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**Research Article**

# THE ROLE OF SOME NUTRIENTS ON IN VITRO POLLEN GERMINATION OF SWEET BROOM WEED (SCOPARIA DULCIS L.)

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**ABSTRACT**

*In vitro* pollen germination of *Scoparia dulcis* L. belonging to Scrophulariaceae has been carried out to study the role of different nutrients like sucrose, boric acid, different salts like calcium nitrate, potassium nitrate and magnesium sulphate on pollen germination and tube growth. Flowers open in the morning (06:30-07:30 hrs.) after which anther dehiscence takes place. Maximum 97 % pollen germination along with 1183 µm pollen tube development was observed in 5 % sucrose solution supplemented with 100 ppm boric acid. Among the salts, maximum 52 % pollen germination along with 325 µm pollen tube development was observed in 300 ppm calcium nitrate solution. Pollen grains which were collected during anthesis (08:00-09:00 hrs.) showed the best results.



## INTRODUCTION

*Scoparia dulcis* L. commonly known as 'sweet broom weed', grows everywhere including crop fields. It's a profusely branched, erect herb belongs to the family Scrophulariaceae having ethno-medicinal importance. The plant bears white flowers with 4 stamens, containing large amount of pollen grains in anther lobes. Pollen grains are unicellular male gametophytes of angiosperms, after release from the anthers reside on a compatible stigma, germinate and develop pollen tubes that elongate through the stylar matrix to reach the ovary and ultimately to the embryo sac, where tube tips bursts and sperm cells are discharged for ensuring fertilization. *In vitro* pollen germination was carried out to study pollen viability in respect to germination and pollen tube growth. Pollen fertility and viability have a paramount importance in breeding programmes. The stigma provides a natural environment for pollen germination, but pollen can also be germinated *in vitro* provided with suitable nutrients, which helps to understand the physiology and biochemistry of pollen germination. The present investigation is aimed to study the effect of sucrose, boric acid, potassium nitrate, calcium nitrate and magnesium sulphate separately or in combinations on *in vitro* pollen germination and tube length of *Scoparia dulcis* L.

## MATERIALS AND METHODS

Fresh and newly opened flowers were collected in the morning (08:30-09:30 hrs.) during anthesis and transferred to polythene bags. Solutions of different concentrations of sucrose (1-50%), boric acid (50-500 ppm) and salts (50-500 ppm) like calcium nitrate [$Ca(NO_3)_2$], magnesium sulphate ($MgSO_4$) and potassium nitrate ($KNO_3$) were prepared. Pollen grains were sown on several grooved slides containing solution of sucrose ($C_{12}H_{22}O_{11}$) and boric acid ($H_3BO_3$) at different concentrations separately or in combinations and salts of potassium nitrate ($KNO_3$), calcium nitrate [$Ca(NO_3)_2$] and magnesium sulphate ($MgSO_4$) were also used to find out their effects on pollen germination and tube development. Slides were then kept in petridishes lined with moist filter paper and examined under microscope at low magnification (10x X15x) at different time intervals to record the germination percentage and pollen tube length following the method of Shivanna and Rangaswamy (1993). Pollen grains were considered as germinated, when the pollen tube length were twice greater than the diameter of the pollen grains (Gupta *et al*, 1989).

## RESULTS AND DISCUSSION

In the present experiment, *in vitro* pollen germination showed that, 86% pollen germination with an average of 988 µm long pollen tube development occurred in 5% sucrose solution after

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4 hrs. of incubation (Table 1, Fig. 1), while solution with 100 ppm boric acid showed 87 % germination along with an average of 849 µm long pollen tube (Table 2). The maximum 97 % germinating pollen along with 1183 µm long pollen tube developed after 4 hrs in 5 % sucrose solution supplemented with 100 ppm boric acid (Table 3).

**Table 1** Effect of sucrose on *in vitro* pollen germination of *Scoparia dulcis* L.

| 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) |
| Dist. $H_2O$ | 1 | 26 | 8 | 78 | 12 | 156 |
| 1 | 3 | 52 | 11 | 104 | 25 | 234 |
| 2 | 37 | 130 | 46 | 598 | 70 | 910 |
| 5 | 28 | 156 | 63 | 520 | 86 | 988 |
| 10 | 10 | 117 | 60 | 429 | 72 | 780 |
| 12 | 10 | 78 | 20 | 182 | 42 | 351 |
| 15 | 9 | 65 | 18 | 169 | 41 | 338 |
| 20 | 9 | 65 | 17 | 143 | 38 | 286 |

**Table 2** Effect of boric acid on *in vitro* pollen germination of *Scoparia dulcis* L.

| Conc. (ppm) | After 1hr. | | After 2 hrs. | | After 4 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Mean tube length(µm) | Germination (%) | Mean tube length (µm) | Germination (%) | Mean tube length (µm ) |
| 25 | 13 | 39 | 32 | 143 | 42 | 325 |
| 50 | 38 | 39 | 43 | 169 | 46 | 433 |
| 100 | 64 | 65 | 78 | 481 | 87 | 849 |
| 200 | 43 | 52 | 65 | 429 | 76 | 780 |
| 300 | 27 | 39 | 31 | 416 | 36 | 676 |
| 400 | 11 | 26 | 17 | 273 | 19 | 351 |

**Table 3** Effect of sucrose and boric acid on *in vitro* pollen germination of *Scoparia dulcis* L.

| Conc. (%+ppm) sucrose + boric acid | After 1hr. | | After 2 hrs. | | After 4 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Mean tube length (µm) | Germination (%) | Mean tube length ( µm) | Germination (%) | Mean tube length (µm) |
| 1+100 | 9 | 52 | 31 | 195 | 52 | 254 |
| 2+100 | 16 | 78 | 52 | 312 | 71 | 448 |
| 5+100 | 55 | 91 | 83 | 832 | 97 | 1183 |
| 10+100 | 53 | 91 | 79 | 495 | 90 | 832 |
| 12+100 | 42 | 65 | 57 | 325 | 63 | 559 |
| 15+100 | 44 | 65 | 53 | 286 | 61 | 494 |

Salts 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. Among the salts, calcium nitrate showed maximum 52 % germinating pollen along with 325 µm pollen tube in 300 ppm solution (Table 4),

**Table 4** Effect of Ca $(NO_3)_2$ on *in vitro* pollen germination of *Scoparia dulcis* L.

| Conc. (ppm) | After 1 hr. | | After 2hrs. | | After 4hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Mean tube length ( µm) | Germination (%) | Mean tube length (µm) | Germination (%) | Mean tube length ( µm) |
| 50 | - | - | 1 | 26 | 2 | 78 |
| 100 | 1 | 52 | 3 | 91 | 5 | 104 |
| 200 | 10 | 86 | 21 | 143 | 26 | 191 |
| 300 | 22 | 98 | 46 | 234 | 52 | 325 |
| 500 | 3 | 26 | 7 | 91 | 22 | 130 |

followed by 41 % germinating pollen along with 195 µm tube length in 200 ppm potassium nitrate solution (Table 5) and 6 % pollen germination with 156 µm pollen tube in 100 ppm magnesium sulphate solution (Table 6) respectively.

**Table 5** Effect of $KNO_3$ on *in vitro* pollen germination of *Scoparia dulcis* L.

| Conc.(ppm) | After 1hr. | | After 2hrs. | | After 4 hrs. | |
|---|---|---|---|---|---|---|
| | Germination (%) | Mean tube length (µm) | Germination (%) | Mean tube length (µm) | Germination (%) | Mean tube length ( µm) |
| 50 | 2 | 26 | 11 | 65 | 24 | 78 |
| 100 | 5 | 32 | 13 | 65 | 30 | 91 |
| 200 | 10 | 91 | 20 | 142 | 41 | 195 |
| 300 | 8 | 37 | 10 | 121 | 17 | 156 |
| 500 | 5 | 39 | 14 | 62 | 16 | 143 |

**Table 6** Effect of $MgSO_4$ on *in vitro* pollen germination of *Scoparia dulcis* L.

| 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) |
| 50 | - | - | 1 | 52 | 1 | 78 |
| **100** | **2** | **91** | **4** | **104** | **6** | **156** |
| 200 | 1 | 26 | 1 | 65 | 3 | 117 |
| 300 | 1 | 26 | 3 | 52 | 3 | 78 |
| 500 | 1 | 26 | 1 | 26 | 1 | 39 |

*In vitro* pollen germination study showed that in addition to moisture, germination of pollen required a carbohydrate and boric acid source for satisfactory germination and tube growth. The pronounced effect of sucrose and boric acid on increasing trend of germinating pollen might be reflected with the views of Johri and Vasil (1961) and Shivanna and Johri (1989), who stated that the externally supplied sucrose maintains the osmotic pressure and acts as a substrate of energy source for pollen metabolism. Though the effect of sucrose or boric acid individually showed good results but boron makes a complex with sugar and this sugar-borate complex is capable of better translocation than non borate, non-ionized sugar molecules (Sidhu and Malik, 1986). Boron plays a crucial role in germinating pollen and growing pollen tubes in vascular plants (Lewis, 1980) and is directly involved in pectin synthesis for pollen tube elongation (Stanley and Loewus, 1964). Boron in the form of boric acid is required for pollen germination at concentration of 100 ppm for most species (Brewbaker and Majumder, 1961).

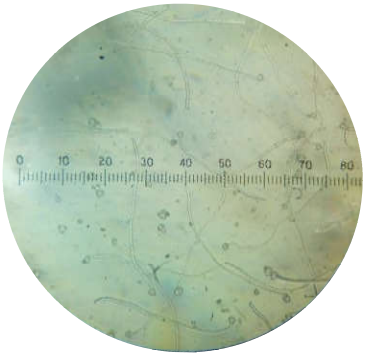


**Figure 1** *In vitro* germinating pollen.

Besides sucrose and boric acid, salts of calcium, potassium and magnesium are also known to have a stimulatory effect on pollen germination and tube growth. Calcium is one of the important cation involved in cell metabolism and maintains membrane permeability (Jones and Lunt, 1967). Kwack (1967) stated that, calcium probably gives rigidity to the pollen tube wall and also induced pollen germination. According to Taylor and Hepler (1997) pollen germination and tube growth are regulated by the transport of inorganic ions, such as $Ca^{++}$ and $K^{+}$ in plasma membrane of pollen tubes. In *Arabidopsis*, K+ ions enhanced the rate of pollen germination and tube growth (Fan *el al*, 2001). According to Moore and Jung (1974), nitrate and magnesium ions enhanced pollen tube growth of sugarcane. In the present investigation, the role of sucrose, boric acid, salts such as calcium nitrate, potassium nitrate and magnesium sulphate in pollen germination as well as pollen tube elongation were studied and findings are corroborated with the works of Mondal *et al* (1991), Bhattacharya *et al* (1997), Biswas *et al* (2008), Mondal and Ghanta (2012), Choudhury *et al* (2013), Biswas and Mondal (2014), Dutta and Mondal (2014), Ghanta and Mondal (2016) and Pal *et al* (2017).

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