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Pollen viability and Pollen Germination Studies in Low Chill Peach [*Prunus persica* (L.) Batsch] Cultivars in the Subtropics of North-West Himalayas

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ABSTRACT: Pollen studies in Peach (*Prunus persica* (L.) Batsch) were carried out at Fruit Research Farm of College of Horticulture and Forestry Neri, Hamirpur, Himachal Pradesh during 2018 -2020 to assess the viability and germination of pollen of six cultivars (Pratap, Earligrande, Flordaprince, Shan-e-Punjab, Glohaven, Royal Paradelux) of Peach. The aim of the study was to find the compatibility behaviour of the cultivars which will be helpful for the breeders in deciding the parental combinations where hybridization can be achieved successfully and to have an understanding of performance of cultivars as pollinizers. Pollen viability of the peach germplasm was determined using 2% acetocarmine while germination studies were conducted with different sucrose concentrations (10%, 15% and 20%) and boric acid (50 ppm, 100ppm, 150ppm). Highest pollen viability was recorded in Flordaprince (97.78 %). In general, most of the cultivars showed maximum germination of fresh pollen with 10 per cent sucrose concentration whether alone or in combination with boric acid as compared to other treatment combinations. The highest germination percentage was recorded in Earli Grande (45.09 %) with 10 per cent sucrose solution. When sucrose and boric acid were used in combination the maximum germination was recorded with 10 per cent sucrose + 100 ppm boric acid in cultivar Flordaprince (96.40 %). There is always need to test the functionality of pollen grains in order to assess the fertility of pollen and hybrids in plant breeding which further helps the breeder to understand the compatibility behaviour among cultivars.

Keywords: Peach, pollen viability, germination, acetocarmine, tricolporate

INTRODUCTION

Peach (*Prunus persica* (L.) Batsch) member of Rosaceae family the subfamily Amygdyloideae, is the most important among stone fruits cultivated in Himachal Pradesh. It is grown successfully in lower as well as mid hills of Himachal Pradesh. Peach is known as "Aaru" in Himachal Pradesh and extensively consumed worldwide. Peach has an important place in human nutrition, and can be used as fresh, dried or processed fruit. The fruit got domesticated around 4000-5000 years ago. The major peach producing countries are USA, Italy, France, Greece and China. In Himachal Pradesh, it is cultivated on an area of 5090 hectares annually producing 7262 MT with productivity of 1.42MT/ha (Anonymous, 2017). In Himachal Pradesh Rajgarh Valley of Sirmaur is called as "Peach Bowl of India". Peaches have relatively performed well at an altitude ranging between 1200-1500 m above mean sea level. Although it is a temperate fruit, low chilling varieties gave been developed which are suitable for subtropical regions also (Turkaman *et al.*, 2004). Due to lack of compatibility, the plant reduces yield and hence pollen and pollination studies helps the breeder to determine the compatibility of varieties (Karmari, 2012).

Pollen viability is significant for plant breeding as an indicator of fertilization efficiency of the male gamete.

The main objective to study the pollen viability and germination studies to use in crop is breeding which create cultivars with high yield, large fruits, resistance to insect pest and diseases and low cost of production. The observation of significant differences between genotypes within the species indicates the existence of genetic variability for the evaluated characteristic. To determine the reproduction biology for optimizing yield, pollen and pollination studies are determined regularly. Some peach cultivars flowers early while the fruit set is inhibited by low temperature at flowering due to poor pollen germination and pollen tube growth.

Therefore synchronized flowering, high pollination and fertilization are critical for fruit set in peach (Semenas and Kouhartchik, 2000). Many cultivars with unfavorable pollens and low germination were reported by breeders and researchers (Yuhu *et al.*, 2006). In artificial pollination the hybridization is carried out with the pollen which is stored, thus for effective controlled pollination the germination and pollen viability studies are required. Du *et al*., (2006) performed experiment in different cultivars of peach with the objectives to determine the longevity, viability, germination and tube growth which helps them to find out the superior genotypes which were effective for controlled pollination. These studies were conducted to identify suitable pollen donors and to investigate the compatibility among the available peach germplasm.

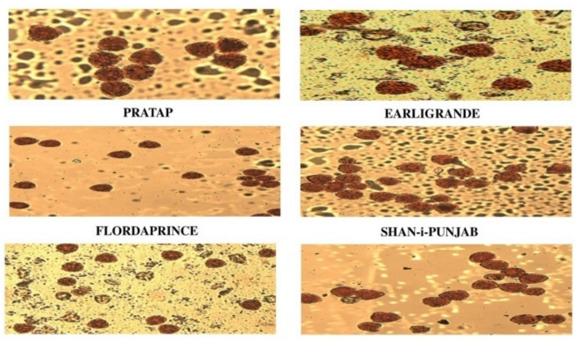

Fig. 1. Pollen viability in different cultivars of peach.

MATERIALS AND METHOD

Place of Experiment: The research experiment was conducted at the College of Horticulture and Forestry, Neri located in the North-western Himalayan region between 31°41' 47.6" N latitude and 76°28' 6.3" E longitude at an elevation of 650 metres above mean sea level in the sub-tropical zone of Himachal Pradesh. Six low chill peach cultivars viz., Pratap, Earligrande, Flordaprince, Shan-e-Punjab, Glohaven and Royal Paradelux being maintained at the Experimental farm of Department of Fruit Science were included in this study so as to assess their suitability as pollen parent in hybridization programme.

Pollen Collection: Flower buds were collected at balloon stage from each variety separately. Anthers were detached from filament with the help of needles and kept in petridishes on a non-sticky paper. This anther mass was kept under partial shade so as to allow dehiscence. As anther dehiscence took place, the pollen were collected when the yellow powdery mass was released. These pollen grains were then transferred to glass vials. Small samples of pollen grains were drawn out from these vials for viability and studies.

Pollen viability: Freshly collected pollen grains of peach cultivars viz., Pratap, Earligrande, Flordaprince, Shan-e-punjab, Glohaven and Royal Paradelux were used to work out pollen viability. The experimental design used was Completely Randomized Design. Fresh acetocarmine solution (2%) was prepared by dissolving 45 ml of glacial acetic acid and 2 g of carmine powder to make final volume 100 ml. The mixture was boiled carefully and then filtered with Whatman No.1 filter paper.

Pollen grains were dusted on a glass slide and one to two drops of acetocarmine were put on pollen grains and then covered with cover slip. Glass slide was left for 4 to 5 min as such so that pollen grains take up the stain properly. The microscopic examination of slides was done with approximately 500– 700 pollen grains for evaluating pollen viability and pollen size. About 10 – 20 fresh slides in each case were prepared from each variety and then analyzed. Well filled pollen grains with stained nuclei were taken as apparently viable, while shriveled and unstained pollen grains were counted as non-viable.

Pollen Germination: The freshly dehisced pollen grains were subjected to *in-vitro* pollen germination tests using hanging drop method. This test was conducted with different concentrations of sucrose (10 %, 15% and 20 %) and boric acid (50ppm, 100ppm and 150ppm) alone and in combination.

The media for pollen germination used were:

A. Sucrose Solution: 10%, 15% and 20%

B. Sucrose + Boric acid in combination:

➢ **S1B1**: 10 % sucrose + 50 ppm boric acid; **S1B2**: 10 % sucrose + 100 ppm boric acid; **S1B3**: 10 % sucrose + 100 ppm boric acid.

➢ **S2B1**: 15 % sucrose + 50 ppm boric acid; **S2B2**: 15 % sucrose + 100 ppm boric acid; **S2B3**: 15 % sucrose + 100 ppm boric acid.

➢ **S3B1**: 20 % sucrose + 50 ppm boric acid; **S3B2**: 20 % sucrose + 100 ppm boric acid; **S3B3**: 20 % sucrose + 100 ppm boric acid.

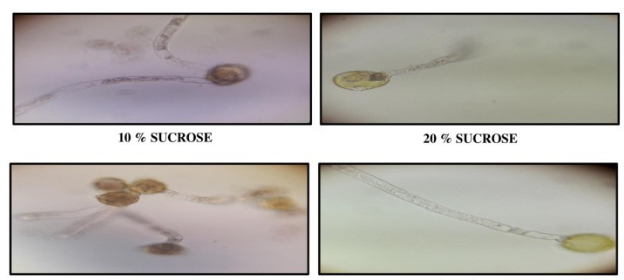

Fig. 2. Germination of pollen grains with different concentrations.

One drop of sucrose solution with desired concentration nalone or one drop of sucrose solution with desired concentration + one drop of boric acid concentration were placed in cavities of different microscopic slides and pollen grains dusted over them as per the treatment. Cover slips were placed after this and their edges were smeared with vaseline. Cavity slides were inverted instantly which led to the formation of hanging drop and then the cavity slides were put into the petridish which contained moist filter paper to ensure uniform moisture conditions. Pollen germination was assessed after 2h, 4h, 6h, 8h, 12h and 24 hrs. The pollen grains having pollen tube at least two times longer than pollen grain was considered as germinated. Average percentage of germination was calculated under three microscopic fields. The experiment was conducted in completely randomized design and analysis of variance was computed separately for each experiment.

RESULTS AND DISCUSSION

Pollen viability: The pollen viability study was done with acetocarmine 2 per cent and maximum percentage of pollen viability was observed in cultivar Earligrande (96.35%) followed by Pratap (95.32 %), whereas minimum pollen viability percentage was observed in Glohaven (90.44 %) (Table 1). The pollen shape was Tricolporate in all the cultivars under study. Among peach genotypes the length of pollen grains ranged from 20 µm- 59.4 µm while the width of pollen grains ranged from 25.7 µm - 62.5 µm. Highest pollen viability was found in flordaprince (97.78 %) and similar results were observed by de Siqueira *et al.*, (2012) in *Psidium guajava* while analyzing pollen viability four times and found 95.6 % viability with no statistaical difference. The lowest pollen viability was found in Royal Paradelux (85.78 %).

Table 1: Pollen viability and morphology of peach cultivars.

Varieties	Pollen viability (%)	Pollen shape	Pollen size (µm)
Pratap	93.90(9.74)	Tricolporate	62.5-46.5 × 37.5-43.8
Earligrande	96.01(9.84)	Tricolporate	52.4-41.5 × 25.0-43.8
Flordaprince	97.78(9.93)	Tricolporate	35.4-31.7 × 25.0-28.6
Shan-i-Punjab	87.83(9.42)	Tricolporate	57.8 – 48.1 × 21.4 – 30.5
Glohaven	95.04(9.80)	Tricolporate	54.9 – 46.5 × 25 – 25.7
Royal Paradelux	85.78(9.31)	Tricolporate	42.1 – 37.7 × 20 – 29.7
Mean	92.72		
$CD_{0.05}$	0.148		

*Figures in parenthesis are square root transformed values.

The maximum length of pollen grains was observed in Earligrande (59.4 µm) while the minimum length of pollen grains was observed in Royal Paradelux (20 µm). The maximum width of fresh pollen grains was observed in Pratap (62.5 µm) while minimum width of fresh pollen grains in Glohaven (25.7 µm). Pollen viability studies have been suggested by Arenas *et al.,* (2016) for better fertilization and it was recommended that it should be performed during pre anthesis when stigma is receptive. Bolat and Pirlak (1999) investigated pollen germination and pollen tube length in apricot and found longest pollen tube in Salak cultivar of apricot (306 µm). It has also been discussed that some cultivars produce high quantity of pollen but not high quality of pollen which affect the fertilization and in turn produces poor fruit set while in some cultivars pollens are sterile or non viable (Nikolic and Milatovic, 2010; Stosser *et al*., 1996). Sulusoglu and Cavusoglu (2014), conducted periodic pollen germination studies and found best results with 15 % sucrose solution. Yao, (2018) studied pollen germination in 48 cultivars of *Ziziphus jujuba* Mill. and found that pollen germination ranged from 0 % to 70 % depending upon the cultivar and year. Perez *et al*., (2019) studied pollen performance in mango in different sucrose concentration (10 %, 15 % and 20 %) and Polyethylene glycol (15 %, 20 %, 23% and 25 %) and best pollen germination was found in 15 % sucrose and 23 % PEG. Barbieri and Nava (2020) studied production and *in vitro* viability of pollen of peach trees grown in subtropical climate and found that pollen germination percentage ranged from 63 % to 77 %.

Pollen germination: In fruit trees pollen germination and tube growth rate are the most important characteristics related to pollen quality and effectiveness for fertilization requires the high rates of germination and fast tube growth. Excessively low rates may lead to low fruit set because of ovule degradation before the pollen tube reaches the ovary (Sharafi and Bahmani, 2011). Among six cultivars of peach, maximum pollen germination was recorded with 10% sucrose (Plate 2) while minimum pollen germination was observed with 20 per cent sucrose solution. Among cultivars Earligrande (42.70 %) followed by Pratap (41.46 %) showed maximum pollen germination while minimum pollen germination was found in Royal Paradelux (36.36 %) Table 2.

Table 2: Pollen germination of peach cultivars with different concentration of sucrose.

Variety	10% sucrose (%)	15% sucrose (%)	20% sucrose (%)
Pratap	37.49 (37.73)	21.59 (27.66)	13.54 (21.87)
Earligrande	45.09 (42.16)	26.43 (30.91)	14.57 (22.72)
Flordaprince	40.71 (39.63)	23.15 (28.74)	11.95 (20.01)
Shan-i-Punjab	41.48 (40.08)	24.15 (29.39)	12.47 (20.66)
Glohaven	38.13 (38.11)	18.93 (25.75)	16.03 (23.59)
Royal Paradelux	35.36 (36.46)	12.46 (20.56)	10.92 (19.28)
Mean	39.71	21.12	12.76
$CD_{0.05}$	2.099	2.616	0.682

*Figures in parenthesis are angular transformed values.

Table 3: Pollen germination of peach in combination with sucrose and boric acid concentration.

Variety	S1 (10 % sucrose)			S2 (15 % sucrose)			S3 (20 % sucrose)		
	B1 (50ppm boric acid)	B2 (100ppm boric acid)	B3 (150ppm boric acid)	B1 (50ppm boric acid)	B2 (100ppm boric acid)	B3 (150ppm boric acid)	B1 (50ppm boric acid)	B2 (100ppm boric acid)	B3 (150 ppm boric acid)
Pratap	68.80 (65.02)	89.51 (71.33)	67.09 (54.99)	42.44 (40.63)	57.4 (49.27)	34.30 (35.81)	47.66 (43.64)	36.31 (39.12)	17.57 (22.56)
Earligrande	75.78 (60.51)	94.96 (77.25)	73.83 (59.25)	50.11 (45.04)	66.51 (54.62)	30.08 (33.23)	45.10 (42.17)	30.76 (29.41)	10.70 (19.51)
Flordaprince	77.29 (61.53)	96.40 (79.49)	62.31 (52.11)	45.99 (42.68)	55.87 (48.36)	43.27 (41.10)	57.69 (49.41)	33.47 (31.38)	15.86 (26.19)
Shan-i-Punjab	70.70 (57.21)	90.64 (72.34)	57.25 (49.15)	40.42 (39.46)	47.11 (43.32)	23.80 (29.18)	39.54 (38.93)	39.52 (28.69)	18.10 (21.00)
Glohaven	64.29 (53.29)	81.09 (64.22)	41.34 (39.99)	37.31 (37.62)	40.36 (39.42)	15.05 (16.61)	27.2 (31.35)	23.94 (27.34)	13.01 (18.30)
Royal Paradelux	61.68 (51.74)	76.79 (61.22)	38.56 (38.36)	30.28 (33.35)	38.72 (38.46)	8.31 (16.61)	16.59 (23.91)	21.19 (20.93)	9.76 (14.97)
Mean	69.75	88.23	56.73	41.09	50.99	25.80	38.96	30.86	14.16
$CD_{0.05}$	1.85	1.28	2.63	3.00	2.49	3.12	3.59	2.41	4.20

*Figures in parenthesis are angular transformed values.

CONCLUSION

In this experiment on pollen viability and pollen germination, different peach cultivars exhibited good results with respect to viability and germination of their pollen. Hence, it can be concluded that breeders should include these low chill varieties which can perform better in hybridization studies. The pollen viability and germination was found best in cultivars Floraprince and Earligrande which shows that these cultivars can act as pollinizers for pollination of commercially growing cultivars. The results indicate that varieties Earligrande and Flordaprince are the best performing varieties and can be utilized as male parents in the cross combinations in various breeding programmes. These studies have demonstrated the suitability of best performing varieties in hybridization and to understand the compatibility behavior of cultivars under study.

Conflict of Interest. Nil.

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