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Morphological and anatomical studies of pollen in peaches

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ABSTRACT

Analysis of the morphology and ultrastructure of pollen grains using scanning electron microscopy (SEM) has shown to be an effective method for identifying related genotypes of peach. The micromorphology of pollen grains of 15 peach genotypes using scanning electron microscopy (SEM) was studied. The anther colour of the accessions examined differed considerably, varying from red (11 Nos), dark red (Selection-12), creamish white (Sharbati Late), purple (Selection-1) and yellow (Selection-2) in colour. All the accessions and cultivars studied showed tricolporated pollens. Pant Peach-1 had the longest pollen (48.68 µm), and Sharbati Late had the shortest pollens (34.44 µm). The highest pollen width was noticed in IC-360680 (38.64 µm) and minimum in Sharbati Late (24.94 µm). All the peach cultivars displayed striate and tectum perforatum ornamentation regarding the surface features. Striae width ranged between 0.25 µm (Pratap) to 0.55 µm (IC-360680), while the highest groove width was noted in Pant Peach-1 (0.60 µm) and minimum in Selection-2 (0.19 µm).

Key words: *Prunus persica*, Anther colour, Pollen micromorphology, SEM

INTRODUCTION

Peach [*Prunus persica* (L.) Batsch] is a species of genus *Prunus* belonging to the family Rosaceae and subfamily Prunoideae and is diploid, with chromosomes number 2n = 2x = 16. It is the most popular stone fruit crop, with the largest number of existing commercial cultivars representing a diverse range of international germplasm (Forcada *et al*., 5). It ranks next to apples and pears in global trade among temperate fruits.

The genus *Prunus* presents many taxonomy challenges due to the species' high polymorphism, broad ecological tolerance, and immense number of cultivars (Donmez and Yildirimli, 3; Geraci *et al*., 6). Besides genetic makeup, the phenotype of any cultivar is influenced by various environmental factors, making taxonomic studies more complicated if there are no reliable biological and biochemical markers associated with that particular cultivar (Martinez-Gómez *et al*., 14). SEM (scanning electron microscopy) has proven to be a powerful tool for analyzing the morphology and ultrastructure of pollen grains to distinguish between related genotypes (Nikolic and Milatovic, 16). SEM plays a major role in understanding any horticultural crop's taxonomy, phylogeny and palaeobotany (Pospiech *et al*., 17; Lallawmzuali *et al*., 11).

Molecular markers and isozyme techniques have differentiated cultivars and hybridity confirmation in peaches (Kumar, 10). However, an integrated approach of utilizing morphological, isozymes and molecular analysis has proven most useful for taxonomic studies (Arzani *et al*., 1). In the current study, efforts were made to complement the lack of information on pollen microstructure concerning peach cultivars grown in India. Based on these considerations, the present investigation was undertaken to examine the size, shape and micromorphology of pollen grains of 15 cultivars of peach using SEM.

MATERIALS AND METHODS

The experimental materials comprised nine low and six high-chill peach cultivars/accessions (Table 1). The intense chill peach cultivars/accessions are maintained at Horticulture Research Center, Patharchatta of G. B. Pant University of Agriculture and Technology, Pantnagar, India, whereas high chill peach cultivars are maintained at Indian Council of Agricultural Research (ICAR)-National Bureau of Plant Genetic Resources (NBPGR), Regional Station (RS), Bhowali, Nainital, India. Pantnagar is geographically located at 29.5º North latitude and 79.3º East longitude in the *Tarai* belt at an altitude of 243.84 m a msl. The climate of the experimental site is humid subtropical with dry summers and cold winters. ICAR-NBPGR (RS), Bhowali is situated at 29.2º North latitude and 79.3º East longitude at 1600 m above MSL, where the climate is cold humid and sub-temperate.

Flower buds of respective cultivars/accessions were collected at the balloon stage in the spring season

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Table 1. List of peach germplasm used in micromorphology study.

Sr. No.	Peach germplasm maintained at Pantnagar	*IC/EC No.	Sr. No.	Peach germplasm maintained at Bhowali	*IC/EC No.
1.	Saharanpur Prabhat	-	10.	Ramgarh Selection	IC-360682
2.	Pratap	-	11.	Nishika	EC-38736
3.	Early Grand	-	12.	Paradelux Chapta	IC-247432
4.	Florda Prince	-	13.	Paradelux Gola	IC-320194
5.	Pant Peach-1	-	14.	Red June	IC-360680
6.	Sharbati Late	-	15.	Red June	IC-360683
7.	Selection-1	-			
8.	Selection-2	-			
9.	Selection-12	-			

*IC-Indigenous collection; EC-Exotic collection

of the year 2022, just before the beginning of anthesis, and their petals and sepals were removed. The colour of the anthers was visually observed after being removed from flower buds and placed in petri dishes. Petri dishes were kept in a desiccator at room temperature for 24 hours to release pollen from anthers. Silica gel was kept in the desiccator for the release of pollen grains. Then, dehisced pollen grains were processed for scanning electron microscopy (SEM).

The freshly collected pollen grains were mounted on the SEM (JEOL JSM-6610LV, Tokyo, Japan) specimen stubs. Pollen dispersion was prevented by placing double-sided sticky tape over the stubs of the SEM specimen. For gold coating, these stubs were kept inside the gold coater (Gold cotter – JSF.1600). Following that, pollen grains along with the stub were examined under the JEOL JSM-6610LV scanning electron microscope for photographic documentation as well as for examining the pollen grain sculpture.

The data were analysed to test the significance of differences between the means for different parameters through Analysis of Variance (ANOVA). Using Duncan's multiple range tests, significant differences were identified between the groups at $p<0.05$. Data analysis and computations were performed using IBM SPSS Statistics 19 (IBM, NY, USA).

RESULTS AND DISCUSSION

The anther colour differed considerably among the different accessions studied, which varied from red (Pant Peach-1, Florda Prince, Early Grand, Pratap, Saharanpur Prabhat, Ramgarh Selection (IC-360682), Nishika, Paradelux Chapta, Paradelux Gola, IC-360680 and IC-360683) to dark red (Selection-12) to creamish white (Sharbati Late), yellow (Selection-2) and purple (Selection-1) in colour (Fig. 1). In most of the accessions, the pollen shape

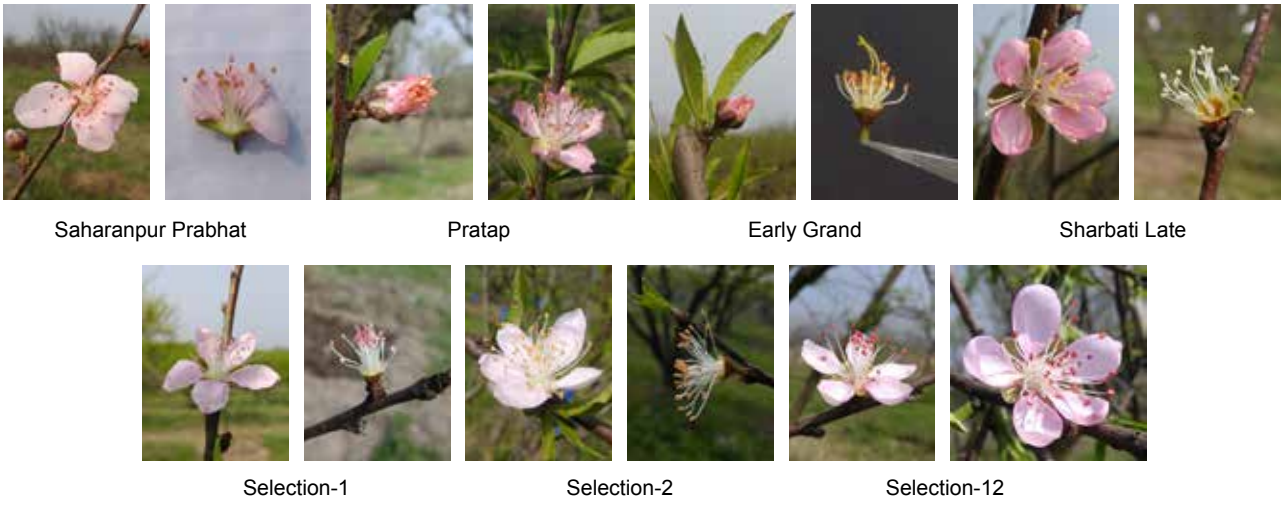

Fig. 1. Variability for anther colour among peach cultivars/accessions.

was tricolporate in the polar view, and elliptical in the equatorial view. In some cases, deviations were also observed (Paradelux Gola) (Fig. 2). Based on the colpus, pollens were categorised as tricolporate or pentocolporate. Colpus, also known as sulcus, refers to the elongated furrow or aperture in the pollen structure, which is the main identification feature of the pollen grains. Lang (12) stated that the pollen grain usually possesses three pores or colpi, which are evenly spaced around either the edge or the equator, depending on the view (polar or equatorial), and also described that in an aperture, exine layer would be either thin (independent of its patterning) or missing. In the case of a more circular aperture, it is referred to as a pore. The pollen with 3 pores, 3 compound apertures or 3 ectocolpi are described as tricolporate (Iversen and Troels-Smith, 8; Evrenosoğlu and Misirli, 4).

Most of the accessions were noted for the tricolporate pollen shape, also known as triporate and tricolpate (Iversen and Troels-Smith, 8). The differences in the pollen shape may also be attributed to the varying magnitude of hydration and sterility of grains in the different cultivars (Radice *et al*., 18). Evrenosoğlu and Misirli (4) noted cylindrical and tricolporate shaped pollen in different peach cultivars under study. Geraci *et al*. (6) examined pollen grains of five *Prunus* species and identified large-sized pollen grains in peaches with trizonocolpate shape. Goswami (7) also noticed tricolporate-shaped pollen grains in low chill cultivars of peaches grown under the Solan conditions of Himachal Pradesh.

The pollen length (polar view) ranged from 34.44 µm in Sharbati Late to 48.68 µm in Pant Peach-1, while the pollen width ranged from 24.94 µm (Sharbati Late) to 38.64 µm (IC-360680) (Table 2). The maximum width of pollen grains noted in IC-360680 differed significantly from Paradelux Gola, Selection-2, IC-360683, Selection-1 and Saharanpur Prabhat. The range of pollen length in the present study substantiates the findings of Goswami (7). However, it was lower than the value registered by Geraci *et al*. (6) and Chwil (2). The width of pollen grains estimated in the present study is in the range reported by Evrenosoğlu and Misirli (4). The length/ width ratio (1.06 to 1.48) noticed in the current study

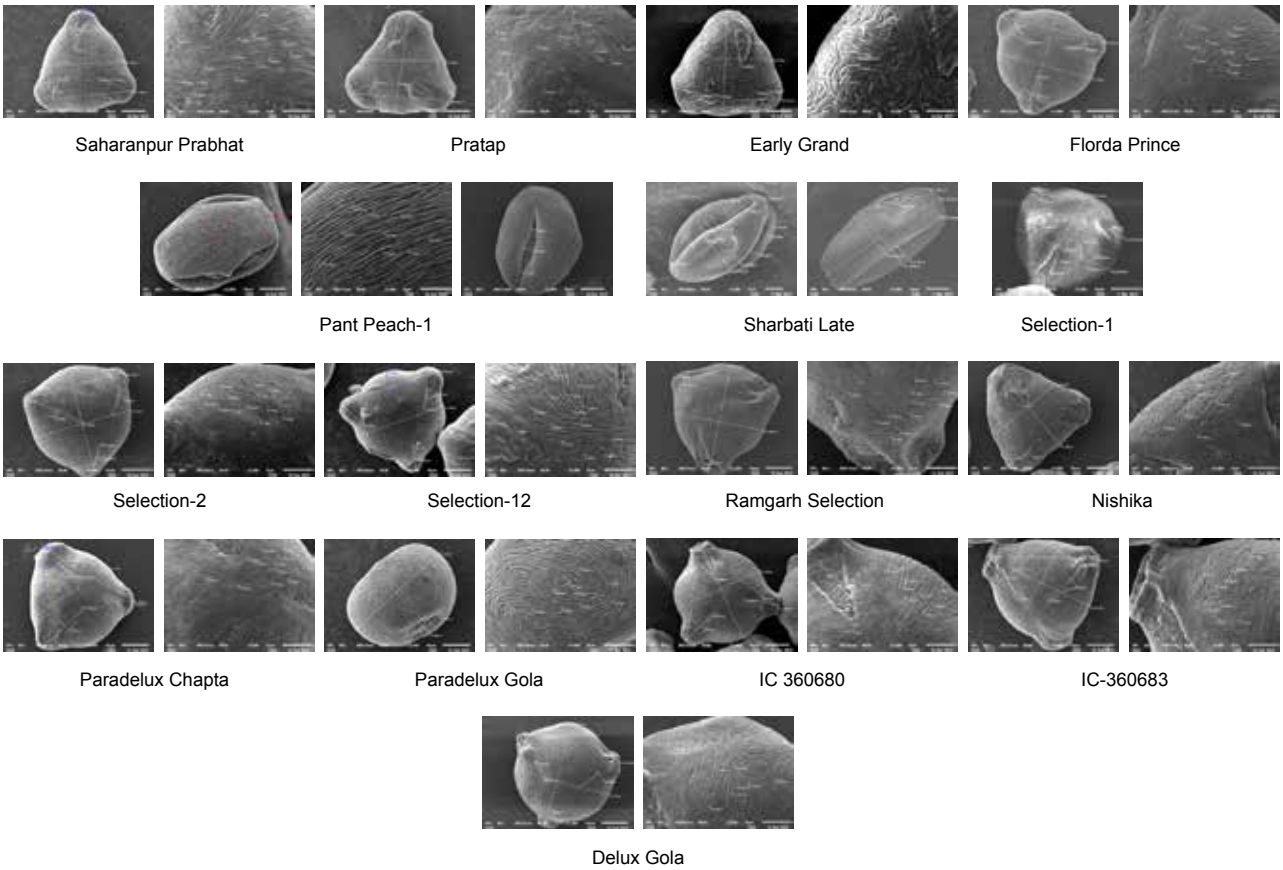

Fig. 2. Scanning electron micrograph of pollen grain of peach cultivars/accessions. Scale bars represent 10 µm for left photomicrographs and 5 µm for right one.

Table 2. Anther colour and micromorphological characters of pollen grain of peach cultivars/accessions.

Sr. No.	Cultivars/accessions	IC/EC No.*	Anther colour	Pollen shape	Pollen grain size (Polar view)		L/W ratio
					Length (µm)	Width (µm)	
1.	Saharanpur Prabhat	-	Red	Tricolporate	43.43^{b**}	35.16^{a}	1.25
2.	Pratap	-	Red	Tricolporate	38.45^{bcd}	31.12^{ab}	1.26
3.	Early Grand	-	Red	Tricolporate	41.19^{bc}	34.14^{a}	1.23
4.	Florda Prince	-	Red	Tricolporate	37.12^{cd}	32.76^{a}	1.15
5.	Pant Peach-1	-	Red	Tricolporate	48.68^{a}	33.21^{a}	1.48
6.	Sharbati Late	-	Creamish white	Tricolporate	34.44^{d}	24.94^{b}	1.39
7.	Selection-1	-	Purple	Tricolporate	37.59^{cd}	35.46^{a}	1.06
8.	Selection-2	-	Yellow	Tricolporate	38.52^{bcd}	35.97^{a}	1.07
9.	Selection-12	-	Dark red	Tricolporate	38.91^{bcd}	33.83^{a}	1.17
10.	Ramgarh Selection	IC-360682	Red	Tricolporate	42.27^{bc}	34.59^{a}	1.24
11.	Nishika	EC-38736	Red	Tricolporate	42.43^{bc}	34.37^{a}	1.26
12.	Paradelux Chapta	IC-247432	Red	Tricolporate	40.51^{bc}	34.25^{a}	1.20
13.	Paradelux Gola	IC-320194	Red	Tricolporate	41.15^{bc}	37.48^{a}	1.11
14.	Red June	IC-360680	Red	Tricolporate	42.43^{bc}	38.64^{a}	1.12
15.	Red June	IC-360683	Red	Tricolporate	42.45^{bc}	35.54^{a}	1.24

*IC-Indigenous collection & EC-Exotic collection, **Means with common letter shows non-significant differences (at p≤0.05) as per Duncan's multiple-range test

was lower than the values (1.76 and 2.02) obtained by Geraci *et al*. (6).

Concerning surface ornamentation, pollen grains of different cultivars exhibited striate sculpture of the exine, which displayed the extending parallel and irregular or sometimes fused pattern. It is evident from the data presented in Table 3 that the cultivars under study differ considerably for striae and groove width. Striae width ranged between 0.25 µm (Pratap) to 0.55 µm (IC-360680,) while the groove width varied from 0.19 µm (Selection-2) to 0.60 µm (Pant Peach-1). The maximum striae width estimated in IC-360680 was statistically *at par* with Selection-1. The minimum groove width was examined in Selection-2 with no significant difference over Paradelux Chapta, Florda Prince, Paradelux Gola, IC-360682, IC-360680 and IC-360683. More or less comparable results concerning the width of striae were documented by Chwil (2), and reported that it varies between 0.31 to 0.60 µm. The groove width (0.19 to 0.60 µm) was also found to be in agreement with the findings of Evrenosoğlu and Misirli (4); however, it was lower than the value determined by Chwil (2).

Nikolic and Milatovic (17) carried out the pollen SEM of five sour cherry cultivars. They revealed the differences among the cultivars for pollen grains length (48.0 to 52.9 µm), width (24.3 to 27.0 µm) and L/W ratio (1.91 to 2.02). In their study, Sotonyi *et al*. (22) demonstrated the importance of micromorphology studies for identifying fruit tree species and cultivars. They stated that the shape and number of pollen grains can differ depending on environmental conditions. Since most pollen micromorphology studies are conducted on dry grains, Radice *et al.* (18) argued that fresh pollen may vary in shape and sculpture from dry grains. Li *et al*. (13) described that pollen grain's polar diameters do not change much when dried, but due to polarity effects on the surface of the pollen grains and aperture depressions, their equatorial diameters may change.

Evrenosoglu and Misirli (4) performed SEM studies on peach cultivars and revealed that pollen grains surface showed tectum perforatum (located in the grooves) and striate ornamentation. Sotonyi *et al*. (19) also studied pollen's size and surface morphology in 87 varieties of 10 fruit species. They inferred that the surface morphology of pollens is controlled by genetic factors and research of this kind may assist in the classification and differentiation of various varieties. Chwil (2) witnessed that the sculpture of peach pollen grains depicts elongated, parallel, regular or irregular striae, which can be dichotomously branched.

Moore *et al.* (15) stated that the ornamentation over the surface of pollen grains is partitioned by grooves, which can be striate when extended and rugulate when elongated elements are arranged in an irregular pattern (longer than 1 µ). In the present

Table 3. Striae and grooves width of pollen grain of peach cultivars/accessions.

Sr. No.	Cultivars/accessions	IC/EC No.*	Width			
			Striae (µm)		Grooves (µm)	
			Min.– max.	Mean	Min.– max.	Mean
1.	Saharanpur Prabhat	-	0.29-0.36	0.33^{cdefg}	0.23-0.37	0.30^{bcd}
2.	Pratap	-	0.20-0.29	0.25^{g}	0.23-0.49	0.35^{bc}
3.	Early Grand	-	0.33-0.53	0.45^{abc}	0.23-0.54	0.39^{b}
4.	Florda Prince	-	0.29-0.32	0.31^{defg}	0.17-0.25	0.22^{cd}
5.	Pant Peach-1	-	0.31-0.43	0.39^{cdef}	0.56-0.68	0.60^{a}
6.	Sharbati Late	-	0.30-0.41	0.34^{cdefg}	0.32-0.37	0.34^{bc}
7.	Selection-1	-	0.50-0.57	0.53^{ab}	0.32-0.35	0.33^{bc}
8.	Selection-2	-	0.23-0.32	0.27^{fg}	0.14-0.23	0.19^{d}
9.	Selection-12	-	0.37-0.46	0.42^{bcd}	0.34-0.47	0.40^{b}
10.	Ramgarh Selection	IC-360682	0.28-0.34	0.32^{defg}	0.22-0.36	0.27^{bcd}
11.	Nishika	EC-38736	0.36-0.45	0.41^{bcde}	0.25-0.40	0.35^{bc}
12.	Paradelux Chapta	IC-247432	0.24-0.31	0.27^{fg}	0.18-0.26	0.21^{cd}
13.	Paradelux Gola	IC-320194	0.24-0.34	0.29^{efg}	0.20-0.31	0.26^{bcd}
14.	Red June	IC-360680	0.45-0.70	0.55^{a}	0.23-0.29	0.27^{bcd}
15.	Red June	IC-360683	0.28-0.52	0.40^{cde}	0.20-0.38	0.29^{bcd}

*IC-Indigenous collection & EC-Exotic collection, **Means with common letter shows non-significant differences (at p≤0.05) as per Duncan's multiple-range test

study, perforation was examined in the tectum of the pollen grains, which corroborates the findings of Geraci *et al.* (6) and Chwil (2). Perforations are the holes (<1 µ in diameter) distributed over the tectum of the pollen grains and is referred to as tectum perforatum, and if there is no perforation, it is termed as tectum imperforatum (Iversen and Troels- Smith, 8; Walker and Doyle, 20).

Significant diversity in pollen grain micromorphology was observed among studied peach cultivars using scanning electron microscopy (SEM). Such micromorphology studies can be useful at the first stage of identification of any crop species and cultivars due to their ease of use and cost effectiveness compared to other more detailed methods. In the present study, anther colour showed a great deal of variation among different cultivars/ accessions, which can be used as a morphological marker for cultivars and species identification across the genus *Prunus*. However, the possible linkage between the anther colour and any trait of interest needs to be further explored for breeding new desirable peach cultivars using the advanced breeding technique.

AUTHORS' CONTRIBUTION

Conceptualization of research (DCD, RK); Designing of the experiments (DCD, RK); Execution of field/lab experiments and data collection (RK, SNS, NC); Analysis of data and interpretation (RK, JSS); Preparation of the manuscript (RK, JSS).

DECLARATION

The authors declare that there is no conflict of interest.

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