

The Essential Role of Different Factors in Pollen Germination and Pollen Tube Growth of *Heliotropium Hirsutissimum* Grauer (Boraginaceae)

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Abstract-

The elongation of the pollen tube in flowering plants is exceedingly rapid and its requirements, in general, seem quite unimpressive, i.e., water, oxygen and a suitable osmotic milieu.

Despite extensive attempts to hasten this growth process with the conventional host of growth factors, few have met with convincing success.

We study the essential role of different factors (temperature, PH, Sucrose, Boric acid, Calcium) in pollen germination and pollen tube growth of *heliotropiumhirsutissimum* (Boraginaceae). We used basal medium (Brewbaker&Kwack, 1963) with different temperatures (25-30-35°C), PH (5-6-7-8), Sucrose (100-140-180-220) g/L, Boric acid (0-100-200-300) mg/L, Calcium (0-300-400-500) mg/L. The best result for each factor in this study was:- Temperature: 30°C, PH: 6, Sucrose: 220 g/L, Boric acid: 100 mg/L, Calcium: 500 mg/L.

Keywords: pollen germination, *heliotropium*, Boric acid, Sucrose, PH

I. INTRODUCTION

In plants, post-pollination sexual selection can occur by competition between pollen grain and by female choice (stephens&Bertin, 1983)

Pollen grains are believed to compete for ovules when there are more pollen grains on the stigma than there are ovules in the ovary.

It is assumed that under conditions of pollen competition, only the fast growing pollen tubes achieve fertilization (MuLChy, 1979)

Selection due to differences between pollen donors can occur either before fertilization, as competition between pollen grains on the stigma and in the style, or after fertilization between the embryos (Herrero&Hormaza, 1996; pasonenet al., 1999)

Pollen grains of many plant species germinate, when placed in an artificial medium, forming a pollen tube that extends by tip growth.

Apart from non-permeant solutes (sucrose, polyethylene glycol, etc...), the medium need only

contain the mineral ions potassium, calcium and borate with appropriate anions such as phosphate and nitrate or chlorine (Weisensee et al., 1975)

The pollen tube is a cellular protuberance formed by the pollen grain, and is characterized by a rapid and unidirectional growth. Pollen tubes grow at very rapid rates that can reach up to 2.75 cm/h (schleiden, 1849)

Liliumlongiflorum, has a pollen tube that grows at 2 mm/h in vivo, while *tobacco* pollen grows at 1.7 mm/h (sanchez et al., 2004)

It should be mentioned that pollen tube growth in vitro is much slower than that of pollen growing in the style of the flower (Daher, 2011)

II. *Heliotropiumhirsutissimum* Grauer.

Annual, covered throughout with ± patent hairs, especially on calyx. Leaves ovate to ovate-Lanceolate, obtuse, rarely subacute. Cymes many-flowered, very dense at first, becoming laxer and elongated later. Stigma squat. Nutlets. Glabrous, densely tuberculate. Distribution area: Bulgaria, Greece, Aegean, N. Africa, Syria, Palestine. (Davis, 1978)

Heliotropium multipotent medicinal herb belonging to the family Boraginaceae has a wide range of medicinal values.

It has been used traditionally, like it is useful for local applications for ulcers, sores, wound, skin infections, stings of insects, and rheumatism (Bagadekar&Jayaraj, 2011; Kumar & Rao, 2007)



Nutlet

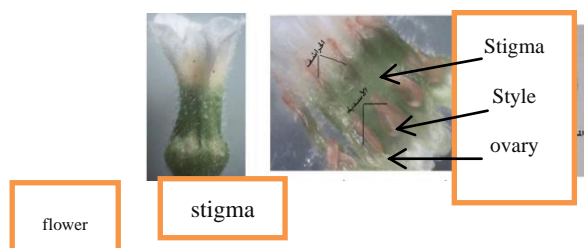


Figure 1: Heliotropium hirsutissimum Grauer. (haddad, 2011)

III. Materials and Methods

1. Plant material

The pollen used in all the essays related came from flowers of *H. hirsutissimum* (Boraginaceae) from Syria (Latakia).

2. Germination medium

We used Brewbaker and Kwach's medium (1963) containing: 100 g/L sucrose, 100 mg/L boric acid (H₃Bo₃), 300 mg/L Ca(NO₃)₂ .4H₂O, 200 mg/L Mgso₄, 100 mg/L KNO₃, 10 g/L Agar. With a PH adjusted to 6.

Agar was added to the mix and heated to dissolve. Hot agar containing medium was poured on to a petri dishes (10 cm diameter) to form a layer with a thickness of a bout (1 cm) and left to cool.

3. Study of different factors on germination, growth rate and pollen tube length

We study the effect:

- Temperature (25-30-35°C)
- PH (5-6-7-8)
- Sucrose (100-140-180-220)g/L
- Boric acid (0-100-200-300) mg/L
- Calcium (0-300-400-500) mg/L

4. Determination of the germination, growth rate and pollen tube length

Pollen grains collected from the freshly dehisced anthers for germination on solid medium (petri dishes)

Pollen was then sprinkled on the surface using (3ml) from liquid growth medium without agar, with moving of dishes.

The dishes were placed in a humid chamber with different temperatures. Pollen grains, which had germinated and produced pollen tubes in different conditions was recorded (15-30-100-120-180-240) 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(Ahmad *et al.*, 2012)

$$\% \text{ pollen germination} = \frac{\text{No. of germinated pollen grains}}{100} \times 100$$

Total no.of germinated and non-germinated pollen grains

For each experiment, at least five images per sample were taken at random, positions and the percentage of germination was quantified. To determine pollen tube length, at least thirty tube were measured for each experiment.

IV. Results and discussion

1. The effect of temperature on pollen germination and pollen tube growth

When pollen was distributed onto the germination medium, differents temperatures affected pollen viability under the three incubation temperatures 25, 30 and 35°C, being 35°C significantly higher.

Table 1: Means of pollen germination and pollen tube growth in agar medium under the effects three different temperatures

Incubation temperature °C	25		30		35	
	1h	2h	1h	2h	1h	2h
Pollen germination (%)	0	0	20	30	9	20
Pollen tube (µm)	0	0	52.6	153.3	49.7	122.12

The optimal temperature for in vitro germination assays can be species dependent. The pollen from many species germinates well at 25°C; however, differences exist. For example, cotton pollen has an optimum germination temperature of 28°C to 31°C (Burke *et al.*, 2004), 25 to 30°C in *Agave* genus (Lopes & Rodrigue, 2008), 30°C in *Erlenmeyer flasks* (Boavida *et al.*, 2007)

Temperature is among the most important environmental factors affecting plant reproductive processes such as pollen germination, pollen tube growth and fruit-set. In the present study, in vitro pollen germination and pollen tube growth of all cultivars were severely reduced under both high and low temperature conditions. Weaver and Timm (1988) suggested that pollen is more sensitive to high temperatures than femal 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 (Buitink *et al.*, 2000)

2. The effect of PH on pollen germination and pollen tube growth

We used the basal medium after fixation the best temperature 30°C, then, we study the effect four PH on pollen germination and pollen tube growth. We observed, there is no germination in a medium with PH 7 and 8. In a medium with PH 6, pollen grains were able to germinate, and the percentage of germination reached a high value that at this point of time was higher than in a medium with PH 5 after 2 hour.

Table 2: Means of pollen germination and pollen tube growth under the effects of four PH after 2h

PH	Pollen germination %	Means pollen tube growth (μ_m)
5	25	119
6	30	154
7	0	0
8	0	0

Medium PH is critical condition for in vitro pollen tube growth. For liliun, solanum and camellia pollen tubes, the optimum PH is situated between 5 and 6. Lower or higher PH values drastically reduce the percentage of germination and are unable to sustain pollen tube growth (Chebli&Geitmann, 2007)

3. The effect of Sucrose on pollen germination and pollen tube growth

We used the basal medium after fixation the best temperature 30°C and best PH, then, we study the effect of sucrose (100-140-180-220 g/L). We observed that in a medium with 220 g/L sucrose, pollen grains were able to germinate faster than in a medium with (100- 140-180) g/L sucrose. (Table 3, Figure 2)

Table 3: Means of pollen germination and pollen tube growth under the effects of sucrose

Sucrose g/L	Pollen germination %				Means pollen tube growth (μ_m)			
	1h	2h	3h	4h	1h	2h	3h	4h
100	20	30	0	0	52.5	154	0	0
140	20	30	30	0	76.9	217.6	217.6	0
180	25	35	38	0	93.1	233.5	248.5	0
220	50	55	65	65	120	261	284	355

Since the pollen tube does not perform photosynthesis, a carbon source is required for energy supply and carbohydrate skeleton formation and metabolized by the pollen grains or tubes (Montaner, 2003)

During its development in the anther, pollen accumulates large quantities of carbohydrates, which constitute a large part of its dry weight (Pacini et al., 2006)

Sugar in the pistil (or in pollen germination media) are necessary to maintain pollen tube growth (Labarca&Loewus, 1972)

Therefore, sucrose is generally added to pollen germination media, but the optimal concentration varies greatly between species.

For instance, optimal *papaver* pollen growth in vitro occurs at 5% sucrose (Bhowmik&Datta, 2012), *Camellia* at 8%, *Lilium* ,*Solanum* and *Tobacco* (*Nicotianatabacum*) at 10% (Loguercio, 2002).

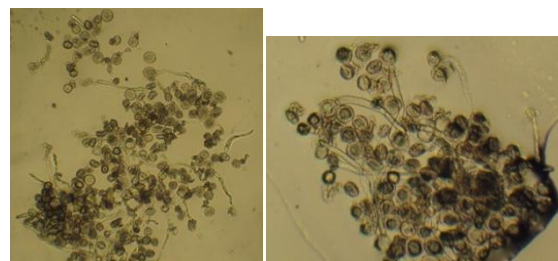


Figure 2: Effect of Sucrose on pollen germination and tubes growth

4. The effect of Boric acid on pollen germination and pollen tube growth

We used the basal medium after fixation the best temperature 30°C, best PH 6 and sucrose 220 g/L then, we study the effect of Boric acid with (0, 100, 200, 300 mg/L)

We observed, In a medium with 100 mg/L, the percentage of germination reached a value 65% with 355 μ_m long pollen tubes.

Table 4: Means of pollen germination and pollen tube growth under the effects of Boric acid

Boric acid mg/L	Pollen germination %				Means pollen tube growth (μ_m)			
	1h	2h	3h	4h	1h	2h	3h	4h
0	40	50	53	53	123	213	313	313
100	50	55	65	65	120	261	284	355
200	50	56	60	60	88	142	213	213
300	50	55	58	58	71	114	142	142

In the pollen tube, Boric acid is involved in cell wall formation and protein assembly into membranes and cell wall (Daher, 2011), though its effect on H⁺-ATPase activity, Boric acid affects pollen germination, tube growth (Wang *et al.*, 2003), it promotes absorption of sugar, and increases oxygen uptake, it is involved in the synthesis of pectic material for the wall of actively growing pollen tube (Ahmad *et al.*, 2012)

100 ppm boric acid was found to be essential for pollen germination (Brewbaker&kwack, 1967), decrease of it concentration in species *Tribulusterrestris*, *peach*, *Almond*, *piceameyeri*, gave a negative results in pollen germination and formation abnormal tubes (Imaniet *al.*, 2011)

5. The effect of Calcium on pollen germination and pollen tube growth

After fixation the best temperature 30°C, PH 6, sucrose 220 g/L Boric acid 100 mg/L, we study the effect of Calcium with (0, 300, 400, 500 mg/L) on pollen germination and pollen tube growth. We found, the percentage of germination in a medium with 500 mg/L of Calcium reached a value 78.5% with 540 μm long pollen tubes. (Table 4, Figure 3)

Table 4: Means of pollen germination and pollen tube growth under the effects of Calcium

Calcium mg/L	Pollen germination %				Means pollen tube growth (μm)			
	1h	2h	3h	4h	1h	2h	3h	4h
0	0	0	0	0	0	0	0	0
300	5	5	6	65	12	26	28	35
	0	5	5		0	1	4	5
400	5	5	6	69	25	28	35	42
	0	8	0		6	4	5	6
500	5	6	7	78.5	34	41	45	54
	0	5	0	5	1	2	5	0

Calcium plays an essential role in pollen tube growth (Zhou *et al.*, 2015), an ionic current of calcium ions enters at the pollen tube tip as a result of the localized positioning of calcium ions channels in the plasma membrane by secretory vesicle fusion at the tube apex. These processes create optimal conditions for growth mediated by the cytoskeleton at the calcium ion-entry site (steer, 1989). The presence of Ca⁺² in the growth medium is known to be required for in vitro pollen germination and tip growth of most plant species. It plays a role in cell wall formation and rigidity, directs vesicle trafficking, controls actin dynamics (chebli&Geitmann, 2007). Normal pollen tube growth can only take place in the presence of a calcium concentration that is situated within a certain

range (picton& steer, 1983), that varies between species (steer & steer, 1989). Within this range, pollen tube tip extension rates are relatively insensitive to small changes in the calcium concentration (picton& steer, 1983), whereas outside of this range, growth is severely hampered.

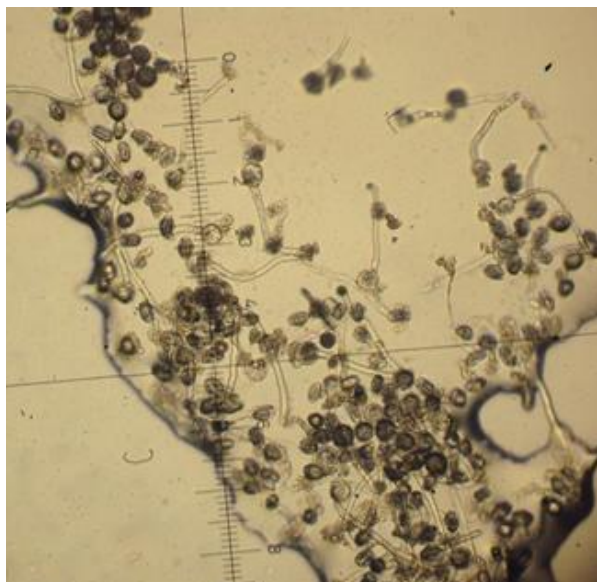


Figure 3: Effect of Calcium on pollen germination and tubes growth

V. CONCLUSION

different factors have an essential roles in pollen germination and pollen tube growth. carbon source is required for energy supply and metabolized by the pollen grains, Boric acid and calcium are involved in cell wall formation and protein assembly into membranes and cell wall, PH and Temperature have positive or negative effect on germination.

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