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# *In vitro* pollen germination study in four species of *dianthus*

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**Keywords:** *Dianthus, germination, pollen, viability, boric acid, light, temperature*

**Abstract: *Dianthus* (Carnation) is a commercially important plant used worldwide as an ornamental flower. Experiments were conducted to investigate pollen tube growth using *in vitro* germination method in four varieties of *Dianthus* (*Dianthus caryophyllus, D. chinensis, D. barbatus* and *D. pulmaris*) under varying physical and chemical parameters. The pollen germination was tested *in vitro* using different concentration of sucrose and boric acid. The germination rates were found to be highest at 10% sucrose and 100ppm boric acid concentration. The effect of temperature and light on pollen tube growth was also evaluated and it was observed that germination is highest at 27°C; and coloured light enhanced the rate of germination many folds. Blue and green light showed the highest percentage of pollen germination.**

## INTRODUCTION

*Dianthus* (Carnations) is genus under Caryophyllaceae family that contains about 300 species of plants (Jurgens *et al.*, 2003). The plant is an annual or a perennial herb, cultivated worldwide as an ornamental plant and culinary purpose. The plant blossoms in cool summers in average, dry to medium moisture, well-drained soil. Flowers are solitary or grow in clusters at the tip of branches. Studies have shown the therapeutic potientials of these herbs (Al- Snafi, 2017, Satish Chandra *et al*., 2016).

The *Dianthus* genus has widely been used to study the diversity in pollen morphology and germination in relation to reproductive success and taxonomical classification (Yildz, 2001, Macukanovic-Jocic *et al.*, 2015, Dyaberi, *et al.*, 2015). Environmental factors play a critical role in pollen viability and germination thereby affecting seed formation and plant propagation. Hence, it is important to study the factors that affect pollen germination rates and to figure out the optimum conditions for pollen germination which would result in optimum reproductive success.

The present study was undertaken to determine pollen fertility using pollen viability tests *in vitro* under varying physical (temperature, light) and chemical (sucrose, boric acid) parameters in different species of *Dianthus namely, D. caryophyllus* (Species 1), *D. chinensis* (Species 2), *D. barbatus* (Species 3) and *D. pulmaris* (Species 4), in Bangalore, India .

*D. caryophyllus* or Dianthus clove pink is a most common species of *Dianthus*. It is native to Asia and South Europe. The plant is an herbaceous perennial with height of upto 18 inches. The flowers are produced singly or upto five in a cyme, having five magenta or dark pink coloured petals with fringed ends (Figure 1a).

*D. chinensis* or Chinese pink is native to China, South Russia, Mongolia and Korea. The plant is a perineal herb with a height

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upto 24 inches. The flower is pink or purple coloured with white border that ends in a fringed-pattern. Flowers appear in 10-15 flowered clusters in summer. *D. chinensis* has been used for centuries to treat various health ailments in Chinese traditional herbal medicine (Satish Chandra *et al*., 2016) (Figure 1b).

*D. barbatus* , commonly known as Red Dianthus or Sweet William, is native to Europe, Africa and Asia. The plant grows upto 18 inches with flowers in a dense cluster of upto 30 at the top. The flowers are large, fragrant and bright red in colour with serrated edges, making it a very popular choice as an ornamental plant. The flowers have a mild flavour and are used as a garnish (Figure 1c).

*D. pulmarius* is native to Europe and Asia. It can grow up to 18 inches and has white flowers with magenta coloured centre (Figure 1d).

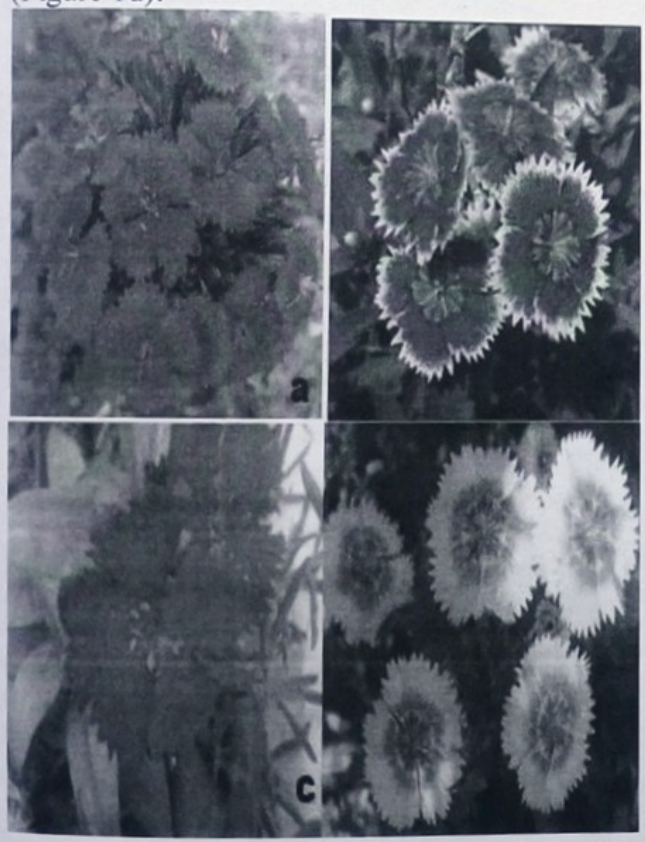


**Figure 1**: Four species of *Dianthus* used in the present study a) *Dianthus caryophyllus* b) *Dianthus chinensis* c) *Dianthus barbatus* d) *Dianthus pulmarius*

## MATERIALS AND METHODS

### Plant Material:

*Dianthus* flowers were collected from the Lalbagh Botanical Garden, Bengaluru. The flowers were plucked early morning between 7 am to 8 am. Four species of *Dianthus* were chosen *D. caryophyllus* (dark pink), *D. chinensis* (Purple with white edges), *D. barbatus* (red) and *D. pulmaris* (white with purple center), were choosen for the present study (Figure 1).

### Methods:

**Viability test under varying sucrose concentration**

Sucrose solutions of different concentration (5%, 10%, 15%, 20%, 25%) were prepared in distilled water. Anthers from the flower were dissected and crushed on the slide to suspend pollen grains in sucrose solution and allowed to germinate for 24 hours. The slides were incubated in a petri plate lined with wet cotton to maintain uniform relative humidity. After 24 hours of incubation, the results were recorded.

**Viability test under varying sucrose and boric acid concentration**

Sucrose solution of varying concentration (2%, 5%, 10%) was prepared in distilled water. Boric acid solution of different concentration (25ppm, 50ppm, 100ppm, 200ppm, 300ppm) was formulated from a 5% stock solution. The pollens were suspended in varying concentrations of sucrose and boric acid medium placed on a glass slide. After 24 hours of incubation, the results were recorded.

**Viability test using modified Brewbaker and Kwack's media**

Basic pollen germination medium by Brewbaker and Kwack (1963) was modified (Dyaberi, *et al.*, 2015). The pollen grains were suspended on a drop of sucrose (8%) on a glass slide. The Brewbaker-Kwack's media was added and the slides were incubated in petri plates

lined with wet cotton. The results were recorded after 5 hr incubation period.

**Viability test under different temperature conditions**

Pollen cells were germinated using Brewbaker and Kwack media in combination with 8% sucrose and incubated for 30 minutes under different temperature conditions (0°C, 4°C, 27°C and 60°C) and then taken out to room temperature. Pollen tube count was taken after 5 hours incubation period and the results were recorded.

**Viability test under different light conditions**

The pollen grains were spread on a glass slide containing 8% sucrose and Brewbaker and Kwack's media. A 50-watt fluorescent tube light was used as the source of light. The pollens were incubated in blue, green, yellow or red light by covering the boxes with the respective coloured cellophane. White light was used as control. The pollen tube count was taken after 5 hours period and the results were recorded.

The slides were observed under light microscope at 45X magnification and the results were tabulated. Pollen grains were scored as 'viable' when the length of the pollen tube exceeded the diameter of the pollen grain. Pollen viability values were calculated as the percentage germination of the total number of pollen grains observed. For each *Dianthus* line, pollen samples were taken from at least 30 different flowers and these samples were pooled. In each experiment, pollen viability was assessed for five optical fields.

## RESULTS AND DISCUSSION

Pollen viability and pollen tube growth is one of the most important factors in influencing fertilization and seed formation. It is well documented that pollen germination and pollen tube growth in plants are influenced by carbohydrate (sugar), amino acids, hormones, enzymes, minerals like boron, calcium, magnesium, potassium, pH and physical factors like temperature and light but are species specific (Johri and Vasil, 1961).

Sucrose plays dual role during tube germination; it acts as the carbohydrate substrate for metabolism in pollen and also acts an osmoticum and maintain the osmotic pressure between the cell and its surrounding. Sucrose in the medium is metabolized by germinating pollen (Stanley and Linskens, 1974). The present study showed that sucrose alone is not sufficient for pollen tube growth. The pollen tube germination is very low (1-2%) when it is incubated in sucrose alone (data not included). High sucrose concentrations lead to an imbalance in osmotic potential in the cell resulting in cell wall rupturing (Figure 2).

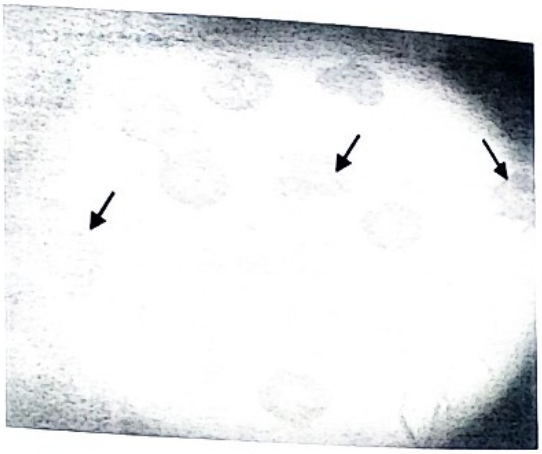

**Figure 2**: Pollen cell wall disintegration at higher sucrose concentrations (>15%) due to osmotic imbalance

Under very low sucrose concentration, a large number of pollen plugs (non-viable) were observed (Figure 3). This may be due to non-availability of sufficient nutrition during tube growth. It has been recorded that for optimal germination of pollen grains 7.5-20% sucrose solution was needed in different cucurbits depending upon species (Acar and Sarpakya, 2010; Mondal and Mandal, 2008) .

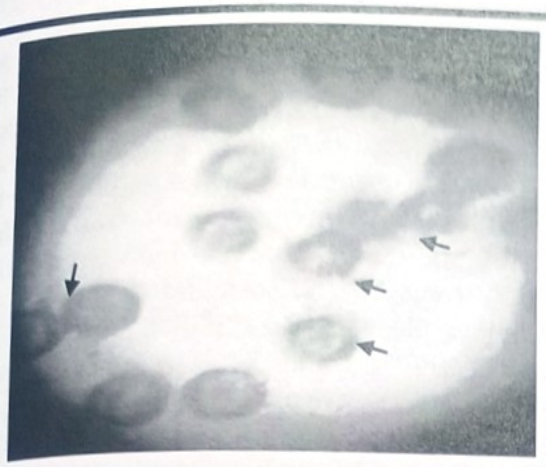

**Figure 3**: Pollen grain develops pollen plugs at low sucrose concentrations (2-5%)

The germination of pollen tubes requires optimum media conditions. Different concentrations of sucrose (2%, 5%, 10%, 15%, 20%) along with and boric acid (25ppm, 50ppm, 100ppm, 200ppm, 300ppm) in the media affected pollen germination. Pollen germination and tube growth were increased with increasing concentration of boric acid in a basic sucrose (10-20%) medium, but only upto a limit. Boric acid concentration more than 200ppm is found to be detrimental to pollen germination. The best pollen tube growth (> 10%) was observed in 50-100ppm of Boric acid concentration in all the four species (Table 1). Sucrose concentration of 10% in combination with 100 ppm boric acid solution was found to be best for pollen germination. A large number of disintegrated cells were seen at 20% sucrose concentration, due to imbalance in osmotic pressure. At the physiological level, boron is believed to control growth, membrane permeability and help in translocation of sugar, while at the biochemical level it controls several enzymes (Khattar and Malik, 1992). A medium containing boric acid and an osmoticum, like sucrose, favours pollen germination in many species (Taylor and Hepler, 1997).

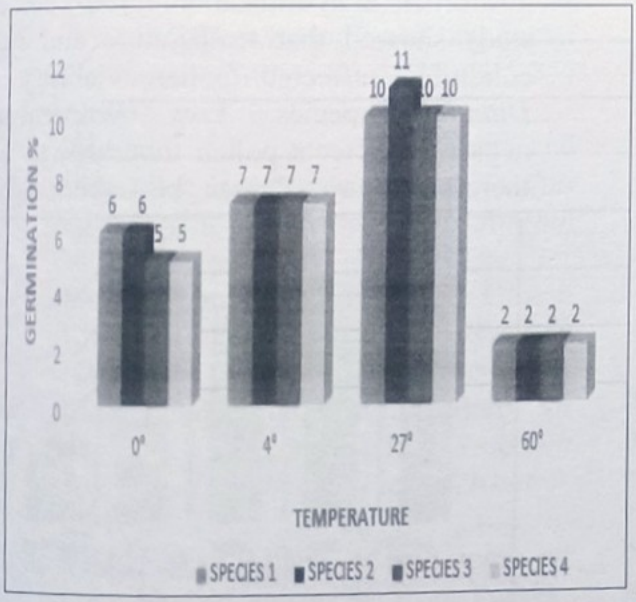


**Figure 4**: Bar graph showing pollen germination percentage in four different species of *Dianthus* at different temperatures

Temperature significantly affected pollen tube growth. Brewbaker-Kwack's media in combination with sucrose (8%) was used and the pollens were incubated at different temperatures for 30 minutes and then taken out and kept at room temperature (5hr). It was observed that pollens at 0°C and 4°C (Figure 4 and 5) showed significant pollen fertility (6-7%), although it is lower than the pollen fertility at room temperature.

**Figure 5**: Pollen growth observed at 4 °C

The highest pollen viability (10.2%) is observed at temperature 27°C (Figure 6). Milatovic and Nikolic (2017), Akond

(2012) reported similar results in cherry and *Lagerstroemia* varieties, respectively. The length of pollen tubes increased at 27°C (Figure 6). Pollen of most plants show optimum germination and tube growth between 20 - 30°C (Johri and Vasil, 1961). At higher temperatures, a significant decrease in pollen viability is observed. At 60°C, the pollen grains disintegrate due to heat and the pollen tube germination does not occur.

The pollen tube growth under different coloured lights using cellophane paper of red, yellow, green and blue colours is represented in Figure 7. A large variation was observed between pollen tube growth under normal (white) light and coloured light suggesting that light of different intensities helps induce pollen tube growth and hence the pollen fertility is increased. Blue and green light enhanced pollen tube growth (Figure 7 and 8). The pollen fertility increased more than three-folds under blue and green light compared to normal light.

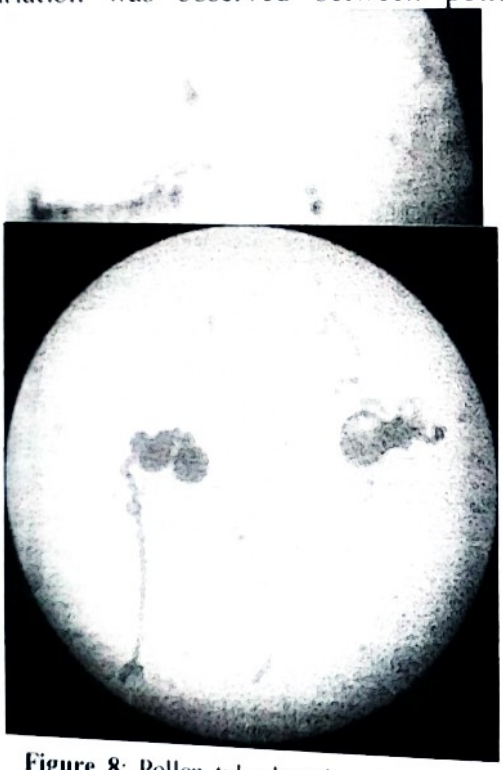

**Figure 8**: Pollen tube length and growth under blue light

The pollen fertility rates among the four species do not show any statistically significant results when incubated under same sucrose and boric acid concentration. However, there were marked differences in the pollen germination rates under different temperature and light conditions. *D. caryophyllus* was found to have a higher than average pollen germination rate at 27°C, whereas *D. barbatus* shows the lowest. Also, *D. caryophyllus* has higher germination rates under all conditions of light- 24% (red), 30% (green), 27% (yellow) and 35% (blue) (Figure 8). Chauhan and Katiyar, (1996) also observed variations in pollen germination and tube elongation in *Schima wallichii* under different coloured light.

## CONCLUSIONS

Study of *in vitro* germination of pollen grains is crucial for various botanical research, taxonomical classification and hybridisation programmes. The evaluation of pollen fertility during the storage of male parents or gametes at low temperature is of paramount importance for artificial hybridisation. The present study showed that temperature and light conditions affected pollen viability in *Dianthus* species. Low temperatures negatively affects pollen tube growth and therefore, care should be taken while

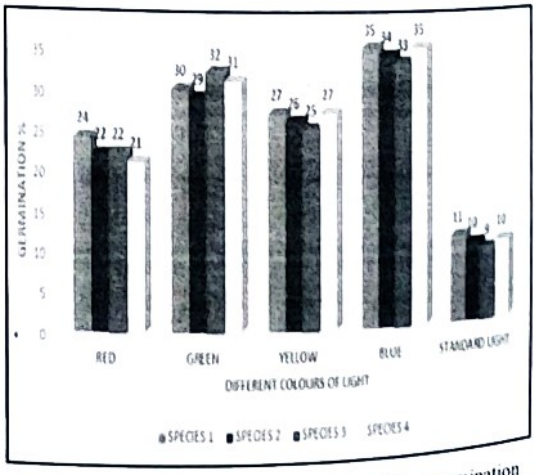


**Figure 7**: Bar graph showing pollen germination percentage in four different species of *Dianthus* under different light conditions

storage of pollens. The optimum temperature for growth of pollen tubes was found to be 25-27° C. It was also observed that light of different intensities (red, yellow, green, blue) helps induce pollen tube germination and are found to increase pollen fertility many folds.

The information collected during these experiments can be utilized for hybridisation and breeding programmes involving *Dianthus* to increase the success rate of the programme. These results also indicate the necessity for more studies concerning the development of a more suitable culture medium to test the *in vitro* fertility of *Dianthus* pollen grains.

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**Table 1:** Pollen fertility (%) in *Dianthus* species in different sucrose and boric acid concentration

| SUCROSE | Boric Acid (ppm) | Pollen Fertility (%)* | | | |
|---|---|---|---|---|---|
| | | *D. caryophyllus* | *D. chinensis* | *D. barbatus* | *D. pulmarius* |
| 2% | 50 | 2 | 2 | 2 | 2 |
| | 100 | 3 | 2 | 2 | 2 |
| | 200 | 2 | 1 | 1 | 1 |
| 5% | 50 | 3 | 3 | 3 | 3 |
| | 100 | 4 | 4 | 4 | 3 |
| | 200 | 3 | 3 | 2 | 3 |
| 10% | 25 | 4 | 4 | 4 | 4 |
| | 50 | 7 | 6 | 6 | 6 |
| | 100 | 12 | 11 | 11 | 11 |
| | 200 | 10 | 10 | 9 | 9 |
| | 300 | 8 | 9 | 8 | 9 |
| 15% | 25 | 2 | 1 | 1 | 2 |
| | 50 | 6 | 6 | 4 | 5 |
| | 100 | 10 | 9 | 9 | 9 |
| | 200 | 8 | 8 | 7 | 7 |
| | 300 | 3 | 3 | 4 | 3 |
| 20% | 25 | 2 | 2 | 2 | 2 |
| | 50 | 3 | 3 | 2 | 2 |
| | 100 | 5 | 5 | 4 | 5 |
| | 200 | 2 | 3 | 2 | 2 |
| | 300 | 2 | 2 | 1 | 1 |

*The above data is an average of 5 experiments each containing 3 replicates.