Effect of key parameters on the pollen germination in an ayurvedic medicinal plant- Nithya Pushpa (*Catharanthus roseus* (L.) G. Don)

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***Abstract*- Nithya Pushpa is a perennial herb extensively grown for its medicinal and horticultural importance. Pollen grains or microspores are the male reproductive structures produced specifically in the anthers of flowers in angiosperms. The present study is an attempt to test the effect of certain key parameters on the germination potential of pollen grains in Nithya Pushpa. The pollen from *the plant* was subjected to germination in mediums containing different concentrations of sucrose and was found that germination medium containing 200 g/L sucrose is most suitable since it gave 100% germination within 20 minutes. The study also tested the effect of boric acid on the germination potential of pollen and found that boron significantly enhances the pollen tube formation. The study also revealed that maximum germination of pollen occurs at neutral pH and at a temperature range between 28-32°C.**

***Index Terms*- Pollen germination, *Nithya Pushpa*, sucrose, boric acid**

I. INTRODUCTION

Nithya Pushpa is an ayurvedic medicinal plant described in Charaka Samhita. The plant is commonly known as Periwinkle and scientifically as *Catharanthus roseus* (L.) G. Don belonging to the family Apocynaceae. The medicinal importance of Nithya Pushpa is linked with the presence of certain vital alkaloids such as vincristine, vinblastine, catharanthine, vindoline and narcotine etc. In Ayurvedic medicine, the plant is used in the treatment of respiratory ailments including cough, bronchitis and asthma etc. and the plant is also known to possess anti-inflammatory properties. The vinca alkaloids vincristine and vinblastine are used as anti-cancer agents in modern medicine as well [1]. The extensive cultivation of the plant is associated with its medicinal, ornamental and horticulture values.

Pollen, a mass of microspores in flowering plants usually appears as a fine dust, produced by the anthers of the stamens in flowers. Pollen grains serve a very important role in the life cycle of flowering plants since the success of fertilization in higher plants is significantly dependent on the pollen viability or the germination potential. Pollen germination is a complex process involving series of events and several factors are known to influence this process of germination and pollen tube growth [2]. However, the effect of individual factors on the germination process vary from plant to plat. Some of the key factors reported to influence the pollen germination includes sugar [3, 4, 5], calcium, boron [6], pH [7], temperature [8]and enzymes etc.

Pollen germination studies marks significance to plant breeders, who often conducts cross pollinations. Crosses are frequently done between plants that have different flowering periods and is effected by means of pollen storage. In such instances of pollen storage, periodic tests for pollen viability is conducted through germination of pollen grains in artificial media. Thus the present study is an attempt to study the effect of various factors on the germination potential of pollen, taking pollen from *Nithya Pushpa* as the material of study.

II. MATERIALS AND METHODS

Materials

Sucrose ($C_{12}H_{22}O_{11}$), Sodium Hydroxide (NaOH), HCl and Boric acid (H3BO3) from Merck India Pvt. Ltd., pH paper (Test strip 1-14; Make: Merck India Pvt. Ltd.)

Germination study

The pollen grains from mature anthers of Nithya pushpa (*Catharanthus*) were transferred into a clean glass slide and added germination medium dropwise, covered with cover slip and observed under compound microscope using 10x eyepiece and 40x objective magnification at specific time intervals.

The germination studies were conducted with three different concentrations of sugar solutions such as 100 g/L, 150 g/L and 200 g/L. Similarly, to test the effect of boric acid, pollens were subjected germination in sugar solutions (200 g/L) containing different concentrations of boric acid such as 0 mg/L, 1 mg/L and 10 mg/L. To test the effect of pH, pollens were subjected to germination in sugar solutions with pH ranges acidic, neutral and basic. The pH of the medium brought to the acidic range using dilute HCl and to basic range using dilute NaOH solution. The pH range was monitored using the pH paper. To test the effect of temperature, the medium temperature was retained at higher and lower ranges and was monitored using glass thermometer.

Calculation of germination percentage

Counts of germinated pollen were made using microscopy after due time and approximately 10-20 pollen grains per slide were counted. Percentage of pollen germination was calculated by following method [9];

$$\%\ germation\ = \frac{No\ of\ germinated\ pollen\ grains}{Total\ number\ of\ germinated\ and\ non-germinated\ pollen\ grains} \times 100$$

III. RESULTS AND DISCUSSION

Selection of germination medium

Pollen germination studies were conducted at three different concentrations of sucrose to test the effect of sucrose concentration on pollen tube formation. The pollen tube formation was monitored at 5 minutes' interval for a period of 20 minutes. The observations were as given in table 1.

Table 1: Pollen tube growth at different time intervals on exposure to various sugar concentrations

Sucrose Concentrations (g/L)	***Total number of pollen grains observed***	***Number of pollen grains with pollen tube growth***			
		5 Min	10 Min	15 Min	20 Min
100	19	4	10	14	14
150	12	6	9	11	11
200	16	11	11	12	16

From the study it became evident that the germination percentage of pollen grains of *Catharanthus* increased with increase in sucrose concentration (Fig. 1). In the germination medium with 100 g/L sucrose, the germination percentage was 73 while that in 150 g/L sucrose was 91%. A 100 % germination was observed when a medium containing 200 g/L sucrose was used. Studies have revealed that sugar in the medium acts as an osmotic controller which regulates the diffusion rate of water from the medium into pollen grains [10]. The results of the present study also correlate to these previous observations [11,12]. Hence, for further studies the medium with 200 g/L sucrose was used as the germination medium.

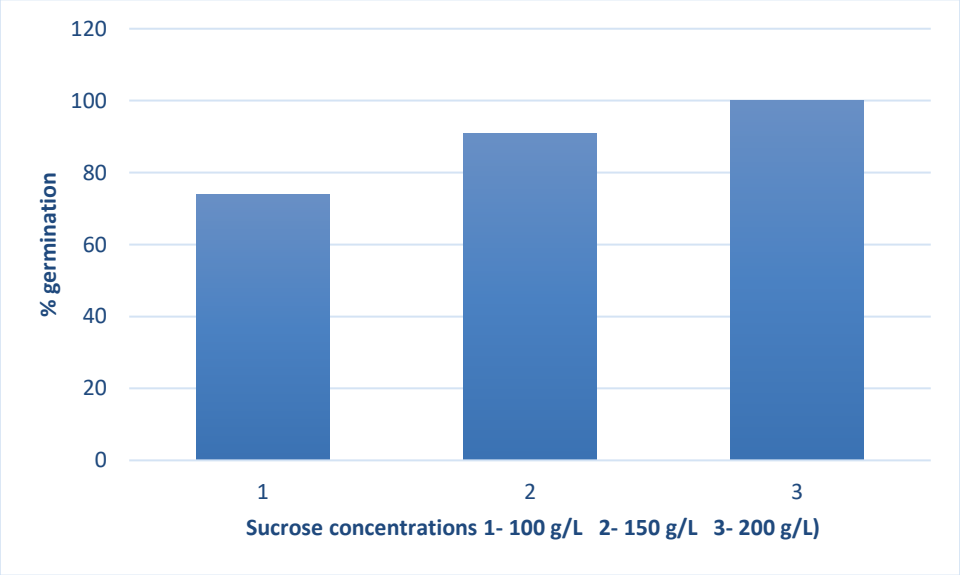

Figure 1: Percentage of pollen germination at different concentrations of sucrose

Effect of boric acid on the pollen germination

The effect of boric acid on the pollen tube growth has been studied using three different mediums with 200 g/L sucrose each and containing a boric acid concentration of 0,1 and 10 mg/L respectively. The pollen tube growth at different time interval were recorded (Table 2).

Table 2: Pollen tube growth at different concentrations of boric acid

Boric acid Concentrations (mg/L)	***Total number of pollen grains observed***	***Number of pollen grains with pollen tube growth***			
		5 Min	10 Min	15 Min	20 Min
0	16	11	11	12	16
1	12	5	9	12	12
10	7	5	7	7	7

The observed results revealed that boric acid has positive influence on the germination of pollen grains. Further, it is noticed that application boric acid could substantially reduce the time for pollen tube growth (Fig. 2). Pollen grains are believed to be deficient in boron, which is normally compensated by high levels of boron present in stigma and style. Boron combines with sugar to form a sugar-borate complex which facilitates translocation of sugar molecules. Boron is reported to be toxic to plants even at, as low as 5.1 ppm [13]. However, pollen seems to tolerate very high concentration of boron. Visser [14] showed that certain species of crop plants require as much as 1200 ppm boric acid for optimal germination and tube growth. Boron deficiency leads to pollen tube bursting as it is required in the pollen wall structure [15]. The enhanced pollen germination observed in the present study also reveals the positive influence of boron in pollen germination.

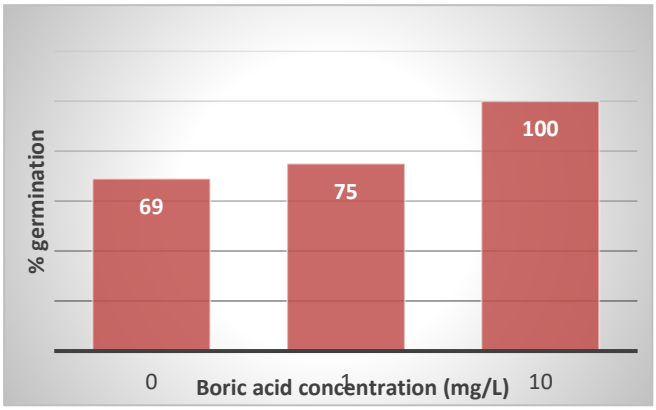

Figure 2: Percentage of pollen germination at different concentrations of boric acid after 10 minutes.

Effect of pH on the pollen germination

The effect of pH on the pollen tube growth has been studied using three different mediums with pH 4, 7 and 9. The pollen tube growth at different time interval were recorded (Table 3).

Table 3: Pollen tube growth at different pH ranges of germination medium

pH	***Total number of pollen grains observed***	***Number of pollen grains with pollen tube growth***			
		5 Min	10 Min	15 Min	20 Min
Acidic	17	0	0	0	0
Neutral	16	11	11	12	16
Basic	24	0	0	0	0

The observed results revealed that the acidic and alkaline pH has a negative influence on the germination of pollen grains of *Catharanthus* (Table 3). It was witnessed that the germination percentage was zero when the medium was retained at pH 4 and 9. Thus, it became evident that the maximum germination of pollen in *Catharanthus* occurs at neutral pH.

From the study, it became evident that medium pH is a critical factor that can influence the in vitro pollen germination. Similar results were also reported in previous studies as well. For instance, the study conducted by Chebli and Geitmann [16]in Lilium, Solanum and Camellia revealed that lower and higher pH values drastically reduce the pollen germination and the optimum pH for germination ranged between 6 to 7. In a study conducted in *Heliotropium hirsutissimum* also showed that, maximum pollen germination occurs at a medium pH of 6 [7].

Effect of temperature on the pollen germination

In order to study the effect of temperature on the germination, the study was performed at higher, lower and normal temperatures of the germination medium. The observed pollen germination at different time intervals are as follows (Table 4).

Table 4: Pollen tube growth at three different temperatures of germination medium

Temperature (°C)	***Total number of pollen grains observed***	***Number of pollen grains with pollen tube growth***			
		5 Min	10 Min	15 Min	20 Min
≈40	19	0	0	0	1
Normal	16	11	11	12	16
≈20	15	0	0	0	2

It was observed that the temperature as high as 38-40°C and as low as 15-20°C had a completely hindering effect on the germination of pollen. And, the optimum temperature where the germination percentage was observed to be maximum ranged between 28-32°C. Temperature is among the most important environmental factors affecting plant reproductive processes such as pollen germination and pollen tube growth. Weaver and Timm [17] suggested that pollen is more sensitive to high temperatures than female reproductive organs, which could account for a lack of fertilization under high- temperatures stress. The low temperature reduces the molecular mobility in the cytoplasm, which may be a controlling factor in pollen longevity [18]. Studies have revealed that the optimal temperature for in vitro germination assays can be species dependent. The pollen from many species found to germinate well at 25°c; however, differences exist. For instance, the study conducted by Burke *et al*. [8] shown that cotton pollen has an optimum germination temperature of 28°c to 31°c. The studies conducted by Boavida *et al*. [19] also revealed that the optimum temperature for pollen germination ranges between 25-30 °c.

IV. CONCLUSION

The present study was an attempt to explore the germination characteristics of pollen grains under varied conditions. Initially, pollen grains were subjected to germination in mediums containing different concentrations of sucrose and the medium containing 200 g/L sucrose was selected as the most suitable one based on the maximum germination potential shown at this particular concentration. The present study further revealed the positive influence of boric acid on the germination potential of pollen grains in *Catharanthus roseus.* The study also explored the effect of pH and temperature on the germination potential of pollen grains and thus act as key factors that can affect germination in pollen viability.

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