Indian J. Applied & Pure Bio. Vol. 35(1), 39-45 (2020).

Studies on the Pollen Germination of *Capparis decidua* (Forsk.) Pax

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Abstract

The investigation on pollen viability indicate that in Capparis decidua, one third of pollen is viable and the optimal germination and maximum tube elongation occurs in a semisolid germinating medium. Application of growth hormones (IAA and GA) also significantly improved pollen germination. Effect of different light shades on pollen germination and tube elongation has also been studied.

Pollen are immature endosporic male gametophytes of seed plants which produce the male gametes or sperm cell and are found in the reproductive organ of the plant which is necessary for the angiosperm species to reproduce sexually[27,17]. Estimation of pollen viability is useful for plant breeders and geneticists in eliminating the time and space problems[11] and can be made using direct methods such as the induction of in vitro pollen germination[1,3]. The assessment of pollen quality by its in vitro germination is a useful method for determining the acceptability of pollen for artificial pollination[4].

In vitro pollen germination provides a novel approach and strategy to accelerate the genetic improvement of tree breeding. It is a very convenient and effective technique for studying many basic and applied aspects of pollen biology[26]. This technique is the best option for selecting viable and potential pollen that can be used for cross-pollination[21]. The conditions required for *in vitro* pollen germination vary across species. It has been reported that pollen germination and pollen tube growth can be inûuenced by many internal and external factors[13,22]. The extrinsic factors which affect the germination of pollen include incubation time, optimal temperature, and medium composition[14]. Organic and inorganic substances such as sucrose, boric acid, calcium nitrate, potassium nitrate, and magnesium sulfate exert an effect on the *in vitro* pollen germination[12,18,18]. In addition, pH has inûuences on in vitro pollen germination[6,20]. Indeed, the quality of pollen, the optimum concentrations of the media and environmental conditions have effects on germination of pollen and tube growth[21]. Various methods and media with different components have been suggested by many plant biologists and researchers[2,14,15,26,28].

Capparis decidua (Forsk.) Edgew. commonly known as Kair, is an important

indigenous shrub found growing along farm boundaries, orans, gochars (local grasslands) and wastelands, widely distributed in arid and semi-arid tracts of India. It is pioneer species of the Arid scrub forest, besides yielding valuable timber, have the potential of being used for ecorestoration of degraded land. Due to urbanization and industrialization, there is a great pressure on this particular species to survive. Therefore, this study was undertaken to examine its pollen viability, pollen germination, pH, temperature and pollen tube growth to contribute understanding and to provide insights to plant physiologists, plant breeders and conservationists on the horticultural practice of its reproduction.

Collection of pollen samples:

Fresh hermaphrodite flowers of *C. decidua* were collected early in the morning from 20 plant individuals of the same population. The collection was done during the anthesis of the flowers. The collected flowers were placed in zip–lock cellophane bags to prevent drying and brought immediately to the laboratory.

The basal medium for germinating pollen was followed after Brewbaker and Kwack[7]. The optimal requirements of pollen germination and pollen tube elongation were worked out by altering one factor at a time. The effects of growth hormone viz. indole acetic acid (IAA), gibberellic acid (GA) and kinetin were also investigated. The concentration of the growth hormone used were 1,5 and 10 ppm. Effect of light of various colour on germination and tube elongation was investigated with the help of cellphone paper of different colours. Two layers of cellphone paper of one colour was used to cover the light source for providing light of a particular colour. The color tried were white (fluorescent), violet, blue green, yellow and red. Pollen were incubated for 3 hour in the germinating medium and then fixed by putting a drop of FAA (formalin: glacial acetic acid: 70% alcohol, 1:1:18) on the incubated pollen. Per treatment 5 slides were recorded by scoring 5 micoscopic fields in a given slide. All the five slides were scored for data recording. Pollen tube elongation was measured in 25 pollen tubes per slide, from five different microscopic fields, 5 slides were scored per treatment.

Statistical analysis:

The data were analyzed either with the help of student's 't' test or ANOVA to find out the significance of the results.

Understanding the requirements for pollen germination and tube growth is of great importance for cross pollination. The requirements of pollen germination vary appreciably from pollen grains of one species to another. In general, each pollen grain has its own requirements and might need different media. The media preparation for in vitro germination play a vital role in pollen germination and tube growth[9]. In general, the media preparation for pollen germination varies according to the plant species and many internal and external factors[9].

Table-1 summarising data on the effect of temperature (20,25,30 & 35^{o}C) on germination and tube elongation reveals that 30^{o}C is the optimum temperature for pollen

germination and pollen tube elongation in the species. Table-2 incorporating data *i.e.* the effect of sucrose (5, 10, 15, 20$), boron – 50, 100, 150, 200ppm, Calcium- 100, 200, 300, 400 ppm, Magnesium- 100, 200, 300, 400 ppm, Potassium 50, 100, 150, 200 ppm and agar (0.4, 0.6, 0.8, 1.0%) exhibited that compared to other concentration, germination of the pollen and tube growth are significantly higher at 10% sucrose, 50 ppm boron, 400 ppm calcium, 300 ppm magnesium, 100 ppm potassium and 0.8% agar. These concentrations at pH 7.0 (Table 3) represent the requirements for optimal pollen germination and pollen tube elongation in *Capparis decidua*. Even at the optimal concentration only 36.03 $ pollen germinated (Table-4).

The Brewbaker and Kwack's[7] medium was modified by incorporation the optimal requirements of *C. decidua* pollen enumerated above. Comparison of pollen germination and tube growth in Brewbaker and Kwack's medium[7] and the modified medium revealed that germination and tube growth were higher in the modified medium (Table-4). However, even in the modified medium the germination was only 36.03 percent.

Effect of growth hormone on pollen germination and pollen tube elongation revealed that 1 ppm of IAA and GA_3 induced significant stimulation of pollen germination. However, 5 and 10 ppm concentration of these growth hormones and all the concentration of Kinetin (1-10 ppm) used caused significant inhibition of pollen germination (Table-5). Thus in *C. decidua*, different growth hormones influenced pollen germination and pollen tube elongation differently. Similar were findings of Setia[24] in *Cicer arietinum*. In the present investigation even where pollen germiatio was stimulated, the germination percentage remained very low. Thus, pollen germination and pollen tube elongation in *C. decidua* are two independent processes governed by separate set of condition a proposed by Malik[16].

The effect of different colours of light on pollen germination and tube elongation in *C. decidua* revealed that white and yellow light had no effect but violet, blue and green light induced significant inhibition of germination and tube elongation vis-a vis white light (Table-6). Red light, however, caused significant stimulation of germination and tube elongation over other light conditions (Table-6). Thus light of different colour influenced pollen germination and tube elongation in *C. decidua*. Similar were the observations of Chhabra *et.al.*[8], Seema and Rajeev[23], Katiyar[10].

Table-1. Effect of temperature on pollen germination and tube elongation in *C. decidua*

S.No.	Temp.(°C)	Pollen germination (%)	Pollen tube elongation
1	20	6.91 ± 2.48	56.25 ± 11.89
2	25	11.44 ± 4.31	59.28 ± 11.77
3	30	24.87 ±3.38	181.20 ± 34.47
4	35	17.14 ± 3.54	129.90 ± 25.76

Table-2. Pollen germination and pollen tube elongation in *C. decidua* (Forsk.) Pax

Factor	Pollen germination (%)	Pollen tube elongation (µ m)
Sucrose (%)		
5	7.3 ± 0.48	85.92 ± 15.98
10	29.49 ± 2.72	293.16 ± 24.49
15	20.37 ± 4.62	218.16 ± 16.12
20	20.52 ±2.63	191.64 ± 23.28
LSD (p = 0.05)	2.85	31.53
Boron (ppm)		
50	43.49 ± 2.28	310.56 ± 3226
100	25.87 ±3.96	237.72 ±8.17
150	17.37 ±2.57	140.46 ±8.13
200	11.01 ±0.68	86.16 ±7.16
LSD (p = 0.05)	2.32	29.35
Calcium (ppm)		
100	7.30 ± 0.2	41.2 ± 3.08
200	9.89 ± 1.75	50.88 ± 3.56
300	19.34 ± 0.88	136.48 ± 13.37
400	26.23 ± 2.36	329.64 ± 17.66
LSD (p = 0.05)	1.53	16.22
Magnesium (ppm)		
100	9.36 ± 0.52	63.22 ± 5.3
200	15.36 ± 0.8	194.04 ±27.14
300	23.78 ± 1.21	306.60 ±18.48
400	13.95 ± 0.76	95.88 ± 9.18
LSD (p = 0.05)	0.71	20.88
Potassium (ppm)		
50	20.1 ± 1.84	193.56 ±27.3
100	30.29 ±2.32	308.04 ± 36.6
150	10.00 ±0.72	52.08 ±4.62
200	00	00
LSD (p = 0.05)	1.53	39.09
Agar (%)		
0.4	13.11 ± 1.14	70.56 ± 1.77
0.6	26.38 ±1.45	432.36± 17.66
0.8	30.95 ±1.09	561.84 ±34.73
1.0	18.96 ±0.99	338.64 ±22.66
LSD (p = 0.05)	1.18	23.95

Table-3. Effect of pH on pollen germination and pollen tube elongation in *C.decidua*

S.No.	pH	Pollen germination (%)	Pollen tube elongation (µ m)
1	6.5	15.3± 109	110.52 ± 14.42
2	7.0	27.12 ± 0.67	470.76 ± 38.27
3	7.3	23.06 ± 0.6	33.52 ± 28.6
4	8.0	16.47 ± 0.76	138.00 ± 9.05
LSD (p = 0.05)		0.81	35.08

Table-4. Comparison of *C. decidua* pollen germination and pollen tube growth in Brewbaker and Kwack (BK) and modified Brewbaker and Kwack (MBK) medium

Medium	Pollen germination (%)	Pollen tube elongation (µ m)
BK	26.13 ± 0.84	230.04 ± 9.62
MBK	36.03 ± 1.88	332.04 ± 22.78

Table-5. Effect of growth hormone on pollen germination and pollen tube elongation in *C. decidua*

Treatment	Pollen germination (%)	Pollen tube elongation (µ m)
Control	21.18 ± 0.54	283.92 ± 30.51
Growth hormone (ppm)		
1	25.33±0.93	275.88±13.24
5	16.32±0.97	254.04±16.94
10	12.38±1.03	130.20±34.37
GA_3		
1	26.07±1.24	297.25±29.82
5	20.09±0.97	144.84±5.72
10	14.95±1.36	136.56±16.13
Kinetin		
1	18.73±1.01	225.00±32.45
5	18.19±0.98	181.56±25.94
10	15.04±0.58	119.64±9.7
LSD (p = 0.05)	1.0	29.55

Table-6. Effect of different colour light on pollen germination and pollen tube elongation in *C. decidua*

Light colour	Pollen germination (%)	Pollen tube elongation (µ m)
Dark	17.51±1.43	198.48±22.03
White	17.71±1.22	201.72±9.93
Violet	9.40±0.77a	129.72±13.15a
Blue	10.34±0.25a	146.16±11.75a
Green	13.78±0.46a	170.04±7.46a
Yellow	16.95±1.15	207.48±14.23
Red	20.71±1.17b	169.28±21.73b
LSD (p = 0.05)	0.91	12.21

Chhabra *et al*.[8] suggested the involvement of phytochrome in pollen germination and pollen tube growth. In the present investigation also red light had significant effect on pollen germination and tube elongation suggesting phytochrome involvement. Sharma and Malik[25] consider that the effect of red light on pollen has two separate stages iz.; synthesis of phytochrome protein and its biological manifestation. Bindra and Malik[5] also suggested that red light induced stimulation of tube elongation in *Crotolaria juncea* is due to its effect on synthesis of membrane components. Blue light inducd inhibition of pollen germination and tube elongation is possibly mediated through its effect on the endogenous level of IAA (Chhabra *et al*.[8]).

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