Indian Journal of Ecology (2024) 51(2): 353-363
DOI: https://doi.org/10.55362/IJE/2024/4242

Manuscript Number: 4242
NAAS Rating: 5.38

Studies on Pollination, Fruit Set and Self-Incompatibility Index in Apple Varieties under Wet Temperate Conditions of Himachal Pradesh

Nikhil Kaushal, Dinesh S Thakur[1], Neena Chauhan[1], R.K. Dogra, Akriti Chauhan[1] and Girish Dangi[*]

Department of Fruit Science, Dr. Y S Parmar University of Horticulture & Forestry, Nauni, Solan-173 230, India
[1]Regional Horticultural Research & Training Station, Mashobra, Shimla-171 007, India
**E-mail: girishdangi@yspuniversity.ac.in*

Abstract: In the present study, floral biology, the extent of fruit set under different modes of pollination, the relationship between fruit shape and seed number, and the compatibility status among different apple varieties were studied. Fruit characters and yield are directly influenced by floral characteristics, flowering time, flowering duration and pollen fertility. Flowering duration varied from 10 days (Scarlet Spur II and Schlect Spur) to 15 days (Granny Smith). Pollen viability ranged from 72.43 per cent (Ace Spur) to 96.83 per cent (Coe Red Fuji) in 1 per cent acetocarmine solution, and from 76.21 per cent (Schlect Spur) to 90.85 per cent (Gale Gala) in 0.04 percent erythrosine B. The findings showed that, Gale Gala and Granny Smith were superior pollinizers. Seed number was maximum (9.40) under hand/controlled pollination and minimum (2.40) under bagging conditions. When the seed number was low, there was a higher percentage of lopsided fruits, and vice versa. All the main varieties were completely self-incompatible, whereas the pollinizing varieties were found to be partially as well as fully self-compatible. It was concluded that a cross compatible variety is required for optimum fruit set in apples, and that the number of seeds in fruit has a direct effect on its shape, size and weight.

Keywords: Floral Biology, Lopsidedness, *Malus × domestica* Borkh, Pollination, Self-incompatibility

The cultivated apple (*Malus × domestica* Borkh.) is a major commercial crop in temperate regions of the world. The total area under apple cultivation in India is 3,15,000 hectares with a production of 25,89,000 metric tonnes, whereas, in Himachal Pradesh the area is 1,15,020 hectares with a production of 6,11,900 metric tonnes (Anonymous 2022). In apple, the pollinizing ability of any variety is determined by its floral characteristics, duration of flowering, fertility and germination of pollen grains. The low productivity of apples might be due to a variety of factors including climate, soil characters, the occurrence of pests and diseases, a lack of pollination and a lack of synchronization among the flowering periods.

In apples, cross pollination is a very important aspect for quality fruit production since it contains a complex biochemical mechanism that prevents the ovules from being fertilized by its own pollen (Hua et al 2008). This gametophytic incompatibility mechanism is genetically controlled by a single locus, i.e., the S-locus, which includes at least two polymorphic and strongly linked S-determinant genes: a pistil-expressed *S-RNase* gene and a number of pollen-expressed *SFBB* genes i.e., S-locus F-Box Brothers (Claessen et al 2019). The self or non-self-recognition process and the acceptance or rejection takes place between the protein products of these genes. The varieties with the same S-genotype are mutually incompatible because self S-RNase can stop the pollen tube expansion, resulting in fertilisation failure, and so their mating will not result in a progeny. Due to this self-incompatibility mechanism, in apples, majority of cultivars exhibit a higher level of allelic heterozygosity, are thus not true to type and ultimately result in fruits of poor size, shape and quality (Webster and Wertheim 2003). Furthermore, the number and distribution of seeds are also important in producing marketable apples with better size and shape. The hormones that are produced by developing seeds influence the growth in the tissues, which ultimately leads to better shape and size of the fruit (Sheffield 2014).

The lack of pollination leads to very less or no fertilization, thus producing lopsided and unmarketable fruits. For these reasons, it has become evident that there is an urgent need for the identification of suitable pollinizing cultivars to counter the problem of inadequate pollination, which ultimately leads to inferior quality and poor yield. Furthermore, knowledge about the floral behaviour of different varieties of apples might be important in relation to different climatic conditions for breeding new varieties suitable for different climatic conditions. So far, very limited data is available with respect to pollen viability and *in-vitro* pollen germination in the varieties undertaken for the present study. Furthermore, the viability and germination of a wide range of varieties across a wide range of environmental conditions also remain to be investigated. The objective of study was to identify variety with better pollinizing abilities to resist the problem of low productivity in apples.

MATERIAL AND METHODS

The present investigations were carried out during the years 2020 and 2021 at the Regional Horticultural Research and Training Station, Mashobra, Shimla (elevation: 2286 m above mean sea level; latitude 31.1°N; and longitude 77.1°E). The six commercial varieties *viz*. Scarlet Spur II, Oregon Spur II, Early Red One, Super Chief, Ace Spur and Schlect Spur along with four pollinizing varieties *viz.* Gale Gala, Granny Smith, Coe Red Fuji and Crimson Gala were taken. Flower length, breadth, length of sepals, petals, pistil and ovary diameter were measured. The colour of sepals, petals, anthers and the flower at balloon stage were recorded using colour chart of Royal Horticultural Society, London. The date on which 5-10 per cent flowers had opened was recorded as the date of initiation of flowering, whereas the date on which about 75 per cent flowers had opened was recorded as the date of full bloom. The date on which the last flower opened in each variety was recorded as the time of opening of last flower and the number of days from date of initiation of flowering to the date of opening of last flower in each variety was recorded as the duration of flowering. Pollen viability was observed in 1 per cent acetocarmine solution and 0.04 per cent erythrosine B solutions and *in vitro* pollen germination was observed in 10 per cent sucrose + 0.5 per cent agar solutions.

To investigate fruit characteristics, a total of 15 representative fruit samples were carefully selected at their peak ripeness. These samples were divided into three sets, with each set containing five fruit replicates. For precise measurements of fruit dimensions, a Digital Vernier Calipers (Model No. CD-6” CS) was employed to record the length and breadth of each fruit in millimeters (mm). Furthermore, a visual inspection of the fruits was conducted to categorize them as either symmetrical or asymmetrical based on their overall shape and appearance. The weight of the fruits resulting from various pollination methods was determined. To assess the fruit's physical characteristics more comprehensively, each fruit was cut in half lengthwise. The depth of the fruit basin was then measured using a measuring scale and categorized as either shallow, medium, or deep based on the depth observed. Seed number in each fruit was assessed by slicing the apples horizontally through the equatorial plane and counting the number of fully developed seeds. The self-incompatibility index was calculated with the values of seed set obtained in self-pollination and cross pollination treatments (Moriya et al 2005). The values of self-incompatibility index range from 0 to 1, where 0 indicates complete self-incompatibility and 1 indicates a fully self-compatible species. A self-incompatibility index of > 0.75 clearly differentiates between fully and partially self-compatible species, whereas an index of < 0.20 is related to fully incompatible species (Castro et al 2016). The cross-compatibility of a particular cross combination was established according to Maliga (1953), cited by Nyeki (1996).

a, b, c, d and e cultivars that can fertilize another to a

a. negligible extent (fruit set ≥0% and <2%)

b. slight extent (fruit set ≥2% and <10%)

c. medium extent (fruit set ≥10% and<20%)

d. high extent (fruit set ≥20% and <30%)

e. large extent (fruit set ≥30%).

RESULTS AND DISCUSSION

Floral biology: The average flower length and breadth were at 38.60 mm and 34.87 mm (Table 1). Majid (2003) also observed variations in flower diameter from 3.6 cm to 5.3 cm. Pandit (2014) recorded flower diameter in the range of 4.00 cm to 5.30 cm. The number of sepals and petals was five in all the varieties under study. The maximum (19.50) number of stamens in all varieties was in Granny Smith and minimum (17.00) in Scarlet Spur II. However, Dennis (2003) reported stamen numbers varying from 20 to 25 among different apple varieties. Pistil length varied from 10.70 mm (Early Red One) to 15.57 mm (Super Chief). Sepal length was maximum (7.66 mm) in Early Red One and minimum (5.99 mm) in Coe Red Fuji. However, the maximum petal length (23.15 mm) was in Super Chief and minimum (14.95 mm) in Ace Spur. Maximum (2.63 mm) ovary diameter was in Oregon Spur II which was statistically at par with Super Chief, Schlect Spur and Crimson Gala and minimum (2.08 mm) in Coe Red Fuji. Anand (2003) observed variations in sepal and pistil lengths from 0.55 - 0.82 cm and 0.63 - 1.36 cm, respectively. Chauhan (2018) also observed variation in pistil length from 0.69 cm to 0.91 cm. These variations in floral parameters might be due to varietal differences (Monteiro et al 2015, Dangi et al 2021).

The flower initiation was earliest in Crimson Gala (26th March), however, Schlect Spur was the last to initiate flowering i.e. on 12th April. Full bloom was earliest in Crimson Gala (31st March) and Schlect Spur was the last to come into full bloom on 16th April. The date of opening of last flower varied from 9th April to 22ndApril, Crimson Gala and Ace Spur being the earliest and Schlect Spur being the last. Earliest

Table 1. Floral characters of different apple varieties

Variety	Flower length (mm)	Flower breadth (mm)	Arrangement of petals	Colour of sepals	Colour of petals	Colour of anthers	Colour of flowers at balloon stage	Number of sepals	Number of petals	Number of stamens	Length of sepal (mm)	Length of petal (mm)	Length of pistil (mm)	Ovary diameter (mm)
Scarlet Spur-II	36.19	33.36	Touching	Green group 139 B	White group 155 B	Yellow group 5 C	Red-Purple group 58 D	5	5	17 (15-19)	6.33	17.21	12.16	2.33
Oregon Spur II	40.46	35.74	Touching	Yellow-Green group 144 B	White group N 155 D	Yellow group 10 D	Red-Purple group 64 C	5	5	18.5 (17-20)	6.73	17.45	11.25	2.63
Early Red One	40.47	36.70	Touching	Yellow-Green group 144 B	Red-Purple group N 74 D	Yellow group 8 C	Red-Purple group 58 C	5	5	17.3 (16-20)	7.66	19.67	10.70	2.21
Super Chief	46.41	39.59	Touching	Green group 138 C	White group N 155 C	Yellow group 10 C	Red-Purple group 59 D	5	5	18 (15-20)	6.24	23.15	15.57	2.45
Ace Spur	35.29	32.91	Touching	Green group 139 D	White group 155 B	Yellow group 4 C	Red-Purple group N 66 C	5	5	18.7 (16-20)	7.27	14.95	10.84	2.25
Schlect Spur	35.88	32.41	Touching	Yellow-Green group 144 B	White group 155 B	Yellow group 5 D	Red-Purple group 64 C	5	5	18.8 (17-20)	7.54	21.06	12.47	2.38
Gale Gala	36.69	34.28	Apart	Green group 139 D	Red-Purple group N 66 C	Greyed-Yellow group 162 D	Red-Purple group 63 B	5	5	18.3 (17-20)	7.18	18.32	13.49	2.17
Granny Smith	43.44	40.84	Touching	Green group 139 D	White group N 155 B	Greyed Orange group 165 B	Red-Purple group 60 A	5	5	19.5 (19-20)	6.60	21.75	15.35	2.33
Coe Red Fuji	36.37	31.02	Apart	Green group 139 D	White group N 155 D	Yellow group 5 D	White group 155 C	5	5	18.3 (17-20)	5.99	15.68	14.25	2.08
Crimson Gala	34.79	31.82	Apart	Green group 139 D	Greyed-Purple group 186 C	Yellow group 2 C	Red-Purple group 58 B	5	5	18.5 (17-20)	7.61	16.76	12.65	2.55
Mean	38.60	34.87						5	5	18.29	6.92	18.60	12.87	2.34
CD at 5 %	0.02	0.03									0.34	0.61	0.38	0.25

petal fall was observed in varieties Crimson Gala and Ace Spur (13th April), however, the last variety to show petal fall was Schlect Spur (25th April). These differences in dates of flowering might be due to their genetic characteristics or their responses to light and prevailing temperature. The longest flowering duration (15 days) was in Granny Smith, whereas, the shortest duration (10 days) was observed in Scarlet Spur II and Schlect Spur (Table 2, Fig. 1). Singh et al (2002) observed flowering duration to vary between 10 and 15 days among different apple cultivars. Sharma et al (2017) reported duration of flowering to vary from 12 to 16 days. The longest (20 days) duration of flowering was observed in Granny Smith and shortest (11 days) in Scarlet Spur II (Verma and Thakur 2019). These variations in time and duration of flowering might be due to differences in cultural practices, environmental conditions and chilling requirements (Dangi et al 2024) of different apple cultivars, which help in breaking bud dormancy and regulating flowering.

Pollen viability and *in vitro* pollen germination: In 1 per cent acetocarmine solution, Coe Red Fuji showed the highest pollen viability (96.83%) and Ace Spur the lowest (72.43%). However, in 0.04 per cent erythrosine B, pollen viability varied from 76.21 per cent (Schlect Spur) to 90.85 per cent (Gale Gala) (Table 2). The observed variations in pollen viability percentage might be due to the prevailing climatic conditions, which may affect the physiology of the pollen grains (Singh et al 2002). Rather et al (2018) observed pollen viability in 1 per cent acetocarmine solution ranged from 64.50 to 100.00 per cent, with Granny Smith achieving 97.94 per cent and Coe Red Fuji achieving 97.53 per cent.

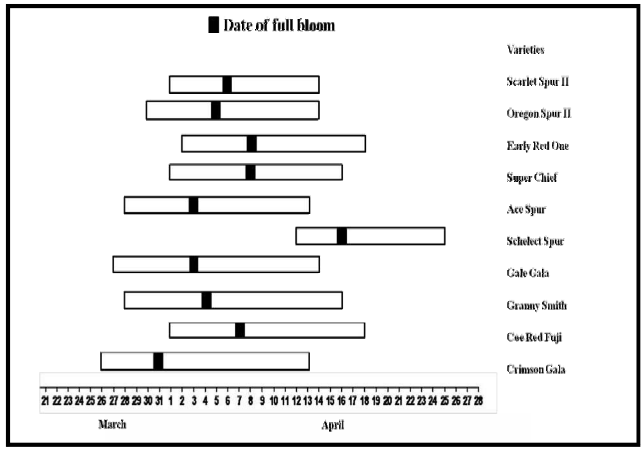

Fig. 1. Time and duration of flowering in different apple varieties (from initiation of flowering to petal fall)

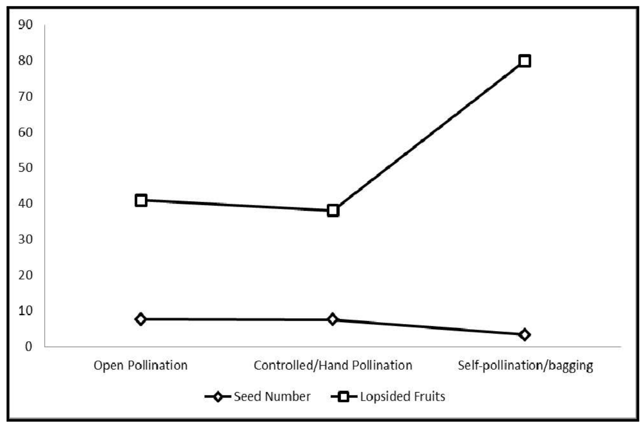

Fig. 2. Relationship between seed number and degree of lopsidedness under different modes of pollination

Table 2. Time, duration of flowering, pollen viability and *in vitro* germination of different apple varieties

Variety	Time of initiation of flowering	Time of full bloom	Time of opening of last flower	Total duration of flowering (days)	Time of petal fall	Pollen viability (%)		*In vitro* pollen germination in 10 % sucrose + 0.5 % agar (%)
						Acetocarmine (1%)*	Erythrosine B (0.04%)*	
Scarlet Spur II	01st April	06th April	11th April	10	14th April	82.03 (9.11)	80.53 (9.03)	39.82
Oregon Spur II	30th March	05th April	10th April	11	14th April	80.37 (9.02)	80.42 (9.02)	40.27
Early Red One	02nd April	08th April	14th April	12	18th April	79.36 (8.96)	77.18 (8.84)	42.51
Super Chief	01st April	08th April	13th April	12	16th April	77.78 (8.88)	78.67 (8.93)	39.24
Ace Spur	28th March	03rd April	09th April	12	13th April	72.43 (8.57)	77.41 (8.86)	44.29
Schlect Spur	12th April	16th April	22nd April	10	25th April	74.52 (8.69)	76.21 (8.79)	45.70
Gale Gala	27th March	03rd April	10th April	14	14th April	95.96 (9.85)	90.85 (9.58)	59.74
Granny Smith	28th March	04th April	12th April	15	16th April	95.66 (9.83)	88.25 (9.45)	64.94
Coe Red Fuji	01st April	07th April	14th April	13	18th April	96.83 (9.89)	88.97 (9.46)	62.29
Crimson Gala	26th March	31st March	09th April	14	13th April	89.40 (9.51)	90.05 (9.54)	57.01
Mean						84.43 (9.23)	82.86 (9.15)	49.58
CD (P=0.05)						(0.05)	(0.18)	02.67

*Figure in parenthesis is square root (*) transformed

Table 3. Pollination treatments applied to main apple varieties under study, fruit set, retention, number of seeds/fruit, SI index and compatibility status

Treatment	Female variety	Male variety	Fruit set (%)	Fruit retention (%)	Number of seeds/fruit	Degree of lopsidedness (%)*	SI Index	Compatibility status
Scarlet Spur II								
Open Pollination	Scarlet Spur II	-	85.28	45.45	7.32	40.00 (39.22)	-	-
Self-pollination/Bagging	Scarlet Spur II	Scarlet Spur II	12.66	0.00	0.00	0.00 (0.00)	0.00	Complete self-incompatible
Cross pollination	Scarlet Spur II	Gale Gala	36.42	66.66	9.00	13.33 (21.39)	-	Cross-compatible to a large extent
Cross pollination	Scarlet Spur II	Granny Smith	65.75	62.50	7.60	33.33 (35.25)	-	Cross-compatible to a large extent
Cross pollination	Scarlet Spur II	Coe Red Fuji	51.88	37.50	8.00	26.67 (31.08)	-	Cross-compatible to a large extent
Cross pollination	Scarlet Spur II	Crimson Gala	21.71	21.67	6.20	46.67 (43.07)	-	Cross-compatible to a high extent
Oregon Spur II								
Open Pollination	Oregon Spur II	-	59.69	69.23	8.10	26.67 (31.08)	-	-
Self-pollination/Bagging	Oregon Spur II	Oregon Spur II	4.91	0.00	0.00	0.00 (0.00)	0.00	Complete self-incompatible
Cross pollination	Oregon Spur II	Gale Gala	0.00	0.00	0.00	0.00 (0.00)	-	Cross-compatible to a negligible extent
Cross pollination	Oregon Spur II	Granny Smith	15.25	100.00	7.33	40.00 (39.21)	-	Cross-compatible to a medium extent
Cross pollination	Oregon Spur II	Coe Red Fuji	0.00	0.00	0.00	0.00 (0.00)	-	Cross-compatible to a negligible extent
Cross pollination	Oregon Spur II	Crimson Gala	4.69	100.00	9.00	13.33 (21.39)	-	Cross-compatible to a slight extent
Early Red One								
Open Pollination	Early Red One	-	75.16	78.95	7.68	40.00 (39.22)	-	-
Self-pollination/Bagging	Early Red One	Early Red One	5.89	0.00	0.00	0.00 (0.00)	0.00	Complete self-incompatible
Cross pollination	Early Red One	Gale Gala	10.78	100.00	8.00	33.33 (35.25)	-	Cross-compatible to a medium extent
Cross pollination	Early Red One	Granny Smith	25.14	66.67	9.00	13.33 (21.39)	-	Cross-compatible to a high extent
Cross pollination	Early Red One	Coe Red Fuji	26.78	20.00	8.00	26.67 (31.08)	-	Cross-compatible to a high extent
Cross pollination	Early Red One	Crimson Gala	51.76	50.00	8.00	26.67 (31.08)	-	Cross-compatible to a large extent
Super Chief								
Open Pollination	Super Chief	-	78.14	74.19	7.25	40.00 (39.22)	-	-
Self-pollination/Bagging	Super Chief	Super Chief	7.53	0.00	0.00	0.00 (0.00)	0.00	Complete self-incompatible
Cross pollination	Super Chief	Gale Gala	57.58	0.00	0.00	0.00 (0.00)	-	Cross-compatible to a large extent
Cross pollination	Super Chief	Granny Smith	11.43	50.00	6.00	53.33 (46.89)	-	Cross-compatible to a medium extent
Cross pollination	Super Chief	Coe Red Fuji	66.88	11.11	6.00	53.33 (46.89)	-	Cross-compatible to a large extent
Cross pollination	Super Chief	Crimson Gala	13.54	0.00	0.00	0.00 (0.00)	-	Cross-compatible to a medium extent
Ace Spur								
Open Pollination	Ace Spur	-	55.61	85.71	6.48	53.33 (46.89)	-	-
Self-pollination/Bagging	Ace Spur	Ace Spur	15.99	0.00	0.00	0.00 (0.00)	0.00	Complete self-incompatible
Cross pollination	Ace Spur	Gale Gala	18.57	0.00	0.00	0.00 (0.00)	-	Cross-compatible to a medium extent
Cross pollination	Ace Spur	Granny Smith	28.45	66.67	6.30	46.67 (43.07)	-	Cross-compatible to a high extent
Cross pollination	Ace Spur	Coe Red Fuji	28.54	50.00	6.50	40.00 (39.21)	-	Cross-compatible to a high extent
Cross pollination	Ace Spur	Crimson Gala	60.82	14.29	8.00	26.67 (31.08)	-	Cross-compatible to a large extent
Schlect Spur								
Open Pollination	Schlect Spur	-	56.13	33.33	7.70	26.67 (31.08)	-	-
Self-pollination/Bagging	Schlect Spur	Schlect Spur	2.75	0.00	0.00	0.00 (0.00)	0.00	Complete self-incompatible
Cross pollination	Schlect Spur	Gale Gala	100.00	50.00	7.00	33.33 (35.25)	-	Cross-compatible to a large extent
Cross pollination	Schlect Spur	Granny Smith	54.94	25.00	9.00	13.33 (21.39)	-	Cross-compatible to a large extent
Cross pollination	Schlect Spur	Coe Red Fuji	57.30	0.00	0.00	0.00 (0.00)	-	Cross-compatible to a large extent
Cross pollination	Schlect Spur	Crimson Gala	62.59	50.00	8.00	13.33 (21.39)	-	Cross-compatible to a large extent

*Figure in parenthesis is angular (*) transformed

Table 4. Pollination treatments applied to different pollinizing apple varieties under study, fruit set, retention, number of seeds/fruit, SI index and compatibility status

Treatment	Female variety	Male variety	Fruit set (%)	Fruit retention (%)	Number of seeds/fruit	Degree of lopsidedness (%)*	SI Index	Compatibility status
Gale Gala								
Open Pollination	Gale Gala	-	70.80	72.13	8.32	20.00 (26.55)	-	-
Self-pollination/bagging	Gale Gala	Gale Gala	22.00	75.00	3.00	80.00 (63.41)	0.38	Partially self-compatible
Cross pollination	Gale Gala	Scarlet Spur II	30.86	100.00	7.33	40.00 (39.21)	-	Cross compatible to a large extent
Cross pollination	Gale Gala	Oregon Spur II	48.43	100.00	6.80	40.00 (39.21)	-	Cross compatible to a large extent
Cross pollination	Gale Gala	Early Red One	12.14	100.00	9.00	6.67 (14.90)	-	Cross compatible to a medium extent
Cross pollination	Gale Gala	Super Chief	50.27	40.00	6.30	46.67 (43.07)	-	Cross compatible to a large extent
Cross pollination	Gale Gala	Ace Spur	27.09	50.00	6.40	46.67 (43.07)	-	Cross compatible to a high extent
Cross pollination	Gale Gala	Schlect Spur	36.46	40.00	7.33	33.33 (35.25)	-	Cross compatible to a large extent
Cross pollination	Gale Gala	Granny Smith	46.78	90.00	9.00	13.33 (21.39)	-	Cross compatible to a large extent
Cross pollination	Gale Gala	Coe Red Fuji	31.56	80.00	9.40	6.67 (14.90)	-	Cross compatible to a large extent
Cross pollination	Gale Gala	Crimson Gala	27.85	16.67	9.00	20.00 (26.54)	-	Cross compatible to a high extent
Granny Smith								
Open Pollination	Granny Smith	-	80.53	50.00	7.72	26.67 (31.08)	-	-
Self-pollination/bagging	Granny Smith	Granny Smith	27.84	40.00	4.50	66.67 (54.72)	0.87	Fully self-compatible
Cross pollination	Granny Smith	Scarlet Spur II	51.91	0.00	0.00	0.00 (0.00)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Oregon Spur II	59.70	0.00	0.00	0.00 (0.00)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Early Red One	45.95	50.00	7.23	40.00 (39.21)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Super Chief	50.53	100.00	6.80	40.00 (39.21)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Ace Spur	33.69	50.00	9.00	6.67 (14.90)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Schlect Spur	45.40	0.00	0.00	0.00 (0.00)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Gale Gala	64.76	55.45	8.25	20.00 (26.54)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Coe Red Fuji	70.42	55.56	7.50	26.67 (31.08)	-	Cross compatible to a large extent
Cross pollination	Granny Smith	Crimson Gala	23.61	50.00	8.00	26.67 (31.08)	-	Cross compatible to a high extent
Coe Red Fuji								
Open Pollination	Coe Red Fuji	-	91.56	77.77	7.85	33.33 (35.25)	-	-
Self-pollination/bagging	Coe Red Fuji	Coe Red Fuji	50.07	66.67	3.20	73.33 (58.88)	0.46	Partially self-compatible
Cross pollination	Coe Red Fuji	Scarlet Spur II	50.10	100.00	6.40	46.67 (43.07)	-	Cross compatible to a large extent
Cross pollination	Coe Red Fuji	Oregon Spur II	75.85	66.67	6.50	40.00 (39.21)	-	Cross compatible to a large extent
Cross pollination	Coe Red Fuji	Early Red One	49.96	100.00	6.40	46.67 (43.07)	-	Cross compatible to a large extent
Cross pollination	Coe Red Fuji	Super Chief	66.86	20.00	7.00	40.00 (39.21)	-	Cross compatible to a large extent
Cross pollination	Coe Red Fuji	Ace Spur	28.79	50.00	6.50	40.00 (39.21)	-	Cross compatible to a high extent
Cross pollination	Coe Red Fuji	Schlect Spur	47.59	83.33	6.60	33.33 (35.25)	-	Cross compatible to a large extent
Cross pollination	Coe Red Fuji	Gale Gala	52.57	75.00	8.60	20.00 (26.54)	-	Cross compatible to a large extent
Cross pollination	Coe Red Fuji	Granny Smith	36.39	85.71	8.75	26.67 (31.08)	-	Cross compatible to a large extent
Cross pollination	Coe Red Fuji	Crimson Gala	23.94	33.33	6.50	40.00 (39.21)	-	Cross compatible to a high extent
Crimson Gala								
Open Pollination	Crimson Gala	-	77.60	85.29	8.00	26.67 (31.08)	-	-

Cont...

Table 4. Pollination treatments applied to different pollinizing apple varieties under study, fruit set, retention, number of seeds/fruit, SI index and compatibility status

Treatment	Female variety	Male variety	Fruit set (%)	Fruit retention (%)	Number of seeds/fruit	Degree of lopsidedness (%)*	SI Index	Compatibility status
Self-pollination/bagging	Crimson Gala	Crimson Gala	21.79	75.00	2.40	86.67 (68.56)	0.41	Partially self-compatible
Cross pollination	Crimson Gala	Scarlet Spur II	56.16	0.00	0.00	0.00 (0.00)	-	Cross compatible to a large extent
Cross pollination	Crimson Gala	Oregon Spur II	41.64	75.00	6.60	40.00 (39.21)	-	Cross compatible to a large extent
Cross pollination	Crimson Gala	Early Red One	45.86	12.50	8.00	20.0 (26.54)	-	Cross compatible to a large extent
Cross pollination	Crimson Gala	Super Chief	47.58	42.86	6.80	46.67 (43.07)	-	Cross compatible to a large extent
Cross pollination	Crimson Gala	Ace Spur	26.83	33.33	8.00	20.00 (26.54)	-	Cross compatible to a high extent
Cross pollination	Crimson Gala	Schlect Spur	64.41	33.33	8.00	20.00 (26.54)	-	Cross compatible to a large extent
Cross pollination	Crimson Gala	Gale Gala	41.56	0.00	0.00	0.00 (0.00)	-	Cross compatible to a large extent
Cross pollination	Crimson Gala	Granny Smith	30.70	71.43	8.80	13.33 (21.39)	-	Cross compatible to a large extent
Cross pollination	Crimson Gala	Coe Red Fuji	67.12	47.12	7.00	33.33 (35.25)	-	Cross compatible to a large extent

*Figure in parenthesis is angular (*) transformed

Table 5. Effect of open pollination and bagging on fruit characters of different apple varieties

Variety	Under open pollination condition				Under bagging condition			
	Fruit length (mm)	Fruit breadth (mm)	Fruit weight (g)	Depth of basin (cm)	Fruit length (mm)	Fruit breadth (mm)	Fruit weight (g)	Depth of basin (cm)
Scarlet Spur II	59.61	65.51	122.37	0.80	0.00	0.00	0.00	0.00
Oregon Spur II	59.59	66.56	124.71	1.00	0.00	0.00	0.00	0.00
Early Red One	60.57	65.43	125.67	0.80	0.00	0.00	0.00	0.00
Super Chief	64.66	68.39	155.45	0.90	0.00	0.00	0.00	0.00
Ace Spur	58.71	58.47	104.11	0.60	0.00	0.00	0.00	0.00
Schlect Spur	66.28	73.37	135.55	1.10	0.00	0.00	0.00	0.00
Gale Gala	63.55	71.31	163.55	0.90	52.58	56.20	98.63	0.60
Granny Smith	64.66	72.59	162.42	0.70	64.24	76.20	154.61	0.60
Coe Red Fuji	57.39	67.23	131.69	0.50	59.44	66.91	130.56	0.30
Crimson Gala	55.37	63.17	139.92	0.60	39.21	61.73	68.73	0.50
Mean	61.04	67.20	136.54	0.79	53.87	65.26	113.13	0.50
CD (p=0.05)	0.48	0.52	0.36	0.001	0.05	1.02	0.57	0.10

Singh et al (2002) observed that pollen viability in 0.04 per cent erythrosine B ranged from 70.70 per cent to 90.32 per cent. *In vitro* pollen germination in 10 per cent sucrose + 0.5 per cent agar was found to range between 39.24 per cent and 64.94 per cent with maximum in Granny Smith and minimum in Super Chief. These variations in pollen germination might be due to the genetic constitution of pollen producing varieties, which influences the hormone level and its distribution which ultimately affects the physiology of pollen grains and thereby cause variations in viability and germination percentage (Kotiyal and Dimri 2017). In agreement with our present findings, Javid (2015) also observed that *in vitro* pollen germination ranged from 49.75 per cent (Manchurian) to 63.00 per cent (*Malus floribunda)*. However, Rather et al (2018) and Ahad et al (2020) reported *in vitro* pollen germination ranging between 44.24 per cent (Oregon Spur) and 64.54 per cent (Granny Smith) in 15 per cent sucrose solution. Higher values of pollen viability and germination in different apple varieties may be attributed to pollen fertility as a result of regular meiosis and the activation of certain enzyme systems present in the pollen itself (Nautiyal and Dimri 2009). Furthermore, viable pollen grains contain higher levels of pigments and free amino acids than non-viable pollens.

Fruit quality: The controlled or hand pollination resulted in the highest fruit length (69.53 mm), breadth (79.71 mm), and weight (196.58 g), followed by open pollination (Table 5, 6). The lowest fruit length, breadth, and weight (39.21 mm, 56.20

Table 6. Effect of controlled/hand pollination on fruit characters of different apple varieties

Cross combinations	Fruit length (mm)	Fruit breadth (mm)	Fruit weight (g)	Depth of eye basin (cm)
Scarlet Spur II × Gale Gala	69.53	78.38	194.89	1.28
Gale Gala × Scarlet Spur II	54.84	63.95	100.78	1.30
Scarlet Spur II × Granny Smith	54.59	63.37	103.17	1.44
Granny Smith × Scarlet Spur II	0.00	0.00	0.00	0.00
Scarlet Spur II × Coe Red Fuji	47.71	72.04	129.51	1.17
Coe Red Fuji × Scarlet Spur II	52.22	63.84	103.67	1.28
Scarlet Spur II × Crimson Gala	49.52	66.10	104.22	1.30
Crimson Gala × Scarlet Spur II	0.00	0.00	0.00	0.00
Oregon Spur II × Gale Gala	0.00	0.00	0.00	0.00
Gale Gala × Oregon Spur II	52.97	64.11	100.98	1.10
Oregon Spur II × Granny Smith	57.65	70.04	140.42	1.27
Granny Smith × Oregon Spur II	0.00	0.00	0.00	0.00
Oregon Spur II × Coe Red Fuji	0.00	0.00	0.00	0.00
Coe Red Fuji × Oregon Spur II	50.55	63.41	99.75	1.16
Oregon Spur II × Crimson Gala	56.03	64.73	108.70	1.20
Crimson Gala × Oregon Spur II	40.24	56.96	79.59	1.35
Early Red One × Gale Gala	44.46	62.15	98.66	1.50
Gale Gala × Early Red One	59.35	69.96	149.64	1.40
Early Red One × Granny Smith	55.44	70.31	151.85	1.35
Granny Smith × Early Red One	54.34	65.59	106.42	1.00
Early Red One × Coe Red Fuji	51.33	60.48	94.71	1.20
Coe Red Fuji × Early Red One	56.70	67.78	120.80	1.30
Early Red One × Crimson Gala	48.38	60.20	90.53	1.28
Crimson Gala × Early Red One	45.51	60.38	84.92	1.10
Super Chief × Gale Gala	0.00	0.00	0.00	0.00
Gale Gala × Super Chief	39.58	61.42	88.06	1.30
Super Chief × Granny Smith	39.84	69.61	83.21	1.10
Granny Smith × Super Chief	62.76	71.13	142.33	1.74
Super Chief × Coe Red Fuji	39.98	60.11	71.63	1.30
Coe Red Fuji × Super Chief	54.51	66.81	115.32	1.50
Super Chief × Crimson Gala	0.00	0.00	0.00	0.00
Crimson Gala × Super Chief	52.33	62.08	98.43	1.10
Ace Spur × Gale Gala	0.00	0.00	0.00	0.00
Gale Gala × Ace Spur	56.50	62.52	132.88	1.10
Ace Spur × Granny Smith	45.65	57.67	82.63	1.60
Granny Smith × Ace Spur	59.99	71.50	145.05	1.80
Ace Spur × Coe Red Fuji	47.81	64.28	114.64	1.10
Coe Red Fuji × Ace Spur	58.47	70.48	136.48	1.30
Ace Spur × Crimson Gala	56.81	62.60	100.07	1.00
Crimson Gala × Ace Spur	48.19	62.28	82.05	1.10
Schlect Spur × Gale Gala	60.85	75.55	146.09	1.30
Gale Gala × Schlect Spur	54.91	68.24	114.15	1.67
Schlect Spur × Granny Smith	62.16	69.77	139.36	1.30

Cont...

Table 6. Effect of controlled/hand pollination on fruit characters of different apple varieties

Cross combinations	Fruit length (mm)	Fruit breadth (mm)	Fruit weight (g)	Depth of eye basin (cm)
Granny Smith × Schlect Spur	0.00	0.00	0.00	0.00
Schlect Spur × Coe Red Fuji	0.00	0.00	0.00	0.00
Coe Red Fuji × Schlect Spur	55.65	56.90	128.60	1.34
Schlect Spur × Crimson Gala	58.63	65.96	113.43	1.50
Crimson Gala × Schlect Spur	45.14	58.92	74.62	1.05
Gale Gala × Granny Smith	59.49	68.84	137.90	1.50
Granny Smith × Gale Gala	63.39	72.16	156.24	1.40
Gale Gala × Coe Red Fuji	69.16	79.71	196.58	1.02
Coe Red Fuji × Gale Gala	53.65	68.56	118.75	1.43
Gale Gala × Crimson Gala	65.42	78.26	196.50	1.40
Crimson Gala × Gale Gala	0.00	0.00	0.00	0.00
Granny Smith × Coe Red Fuji	59.95	70.57	139.68	1.78
Coe Red Fuji × Granny Smith	53.84	65.12	132.27	1.33
Granny Smith × Crimson Gala	64.57	74.24	163.74	1.35
Crimson Gala × Granny Smith	53.56	66.73	115.81	1.20
Coe Red Fuji × Crimson Gala	57.71	75.84	136.08	1.50
Crimson Gala × Coe Red Fuji	46.41	63.72	101.70	1.05
Mean	54.05	66.62	119.74	1.31
CD (p=0.05)	0.13	0.12	0.28	0.001

mm, and 68.73 g, respectively) were observed when bagging was used as the pollination method. These variations might be attributed to both the specific characteristics of the fruit variety studied and the distribution of seeds within the fruits. Dantas et al. (2001) and Chauhan (2018) also reported larger fruit sizes resulting from cross-pollination compared to open pollination. These variations in fruit size are not solely linked to pollination methods but can also be influenced by a combination of genetic and environmental factors. These factors include soil fertility, irrigation practices, nutrient levels, and various agricultural activities, all of which play a significant role in shaping the physical characteristics of the fruit.

Seed number and degree of lopsidedness: Under open pollination conditions, the percentage of asymmetrical fruits (degree of lopsidedness) ranged from 20.00 per cent in Gale Gala to 53.33 per cent in Ace Spur. The average seed number was 7.64, ranging from 6.48 (Ace Spur) to 8.32 (Gale Gala) (Table 3, 4). Under controlled pollination conditions, degree of lopsidedness was maximum (53.33 %) in Super Chief × Granny Smith and Super Chief × Coe Red Fuji and minimum (6.67%) in Gale Gala × Coe Red Fuji, Gale Gala × Early Red One and Granny Smith × Ace Spur. Maximum (9.40) number of seeds was observed in Gale Gala × Coe Red Fuji and minimum (6.00) in Super Chief × Granny Smith and Super Chief × Coe Red Fuji. Under bagging conditions, the percentage of asymmetrical fruits was highest (86.67%) in Crimson Gala and lowest (66.67%) in Granny Smith. The seed number was highest (9.40) under hand/controlled pollination and lowest (2.40) under bagging conditions. The percentage of lopsided fruits was substantially associated with seed quantity. The percentage of lopsided fruits was highest (86.67%) under bagging conditions and lowest (6.67%) under controlled/hand pollination conditions (Fig. 2). There was association between the number of seeds and the proportion of lopsided fruits, i.e., with the percentage of lopsided fruits being higher, the number of seeds was observed to be lower. Buccheri and Vaio (2004) and Sheffield (2014) observed that when the quantity of seeds increased, the percentage of deformed fruits decreased. According to Alabadi et al. (2009) and Balaguera-Lopez et al. (2020) synchronous activity of auxin and gibberellins hormones regulates the development of ovary into fruits. The hormones that are synthesized in seeds (auxins, gibberellins, brassinosteroids, cytokinins, polyamines, ethylene etc.) govern seed development and improve the activity of fruits to act as a sink, and ultimately result in a more uniform shape and better size of the fruit (Sun et al. 2010 and Kang et al. 2013).

Self-incompatibility index: The delicious group of varieties *viz.* Scarlet Spur II, Oregon Spur II, Early Red One, Super Chief, Ace Spur and Schlect Spur were completely self-incompatible followed by Gale Gala, Coe Red Fuji and Crimson Gala which were partially self-compatible, whereas, Granny Smith was completely self-compatible (Tables 3, 4). The cross compatibility of the main varieties varied from being compatible to a negligible extent to being compatible to a large extent, whereas, all the pollinizing varieties were compatible to a higher or larger extent. This indicated the need for a pollinizing variety for optimum fruit set in the case of the delicious group of apple varieties.

CONCLUSION

The current study determined the degree of synchronization of flowering periods among different apple varieties, as well as the extent of fertility of pollen produced by them. All the pollinizing varieties were good source of pollen for the commercial varieties. However, among the pollinizing varieties under study, Gale Gala and Granny Smith were observed to be the best pollinizers, since their duration of flowering was in synchronization with most of the commercial varieties and their pollen viability and *in vitro* pollen germination were considerably higher. The shape of harvested fruits was influenced by the number of seeds present in the fruit. When the seed number was lower, a higher percentage of asymmetrical fruits were obtained. The present study also confirms the self-incompatible nature of delicious varieties of apple and highlights the importance of presence of a suitable pollinizing variety. From the cross pollination experiments, Gale Gala was administered as the best pollinizer, because when used as a source of pollen grains, gave maximum fruit set as compared to other pollinizing varieties.

AUTHORS CONTRIBUTION

Nikhil Kaushal, Girish Dangi and Akriti Chauhan - Designing of the experiments, execution of field/lab experiments, data collection, analysis of data, interpretation and preparation of manuscript; Dinesh S Thakur-Conceptualization and designing of the experiments; Neena Chauhan and RK Dogra -Assisted in the morphological and morphometric work.

REFERENCES

Ahad S, Mir MM, Ashraf S, Mumtaz S, Hamid M and Majid I 2020. Blooming behaviour and pollen characteristics of exotic apple cultivars under high density planting systems of Kashmir. *International Journal of Chemical Sciences* **8**(4): 2998-3001.

Alabadi D, Blazquez MA, Carbonell J, Ferrandiz C and Perez-Amador MA 2009. Instructive roles for hormones in plant development. *The International Journal of Developmental Biology* **53**: 1597-1608.

Anand R 2003. Pollination Studies in Apple (*Malus* × *domestica* Borkh.). M.Sc. thesis. Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan.

Anonymous 2022. *Area and production of horticultural crops for 2021-22.* https://agriwelfare.gov.in/en/StatHortEst.

Balaguera-Lopez HE, Fischer G and Magnitskiy S 2020. Seed-fruit relationship in fleshy fruits: Role of hormones. *Revista Colombiana de Ciencias Horticolas* **14**: 90-103.

Buccheri M and Vaio CD 2004. Relationship among seed number, quality and calcium content in apple fruits. *Journal of Plant Nutrition* **27**: 1735-1746.

Castro DC, Cerino MC, Gariglio N and Radice S 2016. Study of reproductive behaviour in low-chill apples in warmer zones of Argentina. *Scientia Horticulturae* **199**: 124-132.

Chauhan A 2018. *Pollination Studies in Apple (Malus × domestica Borkh.).* M.Sc. thesis. Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan.

Claessen H, Wannes K, De Poel V and Nico DS 2019. Finding a compatible partner: Self-incompatibility in European pear (*Pyrus communis*); molecular control, genetic determination and impact on fertilization and fruit set. *Frontiers of Plant Science* **10**: 407.

Dangi G, Singh D, Chauhan N, Dogra RK, Verma P and Chauhan A 2024. Evaluating genetic diversity of morpho-physiological traits in sweet cherry (*Prunus avium* L.) cultivars using multivariate analysis. *Genetic Resources and Crop Evolution* **11**: 1-36**.**

Dangi G, Singh D, Chauhan N, Dogra RK, Verma P and Sharma S 2021. Characterization of selected sweet cherry (*Prunus avium* L.) varieties using DUS test guidelines. *Indian Journal of Plant Genetic Resources* **34**(2): 290-294.

Dantas ACM, Nunes JCO, Brighenti E, Ribeiro LG and Nodari RO. 2001. Effect of the artificial pollination among apple rootstock (*Malus* spp.) in the effective fruit set and fruit development in Sao Joaquim/SC. *Revista Brasileria de Fruticultura* **23**: 497-503.

Dennis FJ 2003. Flowering, pollination and fruit set and development. In: *Apples Botany Production and Uses*, Ferree DC (Eds), pp.153-166. CAB International, Cambridge.

Hua ZH, Fields A and Kao TH 2008. Biochemical models of S-RNase based self-incompatibility. *Molecular Plant* **1**: 575-585.

Javid R 2015. *Pollination compatibility among different apple cultivars.* M.Sc. thesis. Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir.

Kang C, Darwish O, Geretz A, Shahan R, Alkharouf N and Liu Z 2013. Genome scale transcriptomic insights into early-stage fruit development in woodland strawberry *Fragaria vesca*. *Plant Cell* **25**: 1960-1978.

Kotiyal A and Dimri DC 2017. Reproductive evaluation of different apple (*Malus* × *domestica* Borkh.) cultivars grown in Uttarakhand hills of India. *BioScan* **12**: 1101-1105.

Majid S 2003. *Evaluation of Newly Introduced Apple Cultivars under Kashmir Conditions.* M.Sc. thesis. Sher-e-Kashmir University of Agricultural Sciences and Technology of Kasmir.

Monteiro VM, Silva CI, Pacheco A and Freitas BM 2015. Floral biology and implications for apple pollination in semiarid north eastern Brazil. *Journal of Agriculture and Environmental Sciences* **4**(1): 42-50.

Moriya Y, Takai Y, Okada K, Ito D and Shiozaki Y 2005. Parthenocarpy and self and cross-incompatibility in ten European pear (*Pyrus communis*) cultivars. *Journal of the Japanese Society for Horticultural Science* **74**: 424-430.

Nautiyal P and Dimri DC 2009. Pollination studies in apple (*Malus* × *domestica* Borkh.) cv. Red Delicious. *Progressive Horticulture* **41**(2): 157-163.

Nyeki J 1996. Fertilization conditions. In: *Floral Biology of Temperate Zone Fruit Trees and Small Fruits,* Nyeki J and Soltesz M (Eds), pp. 185-256. Akademiai Kiado, Budapest, Hungary.

Pandit BA 2014 *Pollen compatibility studies of some exotic apple cultivars.* Ph.D thesis. Sher-e-Kashmir University of Agricultural Sciences and Technology of Kasmir.

Rather GH, Javid R, Paray MA, Khursheed R and Yaqoob M 2018.

Investigations on floral phenology, pollen viability and pollen germination in some apple genotypes in climatic conditions of Kashmir. *International Journal of Chemical Studies* **6**: 1249-1251.

Sharma DP, Sharma HR and Sharma N 2017. Evaluation of apple cultivar under sub-temperate mid hill conditions of Himachal Pradesh. *Indian Journal of Horticulture* **74**(02): 162-167.

Sheffield CS 2014. Pollination, seed set and fruit quality in apples: Studies with *Osmia lignaria* (Hymenoptera: Megachilidae) in the Annapolis valley, Nova Scotia, Canada. *Journal of Pollination Ecology* **12**: 120-128.

Singh D, Sharma SD and Kumar K 2002. Flowering and cross-compatibility in apple cultivars growing in the Kullu valley of India. *Journal of the American Pomological Society* **56**(1): 46-50.

Sun X, Shantharaj D, Kang X and Ni M 2010. Transcriptional and hormonal signalling control of Arabidopsis seed development. *Current Opinion in Plant Biology* **13**: 611-620.

Verma P and Thakur BS 2019. Comparative studies on growth, flowering, fruit set and yield of some apple (*Malus* × *domestica* Borkh.) cultivars under mid hill conditions of Himachal Pradesh, India. *International Journal of Current Microbiology and Applied Sciences* **8**(2): 2710-2716.

Webster AD and Wertheim SJ 2003. Genetic Improvement of apple: Breeding, markers, mapping and biotechnology. In: *Apples: Botany, Production and Uses.* Ferree DC and Warrington IJ (Eds), pp.31-32. CABI Publishing, CAB International.

Received 23 September, 2023; Accepted 12 March, 2024