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Original Article

# Effect of Some Nutrients on *In Vitro* Pollen Germination of *Withania Somnifera* (L.) Dunal

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**Abstract:** The present investigation reveals 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 *Withania somnifera* (L.) Dunal, a medicinally important plant belonging to the family of Solanaceae, to know the pollen viability and optimum nutrient requirements for *in vitro* pollen germination. Flowers start to open in the morning at 07.30 hrs.- 08.30hrs., after which anther dehiscence take place. Maximum 75% pollen germination along with 455μm pollen tube development was observed in 15% sucrose solution supplemented with 50 ppm boric acid and among the salts; maximum 56% pollen germination along with 316μm pollen tube was observed in 100 ppm Potassium nitrate solution. Pollen grains which were collected in the morning (08.30-09.30hrs.) showed best results.

**Keywords:** Pollen germination, Sucrose, Boric acid, Salts, *Withania somnifera.*

## Introduction

Germination is the first critical morphogenetic event in the pollen towards fulfilling its ultimate function of discharge of male gametes in the embryo sac. It is therefore important to understand the physiology and biochemistry of pollen germination. The stigma provides a suitable site for pollen germination, however studies on *in vivo* are not easily feasible because of the complications involving in pistillate tissue. 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. Pollen grains, being the sexual reproductive unit and the carrier of male genetic material in higher plants, play a vital role in breeding programme and assists successful fruit-set. High crop yield generally depends on viable pollen grains. Pollen fertility and viability have a paramount importance in hybridization programme. Pollen performance in terms of germinating ability may have the relative importance upon not only fruit-set, but also the flower-flower and flower-pollinator interaction. The basic needs for the improvements of plants before going to the breeding programme are pollen fertility, viability and its longevity. *Withania somnifera* has highly flexible mating systems and is facultative xenogamous (1, 2). The present work 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 *Withania somnifera* (L.) Dunal, a medicinally important plant (3, 4, 5) belonging to the family Solanaceae (Fig.1). It has extensive medicinal properties and one of its products is commonly called as ashwagandha. It is one of the most widespread tranquillizers used in India, where it holds a position of importance similar to ginseng in China (6). It acts mainly on the reproductive and nervous systems, having a rejuvenative effect on the body, and is used to improve vitality and aid recovery after chronic illness (6, 7).

## Materials and Methods

For the study of *In Vitro* pollen germination, newly opened flowers were collected in the morning (08.30-09.30hrs.) and transferred to polythene bags. The fresh pollen samples 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 petridishes lined with moist filter paper and examined under an Olympus microscope at low magnification (10x X 15x) at different time intervals to know the germination percentage and pollen tube length (8). A pollen grain was considered as

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germinated if pollen tube length at least becomes twice greater than the diameter of the pollen (9).

## Results

Studies on *In Vitro* pollen germination after anthesis indicated that 55% germinating pollen along with 317 μm long pollen tube development occurred in 15% sucrose solution (Table-1). Individually, 50ppm boric acid showed 55% germination along with 270 μm long pollen tube (Table-2). The pollen germination as well as tube development decreased in lower concentrations as well as in higher concentrations of sucrose. Same thing happened in case of boric acid also. The highest germinating pollen (75%) along with 455μm long pollen tube developed in 15% sucrose solution supplemented with 50 ppm boric acid (Table-3, Fig.-2).

Among the salts, maximum 56% pollen germination along with 316μm pollen tube development in 100 ppm Potassium nitrate solution following 52% pollen germination along with 296μm pollen tube was observed in 400 ppm Calcium nitrate solution and 29% pollen germination along with 156μm long pollen tube in 200ppm Magnesium sulphate solution (Table-4). Both Potassium and Calcium showed good results, however maximum pollen germination as well as tube development was observed in Potassium nitrate solution. Pollen germination and tube length decreased in lower as well as in higher concentrations (Table-4).

## Discussion

The pronounced effect of the 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 (10, 11). The role of boron in germinating pollen and growing pollen tubes has been confirmed in vascular plants (12, 13). Boron is directly involved in pectin synthesis and thus indirectly involved in development of pollen tube membrane (14). Boron could exert a protective effect in preventing excessive polymerization of sugars at sites of sugar metabolism (15). In nature water, sugar, amino acids are supplied by the style to nourish the growing pollen tube. Boron is also provided by stigmas and styles, facilitates sugar uptake and has a role in pectin production in the pollen tube (16). Boric acid is known to be crucial for pollen germination and tube growth and it is required at concentration of 100ppm for most species (17). Boron plays a role in flowering and fruiting process in pistachio (18) and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth (19). 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 the *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 (20, 21). Boron plays an important role on the localization of pectins and callose in the wall of pollen tubes in *Picea meyeri* (22). The stimulatory effect of boron also reported on *In Vitro* pollen germination of *Pistacia vera* (21). 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 (13, 23, 24).

Salts of Calcium, Magnesium and Potassium 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. The results indicate that Pottasium ion was the most effective to influence the pollen germination followed by Calcium and Magnesium. 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 (25, 26). Calcium probably gives rigidity to the pollen tube wall by binding pectic carboxyl groups and also induced pollen germination (27). Calcium concentration plays a critical role in maintaining the tube growth (28, 29). 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 (30, 31). It is also known that $K^{+}$ is required for both pollen germination and tube growth (30, 32, 33). Both $Ca^{++}$ and $K^{+}$ are interdependent on each other because the inward $K^{+}$ channels are greatly regulated by $Ca^{++}$ as in case of *Arabidopsis* pollen (34) as well as stomatal guard cells (35, 36, 37, 38, 39, 40, 41). Externally supplied $K^{+}$ ion

enhanced the rate of pollen germination as well as pollen tube growth in *Arabidopsis* (34). $NO_3^-$ and $Mg^{++}$ enhanced the tube growth in the case of *In vitro* pollen germination of sugarcane (42). Calcium, Magnesium and Nitrate play a key role in pollen tube growth of *Luffa aegyptica* (43). The role of sucrose, boric acid and different salts like Calcium nitrate, Potassium nitrate and Magnesium sulphate on *In vitro* pollen germination was established (44, 45). Thus, the present findings corroborated by several previous experiments (46, 47, 48, 49, 50, 51, 52, 53, 54).

## Conclusion

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. Sucrose supplemented with Boric acid promoted pollen germination as well as tube development as sugar-borate complex is known to be capable of better translocation than non-borate, non-ionized sugar molecules. Among the salts, Potassium nitrate and Calcium nitrate play a vital role in pollen germination as well as pollen tube development.

**Table.1:** Effect of sucrose on *In vitro* pollen germination *of Withania somnifera* (L.) Dunal

| Conc. (%) | After 1 hr. | | After 2 hrs. | | After 4 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) |
| Distilled water | -- | -- | -- | -- | -- | -- |
| 1 | -- | -- | -- | -- | -- | -- |
| 2 | -- | -- | -- | -- | -- | -- |
| 5 | 10 | 26 | 14 | 58 | 16 | 65 |
| 10 | 21 | 91 | 30 | 158 | 36 | 169 |
| 12 | 32 | 128 | 42 | 245 | 48 | 256 |
| ***15*** | ***38*** | ***142*** | ***48*** | ***298*** | ***55*** | ***317*** |
| 20 | 18 | 91 | 24 | 116 | 28 | 130 |
| 25 | 16 | 65 | 19 | 78 | 21 | 91 |

**Table.2:** Effect of boric acid on *In vitro* pollen germination of *Withania somnifera* (L.) Dunal

| Conc. (ppm) | After 1 hr. | | After 2 hrs. | | After 4 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) |
| 25 | 32 | 128 | 38 | 238 | 42 | 256 |
| ***50*** | ***38*** | ***158*** | ***52*** | ***252*** | ***55*** | ***270*** |
| 100 | 28 | 113 | 32 | 198 | 38 | 216 |
| 200 | 24 | 91 | 21 | 113 | 26 | 123 |
| 300 | 10 | 26 | 12 | 52 | 13 | 65 |

**Table.3:** Effect of sucrose and boric acid on *In vitro* pollen germination of *Withania somnifera* (L.) Dunal

| Conc. (Sucrose+ Boric acid) | After 1 hr. | | After 2 hrs. | | After 4 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) |
| 15%+25ppm | 34 | 195 | 42 | 316 | 45 | 385 |
| ***15%+50ppm*** | ***62*** | ***221*** | ***70*** | ***426*** | ***75*** | ***455*** |
| 15%+100ppm | 46 | 218 | 61 | 385 | 65 | 426 |
| 15%+200ppm | 44 | 218 | 46 | 368 | 54 | 392 |
| 15%+300ppm | 38 | 195 | 40 | 298 | 42 | 346 |

**Table.4:** Effect of $Ca(NO_3)_2$, $KNO_3$ and $MgSO_4$ on *In vitro* pollen germination of *Withania somnifera* (L.) Dunal

| Salts | Conc. (ppm) | After 1 hr. | | After 2 hrs. | | After3 hrs. | |
|---|---|---|---|---|---|---|---|
| | | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) | Germination (%) | Mean tube length (μm) |
| $Ca(NO_3)_2$ | 50 | -- | -- | -- | -- | -- | -- |
| | 100 | 10 | 26 | 12 | 52 | 16 | 65 |
| | 200 | 15 | 52 | 18 | 78 | 26 | 91 |
| | 300 | 23 | 116 | 30 | 142 | 32 | 156 |
| | ***400*** | ***32*** | ***126*** | ***38*** | ***225*** | ***52*** | ***296*** |
| | 500 | 16 | 56 | 24 | 104 | 28 | 128 |
| $KNO_3$ | 25 | 15 | 39 | 22 | 65 | 23 | 78 |
| | 50 | 34 | 126 | 42 | 242 | 48 | 256 |
| | ***100*** | ***42*** | ***148*** | ***54*** | ***298*** | ***56*** | ***316*** |
| | 200 | 25 | 116 | 30 | 156 | 34 | 168 |
| | 300 | 16 | 116 | 24 | 126 | 25 | 136 |
| | 400 | 14 | 65 | 20 | 84 | 21 | 91 |
| | 500 | 10 | 52 | 13 | 72 | 13 | 78 |
| $MgSO_4$ | 25 | 6 | 26 | 8 | 39 | 8 | 55 |
| | 50 | 10 | 42 | 14 | 54 | 16 | 65 |
| | 100 | 18 | 78 | 20 | 113 | 24 | 116 |
| | ***200*** | ***21*** | ***98*** | ***24*** | ***148*** | ***29*** | ***156*** |
| | 300 | 14 | 48 | 14 | 65 | 16 | 72 |
| | 400 | 6 | 16 | 8 | 24 | 8 | 26 |
| | 500 | -- | -- | -- | -- | -- | -- |

**Fig.1:** *Withania somnifera* (L.) Dunal

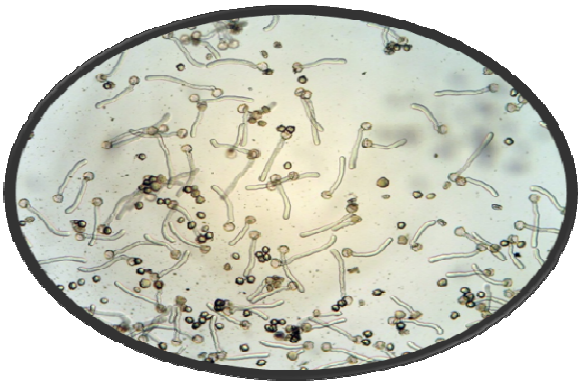

**Fig.2:** *In vitro* germinating pollen grains of *Withania somnifera* (L.) Dunal

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