:30hrs.) showed best results.

Keywords: *Pollen Germination, Pollen Tube, Sucrose, Boric Acid, Salts, Ricinus communis L.*

INTRODUCTION
In flowering plants, the male gametes reside in the pollen grains and on a compatible stigma, the pollen grain germinates and extrudes a tube that elongates through the style to reach the ovary and enter an ovule, where the tube tip bursts, allowing the release of the male gametes for fertilization. The rapid elongation of the pollen tubes over a distance of a few hundred micrometers to several centimeters is therefore an essential process for the sexual reproduction of flowering plants (Wilhelmi and Preuss, 1999). Pollen tube elongation is a dynamic process in which pollen tubes navigate and respond to female tissues to accomplish their mission of delivering the sperm cells for fertilization. Pollen tubes extend exclusively at the cell apex via an extreme form of polar growth, known as tip growth, producing uniformly shaped cylindrical cells (Cheung, 2001).

Pollen fertility and viability have a paramount importance in breeding programme. High crop yield generally depends on viable pollen grains. 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. During the past few years pollen tubes grown *in vitro*, became a popular model system for cell biology studies (Moutinho *et al.,* 2001). The aim of this investigation is to study the effects of different nutrients on *in vitro* pollen germination of an economically important plant *Ricinus communis* L. belonging to the family Euphorbiaceae. In recent years, pollen germination and pollen tube development are used as materials for determining the importance of cytoskeleton in cell growth and differentiation (Ma *et al.,* 2000).

MATERIALS AND METHODS
The newly opened flowers were collected in the morning (09:30-11:30 hrs.) and transferred to polythene bags. The fresh pollen grains 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 petri dishes lined with moist filter paper and examined under an Olympus microscope to record 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 at least becomes twice greater than the diameter of the pollen grains (Gupta *et al.,* 1989).

RESULTS AND DISCUSSION

Results

Studies on *in vitro* pollen germination after anthesis indicated that 80% germinating pollen along with 195µm long pollen tube development occurred in 12% sucrose solution. Individually, 100ppm boric acid showed 40% germination along with 130µm long pollen tube. The highest germinating pollen (90%) along with 260µm long pollen tube developed in 12% sucrose solution supplemented with 100ppm boric acid (Table -1, 2 & 3; Figure 1 A & B).

Among the salts, maximum 40% pollen germination along with 156µm pollen tube development was observed in 300 ppm Calcium nitrate solution following 30% pollen germination along with 143 µm pollen tube was observed in 100 ppm Magnesium sulphate solution and 30%pollen germination along with 104 µm pollen tube was observed in Potassium nitrate solution (Table - 4, 5 & 6).

Table 1: Effect of sucrose on *in vitro* pollen germination of *Ricinus communis* L.

Conc of sucrose	After 1 hr		After 2 hr		After 3 hr	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)
Dis. water	3	26	4	26	5	39
2%	5	39	7	52	10	65
5%	12	65	15	78	20	91
10%	15	65	20	91	25	104
12%	**55**	**130**	**75**	**165**	**80**	**195**
15%	50	26	60	65	65	91
20%	45	39	55	39	60	65
25%	30	26	40	39	50	52
30%	20	26	25	26	50	39

Table 2: Effect of boric acid on *in vitro* pollen germination of *Ricinus communis* L.

Conc of boric acid	After 1 hr		After 2 hr		After 3 hr	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)
25ppm	10	65	15	78	20	91
50ppm	15	78	25	91	30	104
100ppm	**25**	**104**	**35**	**117**	**40**	**130**
200ppm	10	26	15	65	20	78
300ppm	5	26	10	39	12	52

Table 3: Effect of sucrose and boric acid on *in vitro* pollen germination of *Ricinus communis* L.

Conc of sucrose & boric acid	After 1 hr		After 2 hr		After 3 hr	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)
12%+25ppm	30	104	45	130	50	143
12%+50ppm	65	130	70	143	80	182
12%+100ppm	**70**	**182**	**85**	**195**	**90**	**260**
12%+200ppm	60	143	75	156	85	169
12%+300ppm	50	104	60	130	65	143
12%+400ppm	20	91	30	104	40	117

Table 4: Effect of $Ca(NO_3)_2$ on *in vitro* pollen germination of *Ricinus communis* L.

Conc. Of $Ca(NO_3)_2$	After 1 hr		After 2 hr		After 3 hr	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)
50ppm	10	78	15	91	20	117
100ppm	15	91	25	104	35	143
300ppm	**25**	**104**	**35**	**117**	**40**	**156**
500ppm	10	78	12	91	15	104

Table 5: Effect of $MgSO_4$ on *in vitro* pollen germination of *Ricinus communis* L.

Conc. Of $MgSO_4$	After 1 hr		After 2 hr		After 3 hr	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)
50ppm	5	78	10	91	10	104
100ppm	**20**	**104**	**25**	**117**	**30**	**143**
300ppm	10	78	15	104	20	130
500ppm	10	39	12	65	15	91

Table 6: Effect of KNO_3 on *in vitro* pollen germination of *Ricinus communis* L.

Conc. Of KNO_3	After 1 hr		After 2 hr		After 3 hr	
	Germination (%)	Tube length(µm)	Germination (%)	Tube length(µm)	Germination(%)	Tube length(µm)
50ppm	10	26	15	39	20	65
100ppm	**25**	**130**	**35**	**91**	**30**	**104**
300ppm	12	91	17	117	25	130
500ppm	5	104	7	130	15	143

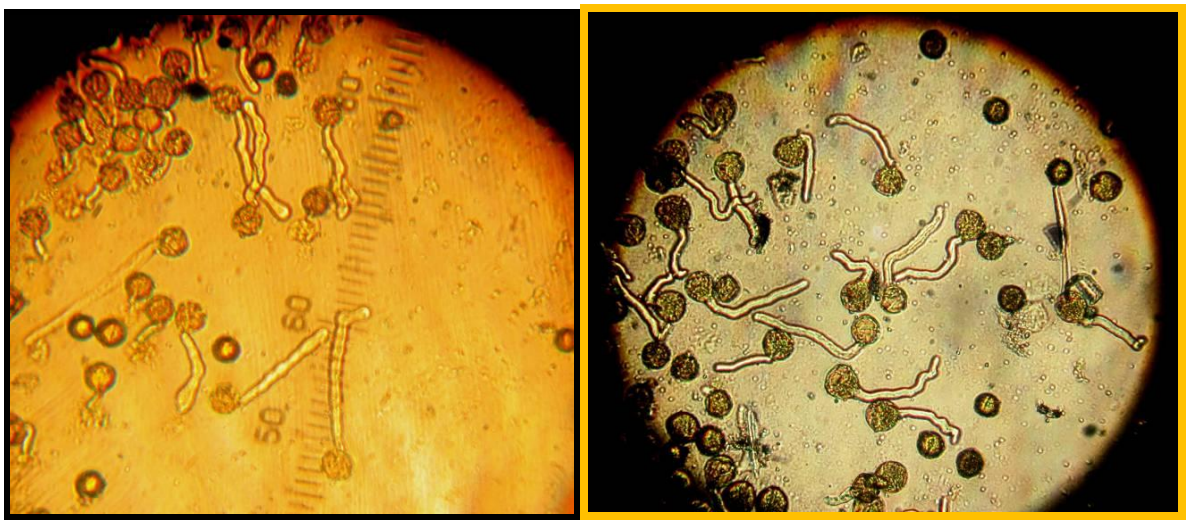

Figure 1A **Figure 1B**

Figure 1A & B: Growing Pollen tubes

Discussion

The results indicated that sucrose and boric acid individually showed good results but sucrose in addition to boric acid yielded better germination and tube development. The pronounced effect of sucrose and boric acid on increasing trend of germinating pollen have been reflected as externally supplied sucrose maintains the osmotic pressure and acts as a substrate for pollen metabolism (Johri and Vasil, 1961; Shivanna and Johri, 1989). The role of boron in germinating pollen and growing pollen tubes has been confirmed 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 could exert a protective effect in preventing excessive polymerization of sugars at sites of sugar metabolism. In nature; water, sugar, and amino acids are supplied by the style to nourish the growing pollen tubes. Boron is also provided by stigmas and styles which facilitates sugar uptake and play a role in pectin synthesis in the growing pollen tubes (Richards, 1986).

Boric acid is known to be crucial for pollen germination and tube growth and it is required at concentration of 100ppm 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 *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; Acar *et al.*, 2010; Wang *et al.*, 2003).

Boron plays an important role on the localization of pectins and callose in the wall of pollen tubes in *Picea meyeri*. The stimulatory effect of boron also reported on *in vitro* pollen germination of *Pistacia vera* (Acar *et al.*, 2010). 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, 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. 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. Calcium is one of the most important cations involved in cell metabolism and also known to be important in maintaining membrane integrity and permeability (Jones and Lunt, 1967; 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; Taylor and Hepler, 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. The role of sucrose, boric acid and salts like Calcium nitrate, Potassium nitrate and Magnesium sulphate on *in vitro* pollen germination were established and thus, the present work gets supports from Vasil (1960, 1964), Gupta *et al.*, (1989), Pal *et al.*, (1989), Mondal *et al.*, (1991), Bhattacharya *et al.*, (1997), Bhattacharya and Mandal (2004), Biswas *et al.*, (2008), Acar *et al.*, (2010), Mondal and Ghanta (2012), Biswas and Mondal (2014) and Dutta and Mondal (2014).

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