EFFECT OF DIFFERENT FACTORS ON GERMINATION AND TUBE GROWTH OF *Solanum sisymbrifolium* LAM. POLLEN

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ABSTRACT

Both germination and tube growth of *Solanum sisymbrifolium* Lam. pollen require an optimum temperature of 25°C and a growth medium containing 100 ppm boric acid, 10% sucrose and 0.6% Bacto-agar. Pollen germination was stimulated by 5 and 10 ppm GA_3, 1-10 ppm kinetin, 1 ppm ethylene, and 1 and 5 ppm ABA. Higher concentrations of these growth hormones reduced pollen germinability. Pollen tube elongation was stimulated by 10 ppm GA_3, 1-10 ppm kinetin, 1 ppm ethylene and 1-10 ppm ABA. IAA at 5 to 25 ppm inhibited both pollen germination and tube growth. The degree of inhibition increased with higher concentrations of IAA.

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KEY WORDS: Pollen physiology. *Solanum sisymbrifolium*. Alkaloid. Germination. Tube growth. Growth regulators.

INTRODUCTION

Solanum sisymbrifolium. Lam. is an undershrub species which is distributed in India within 800 - 1300 m elevation (Balakrishnan, 1983). It is medicinally important since its berries provide a rich source of the alkaloid solasodine (Pandeya et al., 1981), a raw material for the synthesis of steroids. However, berry yield in alkaloid producing *Solanums* is low. Therefore, it is necessary to improve these plants through hybridization (Kaul and Zutshi, 1977).

A successful hybridization program requires an understanding of the factors influencing pollen germination and tube growth (Vasil, 1974). Some of the important factors affecting pollen germination and tube growth are temperature, sucrose, boron and plant growth hormones. However, these requirements are species specific (Johri and Vasil, 1961). Ravindran and Chauhan (1980) described these requirements for germination and tube growth in *S. khasianum*, *S. indicum*, *S. aviculare* and *S. laciniatum* pollen. No information on pollen physiology of *S. sisymbrifolium* is available hence, this study was conducted.

The present investigation deals with the effects of temperature, boron, sucrose, agar, IAA, GA_3, kinetin, ethylene and abscissic acid (ABA) on pollen germination and tube elongation of *S. sisymbrifolium*.

MATERIALS AND METHODS

Pollen was collected only from anthesized flowers with undehisced anthers of *S. sisymbrifolium* plants between 6:00 - 6:30 a.m. Collected flowers were immediately brought to the laboratory and the anther tips were cut off with a sharp scalpel. Pollen grains were inoculated by gently tapping the anthers on cover slips with 0.01 mL of the basal medium. The basal medium consisted of 10% sucrose, 100 ppm boric acid, 300 ppm calcium nitrate, 200 ppm magnesium sulfate, 100 ppm potassium nitrate and 0.6% Bacto-agar (Brewbaker and Kwack, 1963). The pH of the medium was adjusted to 7.3 following the method used by Ravindran and Chauhan (1980). The pollen dusted cover slips were inverted and placed over a metallic ring (11.5 mm thick) prefixed to a glass slide and lined with lanolin.

The effect of temperature on pollen germination and tube growth in the basal medium was studied. The inoculated slides were placed in B.C.D. incubators maintained at 15, 20, 25 and 30°C.

Pollen germination and tube growth as affected by varying concentrations of boron in the form of boric acid (50, 100, 200 and 300 ppm) and sucrose (5, 10, 15%) were also observed. The optimum concentrations for the species were determined by altering the level of boron or sucrose in the basal medium one at a time. The inoculated slides were incubated at 25°C, the optimum temperature as determined earlier.

The effect of gelling was noted by incorporating different concentrations of agar (0.3, 0.6, 1.0%) in the medium.

The plant growth regulators: indole acetic acid (IAA), gibberellic acid (GA_3), kinetin, ethylene releasing Ethrel and abscissic acid (ABA) were used to study pollen germination and tube growth. Different levels (1-25 ppm) of these regulators were incorporated into the basal medium which was modified according to the boron, sucrose and agar requirements of *S. sisymbrifolium* pollen obtained earlier. The pH of all the media was adjusted to 7.3 prior to incorporation of the growth regulators.

Pollen-dusted slides prepared following the method of Ravindran and Chauhan (1980) were incubated at 25°C for 4 hours. The germinating pollen grains and pollen tubes were fixed by flooding with FAA (5 mL formaldehyde + 5 mL glacial acetic acid + 90 mL 50% ethanol). For all treatments, five replicate slides each were maintained. The germinated and ungerminated pollen grains were counted from five randomly chosen microscopic fields per slide. Similarly, at least 20 pollen tubes per slide were selected randomly and measured.

RESULTS AND DISCUSSION

Pollen grains require an optimum temperature for germination (Visser, 1955). In this study, both pollen germination and pollen tube elongation were significantly lower at 20 and 30°C than at 25°C (Table 1). Thus, the optimal temperature for pollen growth is 25°C.

S. sisymbrifolium pollen exhibited higher germination and greater tube growth in media with 100 ppm boric acid (Table 1). Other concentrations of boric acid (50, 200, 300) inhibited both processes. Thus, 100 ppm is the optimal boron level for maximum pollen germination and tube growth. It appears that the role of boron in pollen growth is four-fold, namely: 1) it forms sugar borate complexes which enhance absorption, translocation and metabolism of sugars (Vasil, 1964), 2) it is involved in the synthesis of pectin materials required for wall synthesis of actively growing pollen tubes (Stanley and Loewus, 1964), 3) it stimulates chemotropic activity of Ca^{++} (Mascarenhas and Machlis, 1964), and 4) it increases O_2 uptake by pollen (O'-Kelley, 1957).

The optimum sugar requirement for germination of pollen grains is species specific. Results indicate that 10% sucrose is optimum for growth of *S. sisymbrifolium* pollen. *S. ariculase* pollen has a similar sucrose requirement for optimal pollen tube elongation (Ravindran and Chauhan, 1980). Aside from providing nutrition to the germinating pollen (Johri and Vasil, 1961), sugars maintain proper osmotic balance between the germinating media and the cytoplasm (Mukherjee and Das, 1964).

Gelling of the medium revealed that 0.6% Bacto-agar is the best agar concentration for pollen growth (Table 1). Addition of agar to the sucrose medium improved the germination of the pollen over the liquid medium by causing controlled hydration of the pollen (Shivanna and Johri, 1985). Johri and Vasil (1961) stated that agar incorporated in the sucrose medium improved pollen germination by influencing the osmotic concentration of the germinating medium. These could explain the stimulation of germination and tube growth through addition of 0.6% bactoagar in this study.

Pollen germination and tube growth are influenced by plant growth hormones (Johri et al., 1977). In this study, IAA at 5-25 ppm inhibited pollen germination and the degree of inhibition increased as concentration became higher (Table 2). At 1 ppm, IAA stimulated pollen germination but not tube elongation. Only 25 ppm IAA significantly inhibited tube elongation (Table 3). Pollen of many species contains sufficient auxin for optimal growth (Linskens and Kroh, 1970). Exogenous application of auxin stimulates pollen tube elongation only if auxin content of the pollen is below the threshold level (Brewbaker and Majumder, 1961). Since even low levels of auxin inhibited pollen growth, *S. sisymbrifolium* pollen probably has optimal endogenous IAA such that it does not need exogenous IAA for its germination and tube growth.

Table 1. Germination and tube growth of *Solanum sisymbrifolium* pollen as affected by different treatments.

Treatment	Pollen Germination (%)	Pollen Tube Length[1] (um)
Temperature (°C)		
20	40.9 + 4.9	96.9 + 31.8
25	51.5 + 2.5	209.7 + 36.3
30	35.7 + 3.1	74.6 + 19.1
L. S. D.	6.3	45.7
Boric Acid (ppm)		
50	36.6 + 2.5	198.4 + 63.8
100	51.6 + 3.3	261.2 + 19.4
200	49.0 + 1.1	149.6 + 14.4
300	36.4 + 38	99.3 + 38.7
L. S. D.	3.5	58.7
Sucrose (%)		
5	39.6 + 3.2	104.6 + 28.4
10	51.2 + 2.3	155.7 + 30.3
15	0	0
L. S. D.	40.0	42.8
Agar (%)		
0	61.4 + 0.9	212.4 + 12.9
0.3	63.6 + 3.2	309.2 + 23.4
0.6	68.2 + 3.4	390.3 + 38.0
1.0	51.7 + 0.8	231.0 + 20.2
L. S. D.	3.6	44.8

[1] Means of 100 pollen tubes.
+ S. D.

IAA also inhibited pollen germination and tube growth in *S. khasianum* and *S. indicum,* but *S. sisymbrifolium* pollen is less and more sensitive to IAA than *S. khasianum* and *S. indicum* pollen, respectively (Ravindran and Chauhan, 1986). The differential auxin sensitivity of pollen of various species in a genus is also reported by Yadav (1980) and Ravindran and Chauhan (1986).

Lower concentrations of GA_3 (5 and 10 ppm) promoted pollen germination whereas 25 ppm induced inhibition (Table 2). On the other hand, 10 and 25 ppm GA_3 stimulated and inhibited pollen tube growth, respectively (Table 3). Thus, the response of *S. sisymbrifolium* pollen to GA_3 resembles that of *S. indicum* pollen (Ravindran and Chauhan, 1986). Stimulation of pollen tube elongation is attributed to GA_3 effect on cell expansion and orientation of newly synthesized microfibrils as reported by Malik and Chhabra (1976).

Kinetin concentrations of 1 - 10 ppm enhanced pollen germination and tube growth but the degree of stimulation decreased with increasing concentration (Tables 2 and 3). However, both processes were inhibited by 25 ppm kinetin which probably indicates that it was supra-optimal. Kinetin-elicited stimulation of pollen tube elongation may be due to kinetin-induced promotion of cellulose synthesis (Mehan and Malik, 1975).

Ethrel at 1 ppm was stimulatory whereas higher levels were inhibitory to both pollen germination and tube elongation. Except for occasional stimulation (Buchanan and Briggs, 1969), pollen germination is usually inhibited by ethrel (Mcleod, 1975; Sastri, 1974). Ethrel-induced inhibition of pollen germination at high concentrations is considered to be due to increased acidity of the germinating medium (Johri et al., 1977) However in this study, inhibition

Table 2. Pollen germination of *S. sisymbrifolium* as affected by various growth regulators.

Conc. (ppm)	Pollen Germination (%)				
	IAA	GA_3	Kinetin	Ethrel	ABA
0	50.6 + 1.2	49.8 + 1.3	50.9 + 1.5	50.6 + 1.4	51.2 + 1.0
1	53.0 + 3.1	51.5 + 1.8	64.0* + 1.8	54.9* + 4.5	59.6* + 1.9
5	46.3 + 4.3	56.8* + 6.8	59.3* + 4.5	40.5* + 1.9	56.6* + 2.3
10	4.4 + 3.7	61.3* + 3.2	56.5* + 1.9	32.3* + 3.4	48.9* + 2.7
25	25.4 + 2.9	43.3* + 4.0	40.9* + 2.5	23.2* + 0.9	44.0* + 1.4
L.S.D.	3.7	3.9	3.4	3.4	2.3

+ S.D.
* Significantly different from control

Table 3. Pollen tube growth of *S. sisymbrifolium* as affected by various growth regulators.

Conc. (ppm)	Pollen Tube Growth (um)				
	IAA	GA_3	Kinetin	Ethrel	ABA
0	163.0 + 18.4	211.9 + 8.5	218.1 + 31.9	164.4 + 18.6	200.8 + 13.8
1	179.4 + 14.2	216.2 + 15.2	390.8*+ 16.3	207.4*+ 15.7	282.3*+ 8.3
5	168.8 + 29.8	228.8 + 57.2	300.6*+ 22.1	147.9 + 21.6	277.0*+ 20.3
10	143.2 + 12.8	291.0*+ 36.8	254.1*+ 21.2	112.8*+ 34.8	269.1*+ 37.6
25	77.7*+ 12.1	148.6*+ 14.2	181.9*+ 27.8	56.1*+ 19.3	208.8 + 46.8
L.S.D.	23.4	38.4	29.2	23.6	37.5

+ S. D.
* Significantly different from the control.

was still evident even after the pH of the medium was adjusted to 7.3. Similar findings were obtained by Chauhan and Kharbteng (1986). Therefore, ethrel-induced inhibition of pollen germination might be due to the effect of ethylene rather than of pH as suggested by Mcleod (1975). He further suggested that ethylene may either be an evocator itself or may unmask the evocator of pollen tube growth.

Abscissic acid is an inhibitory growth regulator (Leopold and Kriedmann, 1975). However, lower concentrations of ABA (1 and 5 ppm) used in this study significantly stimulated pollen germination whereas higher levels (10 and 25 ppm) induced significant inhibition (Table 2). Pollen tube elongation was significantly improved by 1-10 ppm ABA (Table 3). ABA increases the permeability of plant cells (Basler, 1974) facilitating quicker hydration, thus influencing pollen tube growth (Mehan and Malik, 1975).

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