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# An investigation on *in vitro* pollen germination and tube development of *Jacquinia ruscifolia* Jacq.

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## Abstract

Pollen grains are prerequisite for pollination, fertilization as well as fruit and seed set and play a vital role in plant reproduction as it transmit the male genetic materials. Germination is the first critical morphogenetic event in the pollen towards fulfilling its ultimate function of discharge of male gametes in the embryo sac. The present investigation has been undertaken to find out the effect of different nutrients like sucrose, boric acid and some salts like calcium nitrate, potassium nitrate and magnesium sulphate on *in vitro* pollen germination of *Jacquinia ruscifolia* Jacq. belonging to the family Theophrastaceae. Maximum 92% pollen germination along with 455 µm pollen tube development was observed in 20 % sucrose solution supplemented with 100 ppm boric acid and among the salts; maximum 12% pollen germination along with 182µm pollen tube development was observed in 100 ppm calcium nitrate solution. Flowers collected during anthesis (08:00 hrs.-09:30 hrs.) showed best germination.

**Keywords:** Pollen germination, sucrose, boric acid, salts, *Jacquinia ruscifolia*.

## Introduction

Pollen transmits the male genetic material during sexual reproduction of angiosperms. Pollen grains are highly reduced male gametophyte consisting of two or three numbers of cells during the time of release from anthers (Borg et al., 2009). In nature, pollen grains germinate on the stigma and develop pollen tubes. For growth and development of a cell, tissues, organs and systems of organism, nutrition are required. Some are needed as respiratory substrates; some are needed for ion transportation while some for regulation of different biosynthetic pathways. Successful fertilization as well as fruit formation depends on the fertility and viability of the pollen grains. To discharge the male gametes in the embryo sac, germination of pollen takes place over the receptive stigmas but studies on *in vivo* are cumbersome due to the complications involving in the pistillate tissues. 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. The pollen tubes are considered as the most rapidly growing cells in the plant world since they are capable of attaining considerable length in a short duration under optimum conditions (Malik, 1977). Thus, pollen germination and growth of pollen tubes are important research materials for morphological, physiological, biotechnological, ecological, evolutional, biochemical and molecular biological studies (Ottavio et al., 1992). In recent years, to determine the importance of cytoskeleton in cell growth and differentiation, pollen germination along with pollen tube development are used as research material (Ma et al., 2000). So, the pollen grains, being single celled structure provide a unique system for *in vitro* studies (Katara, 2013).

The present work is aimed to find out the role of sucrose, boric acid, salts like calcium nitrate, potassium nitrate and magnesium sulphate on *in vitro* pollen germination of *Jacquinia ruscifolia*.Jacq. of Theophrastaceae, which is a medicinally important plant having broad spectrum antifungal activity (Shorma et al., 2008).

## Materials and Methods

Fresh flowers were collected during anthesis in the evening (08:00 hrs.-09:30 hrs.) and transferred to polythene bags. Solution of different concentrations of sucrose (1-50%), boric acid (25-500 ppm), calcium nitrate, potassium nitrate and magnesium sulphate (50-500 ppm) were prepared. Then the fresh pollen samples were sown on several grooved slides containing sucrose and boric acid solution at different concentrations individually as well as in combinations and salts of calcium, potassium, and magnesium. Slides were then kept in Petridishes lined with moist filter paper at room temperature (25°C) and examined under a 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 the pollen tube length atleast becomes twice greater than the diameter of the pollen grains (Gupta et al., 1989).

## Results

Pollen grains start to germinate at 2% sucrose solution showing 2% germination along with 52 µm tube length, while maximum 80% pollen germination along with 312 µm long pollen tube development occurred in 20% sucrose solution (Table 1). Individually, 100ppm boric acid showed 60% germination along with 234 µm long pollen tubes (Table 2). But the highest germinating pollen (92%) along with 455µm long pollen tube developed in 20% sucrose solution supplemented with 100 ppm boric acid (Table 3; Figure1). Among the salts, maximum 12% pollen germination along with 182 µm pollen tube development was recorded in 100 ppm calcium nitrate solution following 10% pollen germination along with 130 µm pollen tube in 200 ppm magnesium sulphate solution and 8% pollen germination along with 130µm long pollen tube in 100 ppm potassium nitrate solution (Table 4). Among the salts, calcium nitrate was the most effective one.

**Table 1:** Effect of sucrose on *in vitro* pollen germination of *Jacquinia ruscifolia* .

| Conc. of sucrose (%) | After 1 hr. | | After 2 hrs. | | After 3 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Pollen tube length (µm) | Germination (%) | Pollen tube length (µm) | Germination (%) | Pollen tube length (µm) |
| Distilled water | -- | -- | -- | -- | -- | -- |
| 2 | 2 | 52 | 5 | 78 | 8 | 130 |
| 5 | 12 | 78 | 15 | 104 | 20 | 156 |
| 10 | 14 | 104 | 18 | 130 | 35 | 169 |
| 15 | 35 | 156 | 52 | 156 | 60 | 195 |
| **20** | **40** | **156** | **60** | **234** | **80** | **312** |
| 25 | 25 | 130 | 42 | 156 | 55 | 182 |
| 30 | 18 | 104 | 25 | 130 | 42 | 156 |
| 40 | 16 | 78 | 22 | 104 | 30 | 130 |
| 45 | 8 | 52 | 12 | 78 | 14 | 91 |

**Table 2:** Effect of boric acid on *in vitro* pollen germination of *Jacquinia ruscifolia*.

| Conc. of boric acid (ppm) | After 1 hr. | | After 2 hrs. | | After 3 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Pollen tube length (µm) | Germination (%) | Pollen tube length (µm) | Germination (%) | Pollen tube length (µm) |
| 25 | 4 | 65 | 6 | 104 | 10 | 130 |
| 50 | 8 | 104 | 10 | 130 | 15 | 156 |
| **100** | **35** | **156** | **45** | **182** | **60** | **234** |
| 200 | 8 | 78 | 12 | 130 | 20 | 156 |
| 300 | 6 | 52 | 8 | 104 | 12 | 130 |
| 400 | 4 | 26 | 6 | 78 | 10 | 104 |
| 500 | -- | -- | 2 | 26 | 4 | 65 |

**Table 3:** Effect of sucrose and boric acid on *in vitro* pollen germination of *Jacquinia ruscifolia*

| Conc. of Sucrose (%) + Boric acid (ppm) | After 1 hr. | | After 2 hrs. | | After 3 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Pollen tube length(µm) | Germination (%) | Pollen tube length(µm) | Germination (%) | Pollen tube length(µm) |
| 2 + 100 | 6 | 52 | 10 | 130 | 12 | 156 |
| 5+ 100 | 10 | 78 | 16 | 156 | 22 | 208 |
| 10+ 100 | 12 | 104 | 22 | 182 | 30 | 234 |
| 15+ 100 | 18 | 156 | 32 | 208 | 45 | 260 |
| **20+ 100** | **52** | **234** | **78** | **364** | **92** | **455** |
| 25+100 | 30 | 182 | 55 | 325 | 80 | 390 |
| 30+100 | 25 | 156 | 48 | 234 | 75 | 286 |
| 40+100 | 18 | 130 | 32 | 195 | 40 | 260 |

**Table 4:** Effect of calcium nitrate, potassium nitrate and magnesium sulphate on *in vitro* pollen germination of *Jacquinia ruscifolia*

| Salts | Conc. (ppm) | After 1 hr. | | After 2 hrs. | | After 3 hrs. | |
|---|---|---|---|---|---|---|---|
| | | Germination (%) | Tube length (µm) | Germination (%) | Tube length (µm) | Germination (%) | Tube length (µm) |
| $Ca(NO_3)_2$ | 50 | 2 | 52 | 4 | 65 | 6 | 91 |
| | **100** | **6** | **104** | **8** | **130** | **12** | **182** |
| | 200 | 2 | 39 | 4 | 78 | 8 | 104 |
| | 300 | 1 | 26 | 3 | 39 | 6 | 65 |
| | 400 | -- | -- | -- | -- | -- | -- |
| $KNO_3$ | 50 | -- | -- | 1 | 26 | 2 | 39 |
| | **100** | **2** | **52** | **6** | **104** | **8** | **130** |
| | 200 | 2 | 39 | 3 | 65 | 4 | 91 |
| | 300 | -- | -- | -- | -- | -- | -- |
| | 400 | -- | -- | -- | -- | -- | -- |
| $MgSO_4$ | 50 | -- | -- | -- | -- | -- | |
| | 100 | -- | -- | 2 | 52 | 5 | 78 |
| | **200** | **5** | **78** | **8** | **104** | **10** | **130** |
| | 300 | -- | -- | 2 | 52 | 3 | 65 |
| | 400 | -- | -- | -- | -- | -- | -- |

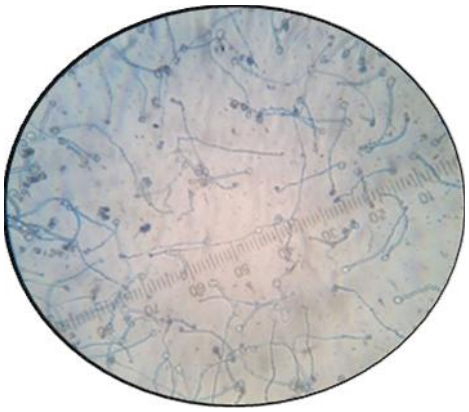

**Figure 1:** Germinating pollen of *Jacquinia ruscifolia* Jacq.

## Discussion

In the present experiment, pollen grains showed germination ability in both sucrose and boric acid solution (Table 1 & 2). However, sucrose in addition to boric acid promoted both pollen germination as well as tube development (Table 3). To nourish the pollen, stylar tissue supplies water, sugar, amino acids etc., to the growing pollen tubes. Boron is also found in style and stigma to enhance sugar uptake and play a key role in pectin synthesis in the growing pollen tubes (Richards, 1986). Sucrose acts as respiratory substrate for pollen grains as well as important to maintain the regulation of osmotic pressure. The conspicuous role of sucrose and boric acid on the *in vitro* pollen germination were reflected with the views (Johri and Vasil, 1961 ; Shivanna and Johri, 1985 ). Boron is an important micronutrient for higher plants (Blevins and Lukaszewski, 1988). Like sucrose, boron also play a vital role on pollen germination and pollen tube growth in vascular plants (Lewis, 1980; Sidhu and Malik, 1986) as it is directly involved in pectin synthesis to the development of pollen tube membrane (Stanley and Loewus,1964). Not only pectin but also it helps the synthesis of callose during pollen tube development which was experimentally proved in *Picea meyeri* (Wang et al., 2003) and important in sugar transport, cell wall synthesis, cell wall development, carbohydrate metabolism, RNA metabolism, indole acetic acid metabolism, membrane transport and respiration (Blevins and Lukaszewski, 1994; Camacho-Cristobal et al., 2008) . Scott (1960) suggested that, boron could exert a protective effect in preventing excessive polymerization of sugars at sites of sugar metabolism. Boron is crucial for pollen germination along with pollen tube development in most species (Brewbaker and Majumder, 1961), thus play an important role in fertilisation of flowering plants towards the successful fruit and seed production. The significant role of boron on *in vitro* pollen germination and tube development in Pistachio was established (Therios et al., 1985; Brown et al., 1994; Acar et al., 2010). In pollen culture medium, 100 ppm boric acid shows the optimal concentration required for germination and pollen tube growth, which agrees with Shorrocks (1997). The boron deficiency affects the pollen viability, pollen germination, and pollen tube growth (Nyomora and Brown, 1997). It is reported that, tube bursting occurred due to elimination of boric acid from pollen culture medium ( Holdaway-clarke and Hepler, 2003; Acar et al., 2010). The deficiency of boron in plants causes carbohydrate accumulation in chloroplasts, may slow the Krebs cycle and accelerates the action of the pentose phosphate cycle (Goldbach, 1997; Lovatt and Dugger, 1984). Thus, the role of sucrose and boric acid in pollen germination and pollen tube development was confirmed, 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; Sidhu and Malik,1986) and involved in the synthesis of pectic substance required for the newly elongating pollen tube wall (Jhori and Vasil,1961).

Salts, like magnesium sulphate, calcium nitrate and potassium nitrate also play a vital role in pollen germination and pollen tube development. The results indicated that among the salts, calcium nitrate was the most effective to pollen germination than magnesium sulphate and potassium nitrate. Pollen was found to germinate at low concentration of calcium nitrate but decreased at higher concentration. Similar observation was also found by Mortazavi et al., (2010) in *Phoenix dactylifera*. In cell metabolism, calcium is one of the most important cation which is involved in pollen germination as well as pollen tube growth. To maintain the membrane integrity and permeability, calcium plays a vital role (Jones and Lunt, 1967; Brewbaker and Kwack, 1963, 1964). It also plays a critical role in pollen tube growth (Picton et al., 1983; Miller et al., 1992) as well as pollen tube development (Kwack, 1967). Intracellular calcium also influenced callose synthesis in growing pollen tubes on *in vitro* condition which was studied in *Oenothera biennis* and agrees with the results (Bednarska, 1989). Transport of inorganic ions such as $Ca^{++}$ and $K^{+}$ across the plasma membrane regulates the pollen germination and pollen tube growth significantly (Feijo et al., 1995; Taylor and Hepler, 1997). In spite of pollen germination and tube development, calcium also plays a role to determine the direction of pollen tube growth which was studied in *Luffa aegyptica* ( Prajapati and Jain, 2010). In case of *Arabidopsis*, rate of pollen germination as well as pollen tube growth is enhanced by externally supplied $K^{+}$ (Fan et al., 2001). Both $Ca^{++}$ and $K^{+}$ are interdependent each other, because the inward $K^{+}$ channels are greatly regulated by $Ca^{++}$ (Schroeder and Hagiwara, 1989; Kelly et al., 1995; Grabov and Blatt, 1997). $NO_3^{-}$ and $Mg^{++}$ also enhanced the tube growth in case of *in vitro* pollen germination of *Saccharum* sp. (Moore and Jung, 1974). Osmotic potential for the swelling of pollen grains are regulated by $KNO_3$ was reported in case of Poaceae by Matsui et al., (2000). Prajapati and Jain (2010) indicated that

calcium, magnesium and nitrate play a key role in pollen tube growth of *Luffa aegyptica* (Acar et al., 2010). Ions, particularly $Ca^{++}$ can modulate the actin cytoskeleton in pollen tubes (Hepler et al., 2006). Thus, the present investigation gets support from the previous studies (Acar et al.,2010; Holdaway-Clarke and Hepler, 2003; Pal et al.,1989; Acar et al.,2010; Mondal et al.,1991, 1997; Bhattacharya *et al.*,1997; Bhattacharya and Mandal, 2004; Biswas et al., 2008; Mondal and Ghanta, 2012; Choudhury et al.,2012, 2013; Ghanta and Mondal, 2013a, 2013b, 2016; Biswas and Mondal, 2014; Dutta Mudi and Mondal, 2014 ).

## Conclusion

It can be concluded that, though sucrose and boric acid individually showed good results but sucrose in combination with boric acid promoted pollen germination as well as tube elongation while salts like calcium nitrate, potassium nitrate and magnesium sulphate also play a key role on *in vitro* pollen germination along with pollen tube development of *Jacquinia ruscifolia* Jacq. Thus, present experiment threw light on pollen viability in terms of germination ability which has great significance on sexual reproduction and productivity.

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