# **Research Article**



# EFFECT OF DIFFERENT MEDIA AND BORIC ACID ON POLLEN GERMINATION AND TUBE GROWTH OF TRIBULUS TERRESTRIS - A TRADITIONAL MEDICINAL PLANT

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#### ABSTRACT

*Tribulus terrestris* is an annual plant, used in folk medicine as urinary-anti-infective. In the present study, effect of different media and boric acid on *in vitro* pollen germination and pollen tube growth was carried out in *Tribulus terrestris*. Pollen germination study was analyzed at different concentration of sucrose and Brewbaker and kwack's medium. The germination started at 10% sucrose solution. Maximum percentage of germination (18 % with 95 µm long pollen tube) was recorded in Brewbaker and kwack's medium. Bursting of pollen grains was observed at 20% sucrose solution. Addition of 0.01% boric acid to different concentration of sucrose solution prevent the pollen bursting and considerably enhanced pollen tube growth. Pollen grains remain viable at the time of anthesis and the viability lasts 24 hours after anthesis. The results reveal that boric acid have a positive role in pollen germination and tube growth in the studied plant.

Keywords: Anthesis, Folk medicine, Pollen germination, Tribulus terrestris, Viability.

## INTRODUCTION

Pollen germination and pollen tube growth are prerequisite for fertilization and seed development. Pollen viability and stigma receptivity are critical factors for the successful completion of post-pollination events<sup>1</sup>. Due to involvement of the pistillate tissues in nature, physiological and biochemical investigations on pollen germination and pollen tube growth in vivo are rather difficult. *In vitro* germination techniques have therefore been used extensively on a variety of pollen systems. Such studies have provided considerable information on the physiology and biochemistry of pollen germination and pollen tube growth <sup>1-3</sup>. Pollen germination and tube growth are generally divided into four phases: imbibitions phase, lag phase, tube initiation phase and rapid tube elongation phase<sup>4</sup>.

*Tribulus terrestris* L. commonly known as puncture-vine, puncture – weed or goathead is the member of family zygophyllaceae which is represented by 22 genera and 220 species. The plant is distributed in warm regions of Asia, Africa, Europe, America and Australia<sup>5-7</sup>. It is used as constituent in Indian ayurveda practice, where it is known by its Sanskrit name, "gokshura". The fruit, roots and the whole plant is used for the medicinal purpose.

Despite of its high medicinal value limited information is available on its pollen physiology. Keeping in view of all these aspects, the main objective of present research was to determine the effect of different media and boric acid on: (1) *in vitro* pollen germination and (2) pollen tube growth.

#### MATERIALS AND METHODS

The pollen grains were collected from freshly dehisced anthers and the germination was tested throughout the flowering period by the following methods:

## Brewbaker and Kwack's Medium

The pollen grains collected from the freshly dehisced anthers were tested for *in vitro* germination by hanging drop culture method after<sup>8</sup>. Pollen grains, which had germinated and produced pollen tubes in the medium, were recorded. Length of pollen tubes in the medium was recorded 45 minutes after the commencement of germination and percentage of pollen germination was calculated and the average length of pollen tube was recorded.

#### **Sucrose Solution**

*In vitro* pollen germination was studied in different concentration of sucrose solution alone (10, 15, 20 and 25%) and in combination with 0.01% boric acid<sup>9</sup>. The pollen grains collected from the freshly dehisced anthers were suspended in different concentration of sucrose solution. Pollen grains, which had germinated and produced pollen tubes in the medium, were recorded. Length of pollen tubes in different concentration was recorded 45 minutes after the commencement of germination and percentage of pollen germination was calculated and the average length of pollen tube was recorded.

Percentage of pollen germination was calculated by the following method.

% Pollen germinatio n =  $\frac{\text{No. of germinated pollen grains}}{\text{Total no. of germinated and non - germinated pollen grains}} \times 100$ 



# RESULTS AND DISCUSSION

# In vitro pollen germination

Effect of different media and boric acid on in vitro pollen germination and pollen tube growth was carried out in Tribulus terrestris. Pollen germination study was analyzed at different concentration of sucrose and Brewbaker and kwack's medium. Pollen germination started in 10% sucrose solution. 9.5% with 48µm and 10.8% with 61µm long pollen tubes respectively was recorded at 10 and 15% sucrose solution. Thereafter as the concentration of sucrose increased a corresponding decline in the germination percentage was noted (Fig. 1). Bursting of pollen grains was observed at 20% sucrose solution. Maximum percentage of germination (18 % with 95 µm long pollen tube) was recorded in Brewbaker and kwack's medium. Pollen germination was also checked at different concentration of sucrose in combination with 0.01% boric acid. With an increase in the concentration of sucrose in combination with 0.01% boric acid, germination percentage increased. This is in accordance with the results reported in Trichosanthes diocia.<sup>10</sup> 77% pollen germination in Trichosanthes dioica in Brewbaker and Kwack's medium was recorded.<sup>11</sup> Similar observations in Brewbaker and Kwack's medium have also been reported in Pyrostegia venusta, Duranta repens and Barleria priontis. 12-12







**Figure 2:** Graph showing effect of different concentration of media on pollen tube length.

## Pollen tube growth

Pollen tube length was more or less found to be corresponding to the percentage of pollen germination. In different concentration of sucrose alone, maximum tube length (61  $\mu$ m) was recorded in 15% sucrose solution. Addition of 0.01% of boric acid to different concentration of sucrose solution considerably enhanced pollen tube growth (Fig. 2). At 10% sucrose + 0.01% boric acid, length of pollen tube was recorded to be 69  $\mu$ m, while 81 $\mu$ m long pollen tube was observed at 15% sucrose + 0.01% boric acid. Maximum tube length (95  $\mu$ m) was measured in Brewbaker and kwack's medium.

# CONCLUSION

In the present study it was observed that percentage of pollen germination was less in sucrose alone. When boric acid was added to the medium, germination percentage as well as pollen tube growth was more. In the absence of boron, pollen tubes showed abnormalities like coiling and bursting. Similar observations have also been made by previous workers<sup>1-3</sup>. The role of boron in pollen germination and pollen tube growth may be 3-fold: (1) it promotes absorption of sugars (2) it increases oxygen uptake and (3) it is involved in the synthesis of pectic material for the wall of actively growing pollen tube.<sup>15</sup>

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