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RESEARCH ARTICLE

EFFECT OF SUCROSE AND BORIC ACID ON *IN-VITRO* POLLEN GERMINATION OF *GUAIACUM OFFICINALE* L.

***Chaitra B. Negalur and H.C. Lakshman**

Department and Studies in Botany, Microbiology Laboratory, Karnataka University, Dharwad - 580 003, India

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ABSTRACT

The evaluation of pollen viability and its germination capacity are two essential criteria for pollinator's characterization. This study was carried out to determine *in vitro* pollen viability and pollen germination in a medicinal plant *Guaiacum officinale* L. belonging to the family Zygophyllaceae. To study the role of boron in pollen germination and pollen tube growth, pollen grains were cultured in standard medium or Boron deficient medium. The maximum 48% pollen germination along with 56.89µm long pollen tube developed in 15% sucrose solution supplemented with 0.01% boric acid compaired to the boron deficient medium. Two pollen viability tests, TTC (2,3,5-triphenyl tetrazolium chloride) and Cotton blue in lactophenol were used and pollen grains showed nearly 39.2% and 40.6% viability respectively. Pollen grains which were collected in the morning (6.00hrs - 8.00 hrs.) showed best results. Stained pollen rate was higher and pollen was well-stained with Cotton blue test and pollen viability estimated with Cotton blue staining test was better than that TTC estimated with the staining test. The result revealed that the boron has a regulatory role in pollen germination and pollen tube growth in *Guaiacum officinale* L. which is necessary for the regeneration and conservation of this highly medicinal plant.

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INTRODUCTION

Pollen viability and pollen germination are prerequisite for fertilization and seed development. 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 development of reliable methods for determining the functional quality of pollen helps in monitoring pollen vigour during storage, genetics and pollen-stigma interaction studies, crop improvement and breeding, and incompatibility and fertility studies (Stanley and Linskens, 1974; Heslop-Harrison and Shivanna, 1984; Shivanna and Rangaswamy, 1992). The physiological and biochemical investigations on pollen fertility and pollen germination *invivo* are rather difficult. Many extensive *invitro* germination techniques are used to study the pollen germination. Such studies have provided considerable information on the physiology and biochemistry of pollen germination and pollen tube growth (Shivanna and Johri, 1985; Heslop-Harrison, 1987; Steer and Steer, 1989). Pollen germination or vigour can be affected by many factors, including temperature, medium osmolarity and the availability of calcium, zinc and boron (Sawidis and Reiss 1995; Taylor and Hepler 1997).

**Corresponding author: Chaitra B. Negalur*
Department and Studies in Botany, Microbiology Laboratory, Karnataka University, Dharwad - 580 003, India.

The effect of either sucrose or boric acid individually showed good results, but 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; Sidhu and Malik, 1986). Keeping in this view, the present work is aimed the effect of sucrose and boric acid on *invitro* pollen germination of *Guaiacum officinale* L.

MATERIALS AND METHODS

Plant materials

For the study of *invitro* pollen germination, newly opened flowers were collected in the morning (6.00hrs - 8.00 hrs.) from Karnataka Science College, Dharwad and transferred to polythene bag.

Pollen germination

Invitro pollen germination was studied by hanging drop method using (Brewbaker and Kwack's, 1963) medium and different concentration of sucrose (10, 15 & 20%) individually as well as in combination with different combination of boric acid (0.1%, 0.01%, 1%).

Table 1. Showing percentage of pollen germination at different time intervals

Plant	Time interval	No. of pollen germinated.	No. of pollen un-germinated.	Percentage of pollen germinated.
Guaiacum officinale L.	2hr	-	-	-
	4hr	-	-	-
	6hr	5	32	15.6%
	8hr	19	46	41.3%

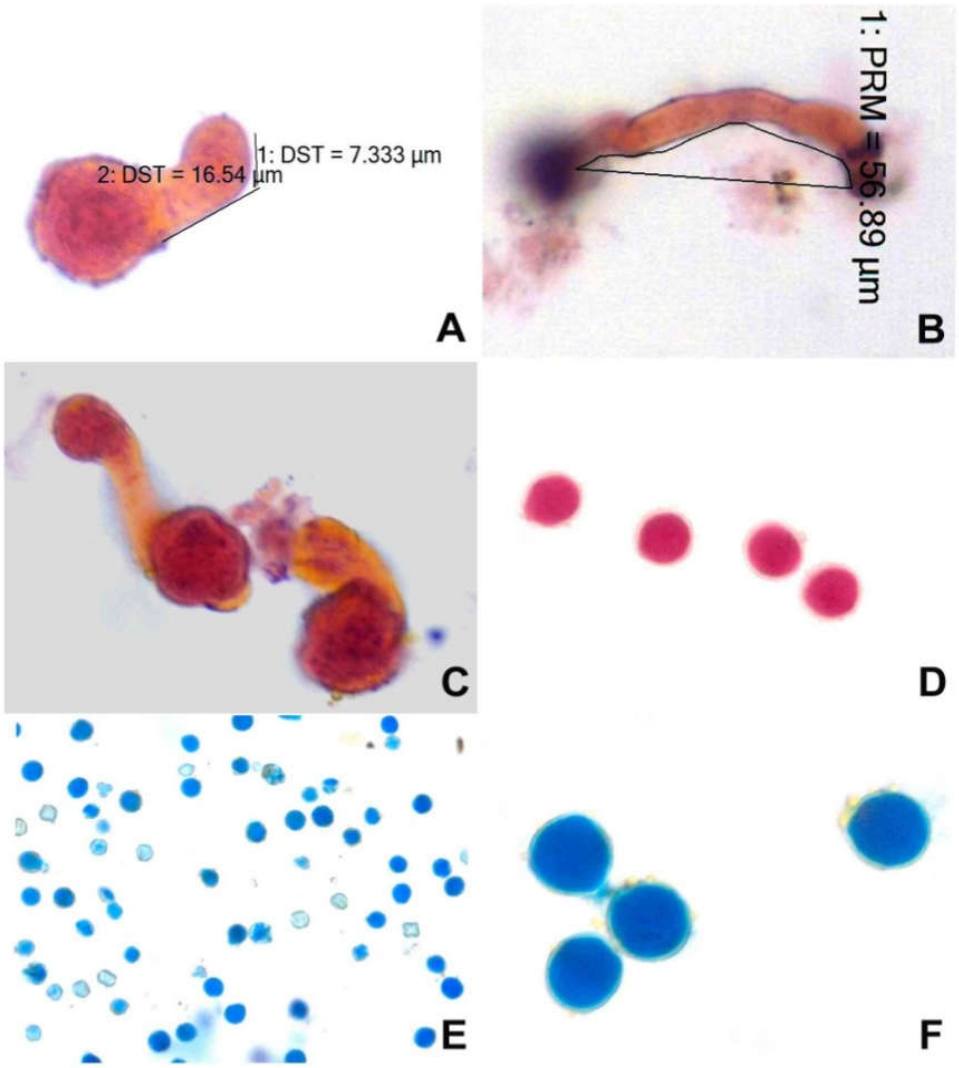

Fig.1. *Invitro* pollen germination and viability test in *G. officinale* L. Fig. A: *In vitro* pollen germination test by using Sucrose solutions (50µm). Fig. B: *In vitro* pollen germination test by using Sucrose + 0.01% boric acid (50µm). Fig. C: *In vitro* pollen germination test in Boron deficient medium (50µm). Fig. D: Pollen viability test by 2,3,5-triphenyl tetrazolium chloride (TTC) (50µm). Fig. E, F: Pollen viability test by Cotton Blue in lactophenol (E-200µ,F-50µm)

The fresh pollen samples were sown on several grooved slides containing solution of sucrose and boric acid at different concentrations separately or in combinations. Slides were then kept in Petri dishes lined with moist filter paper and examined under the microscope at low magnification (150X) 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 pollen tube length at least becomes twice greater than the diameter of the pollen grains (Gupta *et al*., 1989).

Percentage of pollen germination was calculated by the following method:

$$\%\ of\ Pollen\ germination = \frac{No.\ of\ germinated\ pollen\ grains}{Total\ No.\ of\ germinated\ and\ non\ germinated\ pollen\ grains}\ X\ 100$$

Pollen Viability

In order to determine the viability test, the collected pollen grains were subjected to 1% TTC (2,3,5-tri-phenyl tetrazolium chloride) test after (Hauser and Morrison, 1964) and Cotton blue in lactophenol. Small amount of pollen grains were suspended in a drop of TTC solution and slide was covered with a cover glass. After incubation period, pollen grains which turned red were counted as viable.

RESULTS

Studies on *in-vitro* pollen germination at different time intervals after anthesis indicated that 41.3% germinating pollen with a mean of 56.89µm long pollen tube development observed in 15% sucrose solution in combination with 0.01%

boric acid. In boron-deficient medium, pollen germination ranged from 13 to 17% with a mean of 15% with a pollen tube 23.87 µm. In the presence of sucrose with 0.01% boric acid, pollen germination rate increased significantly ($P < 0.05$) compared with that in boron-deficient medium, reaching a maximum of 41.3% with a pollen tube 56.89 µm. However, pollen germination started bursting at higher concentration of 20%, 25% sucrose and also some pollen grains stopped germinating by the addition of 0.1% and 1% boric acid respectively. Pollen tubes cultured in the medium containing sucrose + 0.01% boric acid differed morphologically from the pollen tubes cultured in the boron deficient medium after 10 hrs (Fig.1C). In the Standard medium, pollen tubes seemed healthy with normal length and shape. Lack of boron inhibited pollen tube elongation and caused morphological abnormalities, especially swelling of the tip region of the pollen tubes. The pollen grains were also tested with 1% 2,3,5-tri-phenyl tetrazolium chloride (TTC) and Cotton blue in lactophenol test to check viability. The viable pollen grains show red colour due to the accumulation of formazon, whereas nonviable pollen grains remain colourless (Fig.1D). The pollen grains when tested with cotton blue in lactophenol, the viable pollen grains become purple and non-viable pollen grains remain faint/ colourless (Fig.1)

DISCUSSION

Boron added in the form of boric acid is very crucial for pollen germination and tube growth and it is required at concentration of 100ppm for most species (Brewbaker and Majumder, 1961). In boron deficient medium, it leads to tube bursting (Holdaway-Clarke and Hepler, 2003; Acar *et al*., 2010). The germination and growth of pollen can be considerably improved through the addition of boric acid of appropriate concentrations. The *invitro* pollen germination is less if boric acid is not added or if high concentrations of boric acid are added (Teng *et al.,* 2009). In the present study it was observed that with an increase in the concentration of sucrose (15%) in combination with 0.01% boric acid, germination percentage increased. This is in accordance with the results reported in *Tribulus terrestris* L. by (Ahmad *et al.,* 2012). Percentage of pollen germination was less in different concentration of sucrose alone. When boric acid was added to the medium, germination percentage as well as pollen tube growth increased drastically. In the boron deficient medium, pollen tubes showed abnormalities like coiling and bursting. (Shivanna *et al.,* 1985; Heslop-Harrison ,1987; Steer *et al.,*1985). Sugar can regulate the osmotic potential in pollen tube growth. It is also a source of nutrients and energy. At suitable sucrose concentrations, the balance between the internal and external osmotic pressures of pollen can be maintained, thereby preserving the normal vitality of pollen. Along with the sugar content boron acts as an essential microelement for pollen germination and pollen tube growth. It is believed to promote pollen germination by affecting H+-ATPase activity, which initiates pollen germination and tube growth (Feijó *et al.*, 1995, Obermeyer and Blatt 1995). Boron deficiency also caused morphological abnormalities, including swelling at the tip of the pollen tube (Fig.2A, 2B). Similar findings have been reported for pollen tubes in several angiosperm species (Dickinson 1978, Yang et al. 1999). We note that only low concentrations of H_3BO_3 (0.01%) stimulated pollen germination and pollen tube growth, whereas H_3BO_3 concentrations above 0.01% inhibited pollen grain germination and pollen tube elongation of *Picea meyeri*, which is in agreement with the findings reported for *Eucalyptus* pollen (Potts and Marsden-Smedley 1989). Boron plays a role in flowering and fruiting process in pistachio (Brown *et al.,* 1994) and its deficiency results in low pollen viability, poor pollen germination and reduced pollen tube growth (Nyomora and Brown, 1997). Boron takes part in pollen germination and style tube formation and therefore has a vital function in fertilization of flowering crops. Thus, the present work gets supports from Vasil (1964), Gupta *et al*., (1989), Pal *et al.,* (1989), Mondal *et al*., (1991), Bhattacharya *et al*., (1997) and Bhattacharya and Mandal (2004), Biswas *et al*., (2008) and Acar *et al*., (2010).

Conclusion

In the present study it was observed that 15% Sucrose + 0.01% boric acid can significantly promote the germination and growth rate of *G.officinale* L. pollen. The percentage of pollen germination was less in sucrose alone. When 0.01% boric acid was added to the medium, germination percentage as well as pollen tube growth was more. In the absence of boron *i.e.,* high concentration of sucrose the pollen tubes showed abnormalities like swelling at the tip of the pollen tube. The results also showed pollen viability and germination rates *G.officinale* L. by TTC and Cotton blue in lactophenol. The viable pollen grains showed red and Purple colour and non-viable pollen grains remain faint/colourless respectively. The results presented here are the first observation on the *G.officinale* L. pollen viability and germination rate that will help in *G.officinale* L. reproduction and artificial pollination studies.

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