Research Article

Studies on Pollen Load, Viability and Germination of Raspberry Wild Species (*Rubus macilentus* C.) of Garhwal Himalaya

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Abstract

In most of the temperate fruit crops, pollen load produced from an anther and flower and pollen quality includes pollen viability, longevity, morphology, pollen germination and pollen tube growth rate at different time intervals play vital role in fertilization and fruit set. These properties are useful for plant breeders, geneticists and growers to select the pollinating varieties to ensure high and quality yields. In this study, the investigations were carried out under lab conditions (25°C) on pollen performance and quality of raspberry (*Rubus macilentus* C.), growing as wild species in the foot hills of Himalaya under the climatic conditions of Bharsar, Pauri Garhwal district of Uttarakhand, India. The number of pollens per anther was found to be 900 and per flower were 113400. Difference in the shape and size of the pollen grains were observed under different condition. The maximum pollen length of 31.29µ and width of 28.28µ was recorded under dry condition (Oval shape) and water (Spherical shape) respectively. Acetocarmine staining test showed 100% viability of pollen grains on the day of anthesis and longevity became nil on 9th day of pollen collection. Pollen grains showed early (6 hours) germination and germination percentage was 90.54 with pollen tube length of 1,164.19 µ was recorded after 48 hours of pollen planting in 10% Sucrose + 2ppm Gibberellic acid and 5% Sucrose + 1ppm Gibberellic acid media respectively.

Keywords: Raspberry, Pollen load, Viability, Germination, Pollen tube growth

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Introduction

Rubus is a variable genus comprising about 400 species out of which 57 are found in India. Idaeobatus includes raspberry and Eubatus includes blackberry are horticulturally most important sections of the genus *Rubus*. All the present raspberry cultivars have been developed from the natural hybridization of different species therefore polyploidy exist in the genus *Rubus*. Pollen has vital role in the success of natural hybridization. Pollination is a very essential component for regular and consistent production in many of fruit crops. The pollen viability, pollen tube growth and pollen morphological homogeneity are the most important properties in fruit trees. These properties are useful for plant breeders, geneticists and growers [1]. The positive correlations between viability and pollen germination have been reported by many workers [2].

Variation is the fundamental basis for natural selection, leading to the origin of a new species with new complements of characters. Pollen being the carrier of plant heredity, its variations reflects the change undergone and transmitted to the next generation by the plant. Pollen organization is more or less stable within the same species and when there is a deviation from the general type, it brings about the biological change in the species. It is imperative that the pollen also undergoes change in its morphological characters and that change provide an important tool to analyze and interpret the taxonomic relationship and evolutionary trends of the plants concerned. In recent years, pollen morphology is being put to greater use in phylogeny and evolution.

Pollen germination requirements vary from species to species. Moisture, Carbohydrate, sucrose, calcium, magnesium and potassium plays key role in pollen germination and Pollen elongation, but work has been carried out for only few taxa. Growth hormones affect pollen germination and pollen tube growth. However, the response of pollen to varies from species to species. In present study an attempt was made to investigate the effect of GA_3 on pollen germination and tube growth of *Rubus macilentus* C. Therefore, present investigations carried out on pollen performance (Pollen load) and quality (Pollen morphology, viability and germination) of wild raspberry (*Rubus macilentus* C.) under lab conditions (25˚C) at Department of fruit science, VCSG, Uttarakhand University of Horticulture and Forestry, Bharsar Campus, Pauri-Garhwal district of Uttrakhand, during 2016-17.

Materials and Methods

During the peak period of flowering, floral twigs bearing copious flower buds which were at the balloon stage (likely to open next day) were gathered from the experimental plants on the previous evening of anthesis day and the flower buds were left in vase with water. On the succeeding days as the dehiscence of anthers was ensured, a single gentle tap on the flowers released the pollen grains easily on the petridish.

Number of pollens per anther and per flower

To observe number of pollens per anther the flower buds were placed in a small vial containing 1 ml of glycerine 1%, anthers were smacked and the pollen grains were suspended. From this concentrate, five (10 µl) droplets were taken out in the slides and pollen grains were counted under the microscope and production of pollen grains per flower was estimated by multiplying the number of pollen grains per anther with the number of anthers per flower.

Pollen morphology

The fresh pollen grains were used for making size measurements in different medium, viz., water, acetocarmine, glycerin jelly and without any medium (dry condition). The average size (length and breadth) of 50 pollen grains were measured using compound microscope under each medium with the help of ocular micrometer indexed against stage micrometer to arrive the final value. The data was analyzed with completely randomized design having 4 treatments and 6 replications.

Pollen viability and longevity

The viability of fresh pollen grains of flowers was estimated separately by acetocarmine test. Acetocarmine solution was freshly prepared by taking 55 ml of water, 45 ml of glacial acetic acid and 1 gm of carmine boiled for some time and then filtered. Few drops of acetocarmine solution were placed on the slide and pollen grains were dusted, covered with a cover slip to examine pollens under the microscope. Those grains stained deeply and looked normal under microscope were counted as viable, while, shriveled and poorly stained were considered as nonviable. Care was taken to include data from several microscopic fields to cover the pollen grains laying both at the peripheral and central regions of the cover slips.

The pollen longevity was studied by storing the fresh pollen grains in dry specimen tubes, covered with cotton plugs and maintained at room temperature and pollen viability was calculated by dividing Number of stained pollen grains by Total number of pollen grains observed and multiplying the resulted value with 100.

Pollen germination

The fresh pollen grains were planted in artificial media of sucrose, boric acid and gibberellic acid at different concentration combinations and water served as control. The "Sitting Drop" culture method [3] was employed for pollen germination studies. Slides were examined at 6, 18, 24 and 48 hours (If required number of hours can be increased upto 72 hours), after planting the pollen grains in different media, for recording the observations on germination of pollen grains and pollen tube length from at least ten different microscopic fields for calculating the average values. Data was analyzed with completely randomized designs which have 16 treatments and 3 replications (Tables 4 and 5). Pollen germination percentage of each treatment was calculated by dividing Number of germinated pollen grains to Total number of pollen grains observed and multiplying the resulted value with 100.

Statistical analysis

The experiment was laid out in the Randomized Block Design with three replications for pollen load, viability, longevity, Pollen germination and pollen tube growth, while six replications were used for pollen morphology. The data recorded on various parameters were analyzed using OPSTAT software to find out the significance of variation resulting from the experimental treatments. The mean values of data were subjected to analysis of variance as per Statistical Procedure for Agricultural Research [4] for Randomized Block Design.

Results and Discussion

Number of pollens per anther and per flower

On an average number of pollens per anther and per flower in wild raspberry (*Rubus macilentus* C.) was found to be

around 900 and 113400. The values are given in **Table 1**. The present results are in accordance with the studies on pollen load of pear Xiangli (*Pyrus bretschneideri* R.) was about 4000 in each anther [5].

Table1 Number of pollens per anther and per flower

Observation	*Rubus macilentus* C.
Number of pollens per anther	900±120
Number of pollens per flower	113400±180

Morphology of pollen grains in different conditions

Mature anthers were collected from freshly opened flowers which dehisced in sunshine and were examined under microscope. Individual pollen grains were pale yellow in color whereas in mass the pollen grains appeared golden yellow in color. The shape of pollen grains in glycerine and dry conditions were oblong, elliptic and tricolpate, while in acetocarmine and water, the pollen grains appeared to be round in shape. The values are given in **Table 2**. The pollen grains had the maximum average size of 31.29 µ x 16.12 µ (length x width) in dry conditions, followed by glycerine (30.74 µ x 17.68 µ). The minimum size (25.23 µ x 23.51 µ) was recorded under the acetocarmine conditions, followed by water (28.61 µ x 28.28 µ).

Similar observations were recorded on freshly dehisced anthers of apple pollen grains, the pollens were elliptic and tricolpate and in acetocarmine solution the pollen grains assumed the triangular shape. The average length of fresh pollen ranged from 33.96 µ to 47.95µ in different cultivar, whereas the breadth ranged from 23.97 µ to 29.97 µ. The average length and breadth of pollen grain in aniline oil ranged from 38.29 µ to 47.95 µ and 21.31 µ to 26.64 µ respectively in some apple cultivars [6].

Table 2 Morphology of pollen grains in different conditions

Treatment	Pollen size ± SE(m)	
	Length (µ)	Width (µ)
T1 - Control (Water)	28.61±0.16	28.28±0.11
T2- Glycerine	30.74±0.19	17.68±0.24
T3 - Acetocarmine	25.23±0.15	23.51±0.12
T4 – Dry condition	31.29±0.34	16.12±0.11
SE(d)	0.31	0.22
C.D.	0.65	0.46
C.V.	1.87	1.78
µ - micron (unit of pollen tube length)		

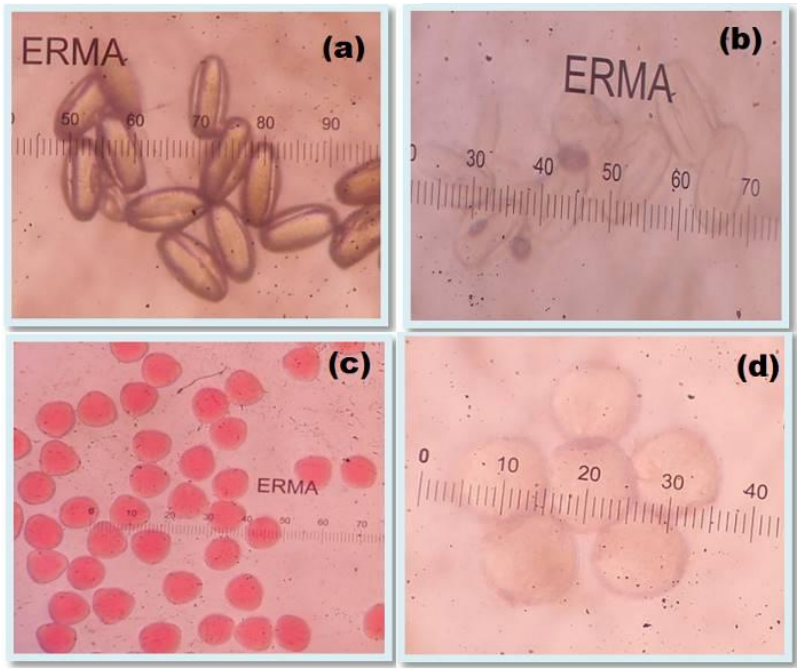

Figure 1 Morphology of pollens under different conditions
(a. Under dry condition, b. Glycerine, c. Acetocarmine, d. Water)

Pollen viability and longevity

It was evident from the **Table 3** that the viability of pollen grains of *Rubus macilentus* C. was high. The viability of fresh pollen grain was recorded 100%. The germinability of pollen grains, stored under room conditions (25°C) is presented in Table 3. The results indicate that after seven days of storage, the viability of the pollen grains totally declined. The sharp decline was seen between third and fifth day of observation. From the Table3, we concluded that the pollen viability became nil on ninth day of storage under room condition.

Similar observations were recorded during studies on determinations of pollen viability and germination ratios for eight apricot cultivars. The results indicated that viable, semi viable and dead pollen rates differed significantly among the cultivars, where, Roksana cultivar had minimum viable pollens (41.50%) [7]. High temperature resulted in rapid loss of viability of raspberry pollen grains [8]. The germinability of pollen grains stored under room conditions indicated that after three days of storage, the pollen grains were quite normal and showed 46.66% germination. But after seven days of storage, the germinabilty was decreased rapidly and after nine days of storage, the germination percentage totally declined.

Table 3 Viability and longevity of pollens

Species	**Longevity of pollens at different days intervals**				
	1st day	**3rd day**	**5th day**	**7th day**	**9th day**
Rubus macilentus C.	100%	94.00%	56.00%	32.66%	0.00%

Pollen germination

The results indicated that, *Rubus macilentus* C. showed best results for pollen germination and pollen tube length. The pollens showed early germination, hence, the observations of pollen germination were taken at 6, 18, 24 and 48 hours after planting of pollen grains in artificial media of sucrose, boric acid and gibberellic acid at different concentration combinations and water served as control.

Table 4 Pollen germination percentage of wild raspberry (*Rubus macilentus* C.) after different time of incubation

Treatments	**Pollen germination (%)±SE(m) (6hours)**	**Pollen germination (%)±SE(m) (18hours)**	**Pollen germination (%)±SE(m) (24hours)**	**Pollen germination (%)±SE(m) (48hours)**
T1- Water (Control)	12.63±0.27	45.83±0.27	64.41±0.21	73.76±0.18
T2- 0.1% Boric acid + 5% Sucrose	35.43±0.33	68.29±0.24	46.57±0.23	46.45±0.22
T3 - 0.2% Boric acid + 10% Sucrose	46.69±0.40	81.81±0.25	85.68±0.19	86.68±0.18
T4- 0.3% Boric acid + 15% Sucrose	41.53±0.27	85.71±0.26	86.2±0.17	87.63±0.21
T5- 0.4% Boric acid + 20% Sucrose	37.82±0.26	83.87±0.15	78.75±0.11	73.78±0.18
T6 - 0.5% Boric acid + 25% Sucrose	37.06±0.10	77.43±0.26	62.75±0.15	40.49±0.16
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	24.53±0.29	76.12±0.18	42.73±0.16	40.64±0.22
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	41.19±0.21	61.17±0.13	62.48±0.24	64.86±0.14
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	58.54±0.31	66.92±0.53	68.11±0.09	69.97±0.13
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	52.91±0.14	61.85±0.12	56.33±0.11	53.48±0.23
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	34.73±0.32	55.12±0.40	49.35±0.12	49.13±0.12
T12 - 5% Sucrose + 1ppm Gibberellic acid	56.82±0.29	85.24±0.17	85.72±0.13	87.12±0.12
T13 - 10% Sucrose + 2ppm Gibberellic acid	68.11±0.11	80.71±0.26	88.84±0.11	90.54±0.15
T14 - 15% Sucrose + 3ppm Gibberellic acid	48.71±0.16	71.33±0.12	76.57±0.24	79.67±0.23
T 15 - 20% Sucrose + 4ppm Gibberellic acid	43.75±0.27	67.87±0.14	72.88±0.17	75.79±0.21
T16 - 25% Sucrose + 5ppm Gibberellic acid	40.14±0.29	64.87±0.14	72.89±0.14	73.77±0.14
SE(d)	0.38	0.36	0.24	0.26
$CD_{0.05}$	0.77	0.73	0.49	0.52
C.V.	1.09	0.62	0.42	0.46

The pollen germination was started from 6 hours and continued upto 48 hours (**Table 4**). The maximum pollen germination (90.54%) and pollen tube length (1,164.19 µ) (**Table 5**) was recorded 48 hours after planting, followed by 24 hours after planting with the pollen germination of 88.84% and pollen tube length of 1,084.61 µ, while the minimum pollen germination and pollen tube length (12.63% and 42.17µ) was recorded 6 hours after planting of pollen grains. Among all the treatments T_{13} (10% Sucrose + 2ppm Gibberellic acid) and T_{12} (5% Sucrose + 1ppm

Gibberellic acid) ranks first with maximum pollen germination (90.54%) and pollen tube length (1,164.19µ), followed by T_4 (0.3% Boric acid + 15% Sucrose) and T_{13} (10% Sucrose + 2ppm Gibberellic acid) with pollen germination (87.63%) and pollen tube length (1,051.85µ), while the minimum value (pollen germination 68.11% and pollen tube length 92.27µ) was recorded after 6 hours of pollen planting with T13 (10% Sucrose + 2 ppm Gibberellic acid) and T_{12} (5% Sucrose + 1ppm Gibberellic acid).

Table 5 Pollen tube growth of wild raspberry (*Rubus macilentus* C.) after different time of incubation

Treatments	Pollen tube length (µ)±SE(m) (6hours)	Pollen tube length (µ)±SE(m) (18hours)	Pollen tube length (µ)±SE(m) (24hours)	Pollen tube length (µ)±SE(m) (48hours)
T1- Water(Control)	42.17 ± 3.33	68.04 ± 3.03	98.52 ± 0.65	77.463 ± 12.68
T2- 0.1% Boric acid + 5% Sucrose	51.53±2.66	78.857 ± 3.04	405.587 ± 4.14	380.12 ± 8.65
T3 - 0.2% Boric acid + 10% Sucrose	60.66 ± 1.63	150.123 ± 7.53	419.72 ± 2.32	404.5 ± 6.27
T4- 0.3% Boric acid + 15% Sucrose	55.61 ± 2.16	126.19 ± 5.60	516.263 ± 1.36	532.667 ± 6.76
T5- 0.4% Boric acid + 20% Sucrose	51.47 ± 3.23	117.493 ± 6.44	487.843 ± 2.64	531.013 ± 16.25
T6 - 0.5% Boric acid + 25% Sucrose	46.427 ± 1.69	114.52 ± 6.54	437.493 ± 2.93	410.497 ± 6.69
T7 - 0.1% Boric acid + 1ppm Gibberellic acid	57.207± 2.35	94.527 ± 6.66	615.503 ± 2.62	785.49 ± 7.43
T8 - 0.2% Boric acid + 2ppm Gibberellic acid	64.147 ± 2.28	101.14 ± 10.10	691.49 ± 3.32	806.097 ± 1.93
T9 - 0.3% Boric acid + 3ppm Gibberellic acid	70.257 ± 3.33	157.49 ± 4.69	816.253 ± 3.71	926.023 ± 9.65
T10 - 0.4% Boric acid + 4ppm Gibberellic acid	76.443 ± 1.69	137.203 ± 6.38	729.867 ± 4.63	861.057 ± 11.14
T11 - 0.5% Boric acid + 5ppm Gibberellic acid	70.28 ± 2.77	164.73 ± 5.29	785.487 ± 5.81	921.83 ± 10.14
T12 - 5% Sucrose + 1ppm Gibberellic acid	92.277 ± 3.32	244.74 ± 3.89	1,084.61 ± 7.68	1,164.19 ± 14.77
T13 - 10% Sucrose + 2ppm Gibberellic acid	90.413 ± 2.70	234.893 ± 8.86	994.97 ± 4.06	1,051.85 ± 10.67
T14 - 15% Sucrose + 3ppm Gibberellic acid	85.157 ± 3.28	218.07 ± 4.65	913.74 ± 1.76	928.43 ± 6.49
T 15 - 20% Sucrose + 4ppm Gibberellic acid	81.457 ± 3.23	215.307 ± 6.43	707.76 ± 3.43	825.223 ± 6.41
T16 - 25% Sucrose + 5ppm Gibberellic acid	75.963 ± 2.93	206.43 ± 5.01	591.363 ± 3.40	795.803 ± 5.99
SE(d)	3.86	8.73	5.35	13.51
$CD_{0.05}$	7.89	17.87	10.96	27.65
C.V.	7.06	7.04	1.01	2.32

µ - micron (unit of pollen tube length)

The data on raspberry (*Rubus macilentus* C.) pollen germination and pollen tube length after 6 hours of planting is presented in Tables 4 and 5. The data revealed that all treatments for pollen germination were significant and for pollen tube length, all treatments were significant except T_6 (0.5% Boric acid + 25% Sucrose), when compared to control (water). The maximum pollen germination percentage (68.11%) was recorded with T_{13} (10% Sucrose + 2ppm Gibberellic acid) and the minimum (12.63%) was recorded with T_1 (Water). The maximum pollen tube length (92.277 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid) which was statistically at par with treatment T_{13} and T_{14} recording 90.41 µ and 85.15 µ respectively. Minimum pollen tube length (42.17µ) was recorded with T1 (Water).

After 18 hours of pollen planting the results (Tables 4 and 5) indicated that all the treatments were significant for pollen germination, the treatment T_2 was not significant for pollen tube length as compared to water. Maximum pollen germination percentage (85.71%) was recorded with T_4 (0.3% Boric acid + 15% Sucrose) which was statistically at par with treatment T_{12} (85.24%). The minimum pollen germination percentage (45.83%) was recorded with T_1 (Water). The maximum pollen tube length (244.74 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid) which were statistically at par with treatment T_{13} (234.89 µ). Minimum pollen tube length (68.04 µ) was recorded T_1 (Water) which was statistically at par with treatment T_2 (78.86 µ).

The Tables 4 and 5 indicated that after 24 hours of germination the treatments T_2, T_6, T_8 and T_{11} were non-significant for pollen germination and remaining treatments were significant, all the treatments for pollen tube length were significant when compared to control (water). Pollen germination percentage (88.84%) was recorded with T_{13} (10% Sucrose + 2ppm Gibberellic acid) was recorded to be maximum and the minimum pollen germination percentage (42.73%) was recorded with T_7 (0.1% Boric acid + 1ppm Gibberellic acid). The maximum pollen tube length (1,084.61 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid). Minimum pollen tube length (98.52 µ) was recorded T_1 (Water).

The results obtained after 48 hours of planting of pollen grains for germination (Tables 4 and 5) indicated that there was no significant difference among the treatments T_2, T_5, T_6, T_7, T_8, T_9, T_{10} and T_{11}. While, the treatments T_3, T_4, T_{12}, T_{13}, T_{14}, T_{15} and T_{16} showed significant differences when compared to control. The maximum pollen

germination percentage (90.54%) (**Figure 2**) was recorded with T_{13} (10% Sucrose + 2ppm Gibberellic acid). The minimum pollen germination percentage (40.49%) was recorded with T_6 (0.5% Boric acid + 25% Sucrose) which was statistically at par with treatment T_7 (40.64 µ). The maximum pollen tube length (1,164.19 µ) was recorded with T_{12} (5% Sucrose + 1ppm Gibberellic acid) which were statistically at par with treatment T_{13} and T_{14} recording 90.41 µ and 85.16 µ respectively. Minimum pollen tube length (77.46 µ) was recorded T_1 (Water).

The results presented above are inline with the results of pollen germination studies in apple cultivars. 13 to 89 per cent pollen germination took place in different apple cultivars in a solution containing 15 per cent sucrose + 150 ppm calcium nitrate + 15 ppm boric acid [9]. The sucrose solution of 9 to 10 per cent concentration was the most effective media for pollen germination of apple cultivars [10]. 73.30 to 86.10 per cent pollen germination was recorded in 15 per cent sucrose solution in three apple cultivars. The best pollen tube length was observed with 25% sucrose + 0.4% boric acid solution which ranged from 26.66 µ to 284.37µ [11].

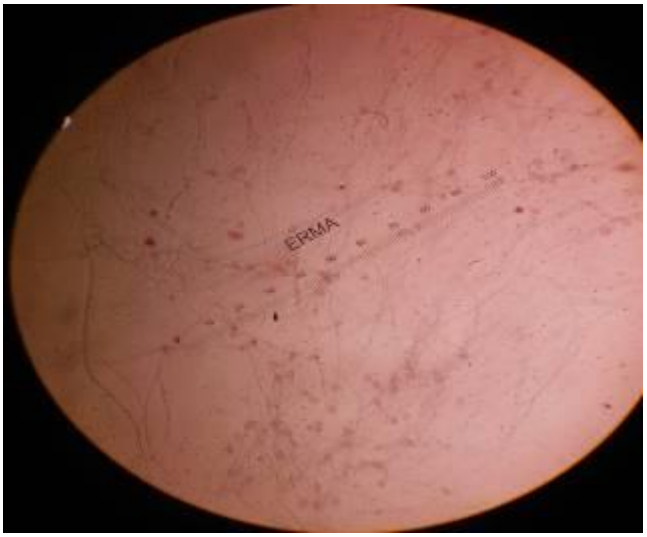

Figure 2 90% pollen germination of *Rubus macilentus* C

Conclusion

Pollination is important biological factor, especially in aggregate fruits. Sufficient amount of pollen load (900 pollen grains/ flower and 113400 pollens / flower) with good viability (100%) and longevity (viability nil on 9th day) was reported to be good for successful fertilization of multiple ovaries of raspberry fruit. Difference in the shape and size of the pollen grains was observed under different condition. The maximum pollen length of 31.29µ and 30.74µ was recorded under glycerine and dry condition respectively. whereas the maximum pollen width of 28.28µ and 23.51µ was recorded under water and acetocarmine respectively. When the pollen grains were hydrated or placed in acetocarmine they took the spherical shape. Media without Sucrose, Boron and Gibberellic acid (Control) showed minimum pollen germination and tube growth when compared with results of T_{12} (5% Sucrose + 1ppm Gibberellic acid) and T_{13} (10% Sucrose + 2ppm Gibberellic acid) for pollen germination (90.54%) and Pollen tube growth (1,164.19 µ) respectively up to 48 hours after pollen planting, indicating the essentiality of sucrose as carbon source and Gibberellic acid as growth hormone for better pollen germination and tube growth for effective fertilization and fruit set. Hence the present findings acts as base line for breeders and growers for the selection of potential wild Indian raspberry species in domestication, hybridization and as source for resistance breeding and other pollen based biotechnological approaches.

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