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Studies on *in vitro* pollen germination of *Mitragyna parvifolia* (Roxb.) Korth.

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A B S T R A C T

In vitro pollen germination test was performed on *Mitragyna parvifolia* (Roxb.)Korth**.** belonging to the family Rubiaceae to study the effect of different nutrients like sucrose, boric acid at various concentration separately and in combinations and salts like Calcium nitrate, Magnesium sulphate and Potassium nitrate. The flowers open in the early evening 19.00 to 20.30 hrs. Anther dehiscence takes place before flower opening. Maximum 96% pollen germination along with 1105µm pollen tube development was observed in 10% sucrose solution supplemented with 100ppm boric acid and among the salts maximum 90% pollen germination along with 910µm pollen tube was observed in 50ppm Calcium nitrate solution and in case of Magnesium sulphate 85% pollen germination along with 728µm pollen tube was observed in 500ppm while in case of Potassium nitrate 65% pollen germination along with 897µm pollen tube was observed in 500ppm solution.

Introduction

Pollen grains are reduced, non-motile, microscopic male gametophytes which, upon pollination, produce pollen tubes that grow through the pistil for effective fertilization and seed set. Pollen fertility and viability have a paramount importance in plant reproduction. So pollen fertility, viability, and its longevity are basic aspect for the improvement of plant before going to successful breeding programme. Pollen viability is critical for the study of following aspects of pollination biology: monitoring pollen vigour during storage; genetics and pollen-stigma interaction; crop improvement and breeding programmes; gene bank maintenance; incompatibility and fertility studies; evaluation of pollen germ inability after exposure to certain conditions, and evaluation of dispersal and gene flow (Stanley and Linkens 1974, Heslop-Harrison *et al*. 1984, Heslop-Harrison 1992, Dafni 1992, Mulugeta *et al*. 1994,Shivanna and Rangaswamy 1993, Shivanna and Heslop-Harrison 1981). Viability has been

defined as having the capacity to live, grow, germinate or develop (Lincoln *et al*. 1982). The term viability has also been used to describe pollen grains capable of germinating on the stigma (Morse 1987, Preston 1991, Vaughton and Ramsey 1991, Niesenbaum 1992).germination *in vitro* (Shchori *et al*. 1992, Beardsell *et al*. 1993, Lindgren *et al*. 1995).

Pollen grains are simple structure of plant cells and pollen tube formation is a good and appropriate model of growth and development (Taylor and Hepler, 1997). Thus pollen germination and pollen tube growth are important research material for morphological, physiological, biotechnological, ecological, environmental, evolutional, biochemical and molecular biological studies (Ottavio *et al.*, 1992). Pollen tube elongation is a lively process in which pollen tubes navigates and respond to female tissues to accomplish their mission of delivering the sperm cells for fertilization. Pollen tube extents exclusively at the cell apex via an extreme form of polar growth, known as tip growth, producing uniformly set cylindrical cells (Cheung, 2001). Pollen tubes are excellent system for the study of polarized tip growth, cell movement, cell to cell communication, cell to cell recognition and signalling in plants. 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). Pollens normally germinate on stigma and the required environment for *in vitro* pollen germination is related to genetic composition and also the quality of nutrient reserves of pollen (Baker and Baker, 1979). During the past few years pollen tube growth *in vitro* becomes a popular model system for cell biology studies in plant cell (Moutinho *et al.*, 2001). The present investigation 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 *Mitragyna parvifolia (*Roxb.) Korth..,an economically and medicinally important plant belonging to the family Rubiaceae popularly knowen as Guli-Kadam.

Materials and Methods

For the study of *in vitro* pollen germination, newly opened flowers were collected in the evening (19.00-20.30 hours) and transferred to polythene bags. *In vitro* pollen germination was studied to know the effect of nutrients like sucrose and boric acid at different concentrations individually as well as in combinations and salts of Calcium nitrate, Magnesium sulphate, Potassium nitrate. The fresh pollen samples were shown on several grooved slides containing solution of different concentrations separately or in combination. Slides were then kept in Petri dishes lined with moist filter paper and examined under the microscope, at different time intervals 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 at least as long as pollen grain diameter (Stanley and Liskens 1974).

Results and Discussion

Studies on *in vitro* pollen germination at different time intervals after anthesis indicated that 92% germinating pollen with a mean of 1014µm long pollen tube development was observed in 10% sucrose solution (Table-1). Individually, 100 ppm boric acid showed 90% germination along with 871 µm long pollen tube (Table-2). The maximum 96% pollen germination along

with 1105 µm long pollen tube developed after 3 hours in 10% sucrose solution supplemented with 100 ppm boric acid (Fig-1 and Table-3). The maximum 90% pollen germination along with 910µm long pollen tube developed after 3 hours in 50ppm Calcium Nitrate solution while 85% pollen germination along with 728 µm long pollen tube development was observed in 500ppm Magnesium Sulphate solution and 65% pollen germination along with 897 µm long pollen tube developed in 500ppm Potassium Nitrate solution (Table-4). Though the effect of either sucrose or boric acid individually showed good results, but sucrose in combination with boric acid promoted pollen germination as well as tube development (Table-1, Table-2, Table-3), 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; Sidhu and Malik, 1986. *In vitro* germination measures pollen germinability under the specific conditions of the medium and temperature conditions reveals the state of the reserves, the condition of the membranes and the sub sequent rate of reserve conversion (Heslop-Harrison *et al*. 1984).

Shivanna and Johri (1989) 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 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 and facilitates sugar uptake and play a vital role in pectin production in the pollen tubes (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).

Environmental factors and especially desiccation risks are considered a main selective force leading to better protection of the pollen grain and from the evolutionary ecology view-point, the possible relation between pollen longevity and pollination chances,pollen competition, and breeding system is noteworthy. Even if pollen is delivered successfully into the proper receptive stigma, there is no guarantee that it is still viable and one may point out that pollen longevity on the vector body even at the right location to meet the stigma may also be a crucial factor in pollination efficiency (Dafni and Firmage 2000).

The pronounced effect of sucrose and boric acid on germinating pollen might be reflected with the views of Johri and Vasil (1961). The induced role of Calcium and boron on *in vitro* pollen germination was reported by Brewbaker and Kwack (1964). The role of boron in flowering and fruiting process has been established (Brown *et al.*, 1994) and its deficiency resulted in low pollen viability, poor pollen germination and reduced pollen tube growth (Nyomora and Brown, 1997). Boron takes part in pollen germination and style 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 also reported 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) 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*.

Table.1 Effect of Sucrose on *In Vitro* Pollen Germination of *Mitragyna parvifolia* (Roxb.) Korth

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)
Distilled water	18	195	25	234	30	260
1	25	221	32	273	35	390
2	42	273	52	598	55	650
5	45	377	55	637	75	689
8	55	403	70	897	85	949
10	**80**	**455**	**86**	**962**	**92**	**1014**
12	48	247	54	403	65	455
15	8	78	10	104	15	130
20	5	39	8	52	10	65

Figure.1 Flower of *Mitragyna parvifolia(Roxb.)Korth*

Figure.2 *In vitro* Pollen Germination of *Mitragyna parvifolia(Roxb.)Korth*

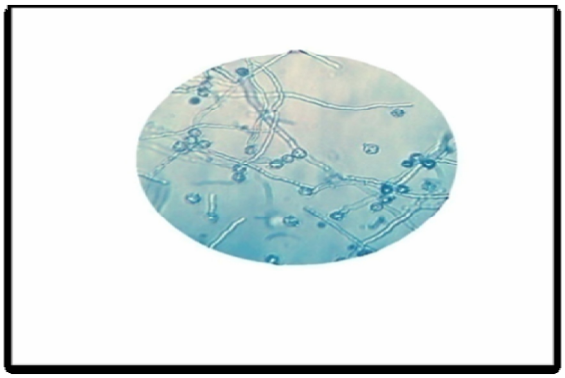

Table.2 Effect of Boric Acid on *In Vitro* Pollen Germination of *Mitragyna parvifolia* (Roxb.) Korth

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)
25	62	260	70	481	80	533
50	65	286	75	611	85	663
100	**72**	**325**	**82**	**845**	**90**	**871**
200	38	286	43	663	50	715
300	26	254	32	365	45	533
400	17	195	22	351	35	403
500	12	130	14	221	20	273

Table.3 Effect of Sucrose and Boric Acid on *In vitro* Pollen Germination *Mitragyna parvifolia* (Roxb.) Korth

	After 1 hr.		After 2 hrs.		After 3 hrs.	
Conc. (Sucrose % + Boric Acid ppm)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)
10+25	58	312	68	533	70	585
10+50	68	351	77	572	85	624
10+100	**85**	**494**	**90**	**1027**	**96**	**1105**
10+200	72	364	80	793	85	845
10+300	63	455	72	611	80	663
10+400	48	182	55	408	65	455
10+500	33	150	38	286	45	338

Table.4 Effect of Calcium Nitrate, Magnesium Sulphate, Potassium Nitrate on *In vitro* Pollen Germination Mitragyna parvifolia(Roxb.) Korth

	After 1 hr.		After 2 hrs.		After 3 hrs.	
Conc. ppm of Ca $(NO_3)_2$	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)
25	42	195	50	390	62	546
50	**78**	**234**	**82**	**858**	**90**	**910**
100	62	221	65	741	70	793
200	46	223	52	481	58	533
300	38	130	42	286	47	338
400	5	39	8	52	13	91
500	6	26	7	39	8	65

	After 1 hr.		After 2 hrs.		After 3 hrs.	
Conc. ppm of ($MgSO_4$)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)
50	15	78	18	104	20	143
100	18	221	25	351	30	403
200	28	286	36	416	45	468
300	38	351	46	546	55	598
400	57	403	68	624	75	676
500	**75**	**429**	**80**	**676**	**85**	**728**
600	12	39	15	65	18	117

	After 1 hr.		After 2 hrs.		After 3 hrs.	
Conc. ppm of (KNO_3)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)	Germination (%)	Mean tube length (µm)
50	18	65	20	104	25	143
100	22	210	25	236	30	288
200	28	195	32	221	40	273
300	32	286	38	416	45	468
400	38	312	45	533	55	585
500	**55**	**325**	**60**	**843**	**65**	**897**
600	8	78	10	91	15	130

Salts of 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 role of all the salts were well marked where Calcium Nitrate was most effective. The results also indicate that Calcium ion was the 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; Brewbaker and kwack 1964). According to Kwack (1967) Calcium propabaly gives rigidity to the pollen tube wall by binding pectic carboxyl groups and also induced pollen germinations. Picton and Steer (1983) and Miller *et al* (1992) demonstrated that calcium concentration play a critical role in maintaining the tube growth. According to Brewbaker and Kwack (1964) Magnesium ions enhance the effect of Calcium ions result in the growth of pollen tube. The role of K^+ was established in pollen germination and tube elongation in *Arabidopsis* and Both the $Ca^{++}$and K^+ are interdependent on each other because the inward K^+ channel are greatly regulated by Ca^{++} while the external supply of K^+ also enhanced the rate of pollen germination as well as pollen tube growth in *Arabidopsis* (Fan *et al, 2001*). Mondal *et al*. (1997) and Choudhury *et al* (2013) studied the role of sucrose, boric acid and difference salt like Calcium nitrate, Potassium nitrate and Magnesium sulphate on *in vitro* pollen germination. Moore and Jung (1971) pointed out that NO^-_3 and Mg^{++} enhance the tube growth in case of *in vitro* pollen germination of *Saccharum officinarum*. 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; 1997), Bhhattacharya *et al* (1997), Holdaway- Clarke and Hepler (2003), Bhhattacharya and Mandal (2004), Biswas *et al* ,(2008), Acar *et al*. (2010), Choudhury *et al* (2012) and Choudhury *et al.* (2013), Mondal and Ghanta (2012), Ghanta and Mondal (2013), Biswas and Mondal(2014),Dane *et al* .(2004),Olaymi *et al*.(2011).

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