IN VITRO GERMINATION OF *PINUS KESIYA* ROYLE EX GORD. POLLEN

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Abstract

The present investigation deals with the temperature, sucrose, boron, pH and gelling requirements for in-vitro germination and tube growth of *Pinus kesiya* pollen. The investigations reveal that *P. kesiya* pollen requires 25°C temperature, 0.03% sucrose, 5 ppm boron and pH 7.3 for optimal germination and tube growth. Gelling of the medium affects germinability of pollen and tube elongation adversely.

Introduction

Pinus kesiya yield pinene rich turpentine oil, a major source of raw material for organic chemicals (Singh & Mehra, 1977). However, due to low yield of oleoresin this species is not exploited commercially (Chadha, 1977); this species therefore needs to be improved. Necessity to understand various factors influencing pollen germination and tube growth is a prerequisite for the success of hybridization programme (Vasil, 1974 ; Mercy *et al.*, 1978). It is axiomatic that some of the factors which control pollen germination and tube growth in plants are temperature, sucrose, boron and pH (Johri & Vasil, 1961). Further, these requirements are species specific. Therefore, an attempt was made to find out these requirements of *P. kesiya* pollen to optimise pollen germination and pollen tube elongation in this species.

Material and Method

Pollen grains were collected in the month of March between 8.00—9.00 a.m. from male cones of *P. kesiya* trees growing in Shillong and stored at -5°C. For evolving optimal conditions of pollen germination and tube growth first optimum temperature requirements were determined by germinating the pollen in 0.03 per cent sucrose solution at varyintg temperatures (15°,20°,25°,30°,35° & 40°C). Having established the optimum temperature condition for pollen germination and tube-growth, the optimum sucrose requirement was determined by incubating pollen at optimum temperautre (25°C) and altering sucrose concentration in the germinating solution. The sucrose concentrations used were 0.01, 0.03, 0.05, 0.1, 0.5, 1.0, 5.0, 10.0 & 15.0 per cent. Next, the boron requirements for pollen germination and tube elongation were determined by incorporating different concentrations of boric acid (5, 10, 50, 100 ppm) in the sugar solution having optimal sucrose concentration (0.03%) and incubating slides at 25°C. Gelling of the medium was done with bacto-agar and the optimum gelling requirement of the medium determined by incorporating various concentrations of bacto-agar (0.25, 0.5, 0.6, 0.75, 1.0, 1.5, 2.0%) in the sucrose-boron nutrient medium devised above. Subsequently the most suitable pH for germination of *P. kesiya* pollen was determined by altering the pH (5, 6, 7, 7.3, 8) of the semisolid medium having optimal sucrose, boron and per cent agar concentration. The nutrient medium

was prepared in double glass distilled water and the chemicals used were of analar grade. For pollen germination, pollen grains were dusted on coverslip with 0.05 ml liquid medium. However, gelling effect was studied by incorporating bacto-agar in the liquid medium 0.05 ml of the bacto-agar incorporated semisolid medium was also used for germinating pollen grains. The pollen dusted coverslip was inverted and placed over a metallic ring prefixed to glass slide and smeared with petroleum jelly. The slides were incubated for 48 hrs. in dark in an incubator maintained at 25±1°C. At the expiry of incubation period the incubated pollen were fixed by flooding with FAA (90 ml 70% ethanol +5 ml glacial Acetic Acid+5 ml formaldehyde) for recording data on pollen germination and tube elongation.

Observations

Per treatment five slides were maintained and data on pollen germination were recorded by counting germinated and ungerminated pollen from five microscopic fields chosen randomly, per slide. For pollen tube elongation 25 tubes were measured per slide, thus for any given treatment 125 tubes were measured. The data were analysed statistically.

Results and Discussion

Temperature is an important factor in pollen germination and pollen tube growth. In the present investigation while no pollen germinated at 15 and 40°C, the pollen germination occurred between 20-35°C. Although maximum pollen germination occurred at 25°C, maximum tube growth was evident at 30°C. Both pollen germination and tube elongation, therefore, have different temperature optima. The optimal temperature for pollen germination differs in different pine species, e.g., 28-30°C in *P. densiflora* (Tanaka, 1955) ; 30-32°C in *P. elliottii* and *P. nigra* (McWilliam, 1959) ; and 25-29°C in *P. mugo* (Nygaard, 1969). The frequency of long pollen tube increases with increasing temperature up to 30°C and declines subsequently (Table-2). But even at sub-optimal temperature satisfactory tube length can be obtained if period of growth is not limiting (Johri & Vasil, 1961).

Table 1 gives data on pollen germination and pollen tube growth at different sucrose concentrations. Table-1 reveals that compared to higher concentrations of sucrose (0.5—15%) both germination and tube growth were more at low sucrose concentrations (0.01—0.1%). Maximum germination and highest tube growth was evident in medium having 0.03% sucrose, however (Table 1). Pollen grains require an optimum concentration of sugar solution for germination (Mukherjee & Das, 1964). Sugars provide nutrition to the germinating pollen and serve as an osmoticum (Johri & Vasil, 1961). It seems 0.03% sucrose meets both the requirements of *P. kesiya* pollen. The incidence of long pollen tubes did not vary much at different sucrose concentrations.

Incorporation of boric acid (5-50ppm) in the germinating medium improved pollen germination and tube growth over control (Table 1). But the degree of stimultaion decreased with the increasing concentration of boric acid (Table 1). Whereas germination differed significantly from control at 5-50 ppm concentrations of boric acid, the pollen tube elongation was different from control only at 5 and 10 ppm boric acid concentrations. Thus 5 ppm of boron is the optimum concentration for both pollen germination and pollen tube elongation. However, optimal boron requirements of *P. patula* (0.001%) boron, Kapoor & Dobriyal, 1980), and *P. roxburghii* (10 mg l^{-1} boric acid, Dhawan &

Table 1—Effect of temperature, sucrose, boric acid, pH and gelling on pollen germination and tube elongation in *P. kesiya*

	Pollen germintion (%)	Pollen tube length (μm)**
Temperature (°C)		
15	—	—
20	25.63±0.83	27.78±1.98
25	51.50±1.84	65.79±2.27
30	34.38±1.75	111.49±6.36
35	26.96±4.57	29.31±2.94
40	—	—
L.S.D.	0.5	9.50
Sucrose (%)		
0.01	53.99±1.53	60.28±2.87
0.03	62.58±2.17	72.83±2.85
0.05	58.77±1.39	70.52±1.88
0.10	62.20±1.48	71.04±4.67
0.50	48.66±2.44	53.12±4.68
1	46.39±4.15	52.35±1.15
5	45.93±1.19	51.18±3.27
10	45.54±2.90	50.19±2.59
15	42.00±3.12	49.79±2.19
L.S.D.	0.40	8.78
Boric acid (ppm)		
0	47.22±0.29	59.64±5.03
5	80.94±1.41	112.00±1.47
10	73.60±3.90	95.49±4.36
50	56.65±3.57	67.71±1.15
100	46.52±1.18	58.37±4.32
L.S.D.	0.70	12.93
Gelling		
Bactoagar (%)		
0	67.90±3.95	93.47±9.59
0.25	37.87±1.00	31.23±1.88
0.50	47.77±1.08	34.30±0.83
0.60	44.79±1.78	31.87±0.78
0.75	38.92±0.92	32.89±1.36
1.00	37.29±0.92	30.72±0.55
1.50	37.54±1.85	30.72±1.42
2.00	29.89±3.30	30.72±1.00
L.S.D.	0.80	10.54
pH		
5.0	4.34±0.65	18.40±0.33
6.0	34.31±3.61	23.80±0.89
7.0	40.66±2.49	27.52±0.83
7.3	53.46±5.10	30.59±1.23
8.0	22.80±2.41	23.68±0.98
L.S.D.	1.00	2.80

±S.E. L.S.D. at P=0.05

**Mean of 125 pollen tubes

Table 2—Effect of temperature on pollen tube size in *P. kesiya* pollen

Temperature (°C)	PER CENT POLLEN TUBES												
	GROUP												
	1	2	3	4	5	6	7	8	9	10	11	12	13
	µm												
	1—16	17—32	33—48	49—64	65—80	81—96	97—112	113—128	129—144	145—160	161—176	177—192	193—208
15	—	—	—	—	—	—	—	—	—	—	—	—	—
20	44±7	44±3	10±2	2±1	—	—	—	—	—	—	—	—	—
25	—	21±3	22±2	23±3	11±2	7±1	7±2	8±2	1±0	—	—	—	—
30	—	1±0	6±1	10±3	20±3	10±2	13±2	11±2	9±2	10±3	2±1	5±1	3±1
35	—	35±5	47±6	16+5	2±0	—	—	—	—	—	—	—	—
40	—	—	—	—	—	—	—	—	—	—	—	—	—

S.E—±

Table 3—Effect of boric acid on pollen tube size in *P. kesiya* pollen

Boric acid (ppm)	PER CENT POLLEN TUBES													
	GROUP													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
	μm													
	1—16	17—32	33—48	49—64	65—80	81—96	97—112	113—128	129—144	145—160	161—176	177—192	193—208	209—224
0	—	28±4	24±1	21±3	12±3	9±2	4±1	2±1	—	—	—	—	—	—
5	—	3±1	6±1	11±3	15±2	11±4	12±2	14±3	3±1	12±4	2±0	6±1	2±0	1±0
10	—	7±4	9±2	12±4	20±2	12±3	17±2	12±1	1±0	7±2	2±0	1±0	—	—
50	—	16±2	26±3	23±3	19±2	7±2	7±2	2±0	—	—	—	—	—	—
100	—	27±3	32±4	18±2	14±3	6±2	2±1	1±0	—	—	—	—	—	—

S.E —±

Malik, 1981), are different from *P. kesiya*. Thus, boron requirement of pollen germination and tube growth differs with the species. Stanley and Linskens (1974) stated that plants requiring high boron for in-vitro pollen germination have high endogenous boron. Boron content of *Pinus* pollen, therefore, seems to be low. Similar were the conclusions of Dhawan and Malik (1981) in *P. roxburghii*. Usually pine pollen do not require boron to germinate in-vitro (Malik, 1979). Although a similar situation is obtained in case of *P. kesiya* pollen, incorporation of boron in the germinating medium improves germination and tube growth. The frequency of long pollen tubes was more in medium supplemented with 5-50 ppm boric acid, compared to control (Table 3). But incorporation of higher concentration of boric acid (100 ppm) in the germinating medium resulted in greater frequency of short pollen tubes in comparison to control (Table 3). Thus, 100 ppm of boric acid has a detrimental effect on pollen tube elongation, being supra-optimal concentration. The effect of boron on pollen germination and pollen tube elongation is many fold : (i) it forms sugar borate complexes which help to increase absorption, translocation and metabolism of sugars (Vasil, 1964), (ii) it is involved in the synthesis of pectin materials required for the wall of actively growing pollen tubes (Stanley & Loewus, 1964), (iii) it stimulates chemotropic activity of Ca^{++} (Mascarenhas & Machlis, 1964), and (iv) it increases O_2 uptake by pollen (O'Kelley, 1957).

Gelling of the medium is not beneficial for *P. kesiya* pollen since incorporation of bacto-agar in 0.03% sucrose medium reduced both germination and tube growth (Table 1). Similar were the observations of Tanaka (1955) in *P. densiflora*. But gelling of the medium improves pollen germination and pollen tube growth in *P. patula* (Kapoor & Dobriyal, 1980). For good germination the osmotic concentration of pollen and germinating medium should be similar (Johri & Vasil, 1961). Incorporation of agar in the germinating medium affects osmotic concentration of the medium (Johri & Vasil, 1961) which could be the reason for bacto-agar induced inhibition of germination and tube growth in the present investigation.

Table 4—Effect of pH of the medium on pollen tube size in *P. kesiya* pollen

pH	PERCENT POLLEN TUBES			
	GROUP			
	1	2	3	4
	μm			
	1—16	17—32	33—48	49—64
5	74±3	26±3	—	—
6	55±4	42±3	3±0	—
7	38±3	53±2	9±2	—
7.3	27±5	54±2	18±4	1±0
8	53±6	47±6	—	—

S.E.±

In the present investigation both pollen germination and tube growth increased with the increasing pH between pH 5-7.3 and thereafter declined (Table 1). McWilliam (1960) stated that optimum pH for both pollen germination and pollen tube elongation ranges between 5-7. However, different pine species differ in their pH requirements for optimal germination and tube-elongation, e.g., *P. densiflora* 4.5-6.5 (Tanaka, 1955), *P. mugo* 4.8-6.2 (Nygaard, 1969) and *P. kesiya* 7.3 (present study). The frequency of long pollen tubes was more between pH 6-7.3 compared to pollen tubes obtained at pH 5 and 8 (Table 4).

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