

Original Research Article

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## Studies on Pollen Germination, Pollination and Fruit Set in Raspberry (*Rubus ellipticus*) under Hilly Conditions of Uttarakhand

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### ABSTRACT

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The pollen grains of raspberry exhibited the average size (length x width) of 177.73 x 124.43 $\mu$  in glycerol conditions, followed by (173.29 x 173.29  $\mu$ ) in water suspension. The viability of fresh pollen grains ranged from 32.50 % to 97.70 % in hermaphrodite or bisexual flowers. 25 % sucrose solution + 0.4 % boric acid solution showed the highest pollen germination of 36.66% with the maximum pollen tube length (284.37 $\mu$ ) after 48 hours interval. After 3 days storage, the pollen grains were quite normal and showed 46.66 % germination. The longevity of pollen grains stored under room conditions indicated that on the third day of storage, the pollen grains were quite normal and showed 97.70 % germination. But after that the longevity of pollen decreased rapidly and after 9 days of storage, the viability dropped to nill. The stigmas were 86.66% receptive on the day of anthesis. Bag self-pollination resulted in the highest fruit set (96.66 %) as compared to hand self-pollination and open pollination.

### Introduction

The Himalayan yellow raspberry (*Rubus ellipticus* var. *obcordatus*) is a large, thorny perennial shrub with heavily armed upright canes (Stratton, 1996). The genus *Rubus* is one of the largest genera of the family Rosaceae, consisting of more than 750 species. Focke (1910) divided this variable and taxonomically complex genus into 12 sub genera. At present, the area, production and productivity in India is completely negligible. Raspberries have a wide range of medicinal and nutritional property. Even though raspberry is used widely in preparation of post-harvest products like jam, jelly etc, but it is not under commercial cultivation in India. It is due to poor knowledge about raspberry

that Indian farmers do not cultivate it and rural folks exploit it from the wild. Proper knowledge about flowering and bearing habit of raspberry will contribute in its cultivation efforts. There is little literature about its flowering and fruit set in India, therefore, to enlighten this part of raspberry cultivation present study was conducted. The aim of the present study was to investigate the floral and reproductive biology of *Rubus ellipticus* examining the implications of floral biology and breeding systems on its life cycle. Alternatively, it is expected to give information which would be useful in management strategies for protecting its endangered population.

## Materials and Methods

A field experiment was carried out at VCSG College of Horticulture, Bharsar, Pauri Garhwal, Uttarakhand during 2015 – 2016. The experiment of pollen study of raspberry required dissection box, butter paper, tag, light microscope, glassware, standardized ocular, stage micrometer and chemicals used for different media and other operations.

## Results and Discussion

### Pollen size in different media

The pollen grains of raspberry exhibited the average size (length x width) of 177.73 x 124.43 $\mu$  in glycerol conditions, followed by 173.29 x 173.29  $\mu$  in water suspension. The minimum size of pollen grains 168.84 x 133.31  $\mu$  was recorded under the dry conditions, followed by acetocarmine staining (146.63 x 146.63  $\mu$ ). The shape of pollen grains in glycol and dry conditions were oblong, elliptic and in acetocarmine and water pollen grains looked round in shape. Bisht and Sharma (1986) found that the fresh pollen grains of apple were elliptic and tricolpate and in acetocarmine solution the pollen grains assumed triangular shape. The average length of fresh pollens ranged from 33.96 to 47.95  $\mu$  in different cultivar, whereas the breadth ranged from 23.97 to 29.97  $\mu$ . The average length and breadth of pollen grain in aniline oil ranged from 38.29 to 47.95  $\mu$  and 21.31 to 26.64  $\mu$  respectively in some apple cultivars. Ali (1962) and Chauhan (1966) observed that the shape of the pollen grains in dry state was elliptical. While the shape of the pollen grains was observed to be roundish when mounted in 2% acetocarmine.

### Pollen viability

The viability of fresh pollen grains was estimated by acetocarmine test. The pollen

grains staining deeply and looking normal under several microscopic fields were counted as viable, whereas shriveled and poorly stained were considered as non-viable. Raspberry viability of fresh pollen grains ranged from 32.50 % to 97.70 % in hermaphrodite or bisexual flowers. Gercekcioglu *et al.*, (2000) studied the pollen grains of eight different pome and stone fruit cultivars (plum, peach, sweet cherry and apple cultivars) and observed pollen viability in the range of 71.53 to 81.78 per cent. Otterbacher *et al.*, (1983) showed that high temperature resulted in rapid loss of viability of raspberry pollen (Table 2).

### Pollen germination

The pollen grains were planted in artificial sucrose media (10, 15, 20 and 25 %) and different boric acid solution (0.1, 0.2, 0.3 and 0.4 %). Distilled water served as a control. Slides have been examined at 12, 24 and 48 hours after planting the pollen grains in different media and observation and pollen tube length were recorded at least under different microscopic fields in each medium.

It is evident from table 1 that 25% sucrose solution + 0.4 % boric acid media was recorded to show the maximum pollen germination of 36.66% and pollen tube length (284.37 $\mu$ ) after 48 hours, followed by 25% sucrose solution with 25.00% germination and (253.27 $\mu$ ) pollen tube length. The control showed the minimum pollen germination of 4.33% with (13.33  $\mu$ ) pollen tube length after 48 hours (Table 1).

Similar observations were recorded by the Asma (2008) who studied the pollen viability and germination ratios for eight apricot cultivars. The results indicated that viable, semi viable and dead pollen rates differed among cultivars. Amongst the four concentration of sucrose solution studied, best

pollen grain germination was obtained with 25% sucrose solution + 0.4 % boric acid solution which ranged from 9.66 to 36.66%. Similar observations were recorded by Wertheim (1996) who reported that 13 to 89 % pollen germination took place in different apple cultivars in a solution containing 15 % sucrose + 15 ppm boric acid + 150 ppm calcium nitrate.

Kumar (1996) reported that the sucrose solution of 9 to 10 % was reported to be the most effective for pollen germination of apple cultivars. Abdel (1999) reported 73.30 to 86.10 per cent pollen germination with 15 % sucrose solution in three apple cultivars. The best pollen tube length (284.37  $\mu$ ) was

observed with 25% sucrose + 0.4% boric acid solution at 48 hours interval.

**Pollen longevity**

For longevity study, fresh pollen grains were stored in dry petri dishes and maintained at room conditions without controlling the humidity.

The longevity of pollen grains stored under room conditions indicated that on the third day of storage, the pollen grains were quite normal and showed 97.70 % germination. But after that the longevity of pollen decreased rapidly and after 9 days of storage, the viability dropped to nill (Table 2).

**Table.1** Pollen germination and pollen tube growth of raspberry in different media

Treatment	Percentage of pollen germination (%)			Pollen tube length ( $\mu$ )		
	12 hours	24 hours	48 hours	12 hours	24 hours	48 hours
<b>T<sub>1</sub>- 10% sucrose solution</b>	8.33	13.00	20.66	62.20	26.66	48.87
<b>T<sub>2</sub>- 15% sucrose solution</b>	6.33	14.00	22.00	44.43	48.87	137.74
<b>T<sub>3</sub>- 20 % sucrose solution</b>	7.66	15.66	21.66	66.65	44.43	133.30
<b>T<sub>4</sub>- 25% sucrose solution</b>	8.66	19.33	25.00	88.86	159.96	253.27
<b>T<sub>5</sub>- 0.1% boric acid</b>	4.66	9.00	17.00	31.10	13.33	31.10
<b>T<sub>6</sub>- 0.2% boric acid</b>	5.33	10.00	11.33	31.10	17.77	48.87
<b>T<sub>7</sub>- 0.3% boric acid</b>	5.33	11.33	11.33	17.77	13.33	26.66
<b>T<sub>8</sub>- 0.4% boric acid</b>	6.00	12.00	9.66	35.54	31.10	35.54
<b>T<sub>9</sub>- 10% sucrose solution + 0.1% boric acid</b>	7.00	11.66	14.00	26.66	57.76	53.32
<b>T<sub>10</sub>- 15% sucrose solution + 0.2% boric acid</b>	8.00	16.00	17.66	44.43	84.42	57.76
<b>T<sub>11</sub>- 20% sucrose solution + 0.3% boric acid</b>	7.33	17.00	24.66	57.76	53.32	133.30
<b>T<sub>12</sub>- 25% sucrose solution + 0.4% boric acid</b>	8.66	20.00	36.66	88.86	168.84	284.37
<b>T<sub>13</sub>- (control) Water</b>	0.00	0.00	4.33	0.00	0.00	13.33
<b>CD</b>	1.93	1.89	1.82	32.01	40.59	53.43
<b>SE(m)</b>	0.66	0.64	0.64	10.93	13.88	18.27

**Table.2** Pollen viability and longevity of raspberry

Date	Total pollen grains	Pollen grains stained	Percentage of pollen grains stained
27/1/16	120	120	100%
29/1/16	450	440	97.70%
1/2/16	120	56	46.66%
3/2/16	80	26	32.50%

**Table.3** Stigma receptivity of raspberry by fruit set method

Age of stigma	Number of buds pollinated	Number of fruit set	Percentage of fruit set
Two days before anthesis	15	5	33.33%
One day before anthesis	15	8	53.33%
Day of anthesis	15	13	86.66%
One day after anthesis	15	9	60.0%
Two days after anthesis	15	7	46.60%

**Table.4** Mode of pollination in raspberry

Mode of pollination	Number of flowers pollinated	Number of fruit set	Percentage of fruit set
Self-pollination	30	29	96.66%
Cross pollination	30	18	60.00%
Natural pollination	30	23	76.66%

**Plate.1** Pollen germination and pollen tube length



**Stigma receptivity**

To find out the receptivity of stigma by visual observation, the stigmas of different age groups varying from 2 days prior up to 2 days after anthesis have been examined daily by hand lens.

The stigma looking shiny, sticky, fresh and attractive were considered to be receptive while dull, dried, faded and brownish stigmas were considered non receptive. The stigmas were green, dark cream in color, less shiny and style length was short two days before anthesis. They were dark cream in color,

shiny, style was healthy one day after anthesis. On the day of anthesis, stigma turned into brown color, very shiny and sticky with more stigmatic secretion. The ovary size increased with brown colored stigma and stigmatic surface was bilobed one day after anthesis. The basal portion was swollen, style constriction at the middle portion and shininess disappeared.

### **Fruit set**

In order to study the receptivity of stigma by fruit set method, different flower buds of different age group were emasculated and pollinated with fresh pollen. It is evident from Table 3 that 33.33 % fruit set was observed two days before anthesis while 86.66 % was recorded when stigma were pollinated on the day of anthesis while after one day anthesis the fruit set percentage was 60.10 %. The receptivity was almost equally good one day before and one day after anthesis. Kumar (1988) observed the maximum fruit set with cross pollination whereas, minimum fruit set was obtained with hand self-pollination. Abdel *et al.*, (1999) reported highest fruit set under open pollination in cultivar Anna of apple.

### **Mode of pollination**

To determine the extent of self-fruitfulness, selfing by bagging was done and fruits were assessed. The data presented in Table 4 revealed that the maximum fruit set (96.66%) was recorded with self-pollination, followed by natural pollination. The average fruit set in raspberry cultivars under study was 76.66%. Cross pollination showed the minimum fruit set (60.00%). Daubeney (1971) found that flowers pollinated only with their own pollen or that with adjacent flowers set nearly 57 to 77 % as open pollinated flowers. Most commercially grown cultivars of red raspberry are self-fertile (Gregor, 1976).

Rajput and Singh (1967) reported that the cross pollination resulted in the highest percentage of fruit set and self-pollination in the lowest fruit set in strawberry cultivars. Shanks (1969) observed that without any additional pollination a berry developed frequently in raspberry, although it was relatively small and had a tuft of unpollinated pistils at its center.

In raspberry plants the pollen size (length × width) of pollen grains was maximum (177.73 X 124.43 μ) in glycerol, followed by water (173.29 X 173.29 μ). The highest pollen germination percentage (36.66%) and maximum pollen tube length (284.37μ) was recorded with 25% sucrose solution + 0.4 % boric acid solution. The pollens were observed viable up to seven days with 46.66% germinability, which decreased rapidly and after three days of storage it remained only 32.50 %. The stigma continued to be receptive from two days prior to two days after anthesis. However, the best receptivity of stigma (86.66%) has been witnessed on the day of anthesis by the fruit set method. The maximum fruit set (96.66%) has been recorded under self-pollination, followed by natural pollination (76.66%). With these findings of present study it has been suggested that the hybridization should done in raspberry plants when the pollen completely mature and stigma shows maximum receptivity.

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