AATCC Review

06 February 2023: Received
08 June 2023: Revised
13 August 2023: Accepted
16 October 2023: Available Online

www.aatcc.peerjournals.net

Research Article Open Access

Crossability studies of inter-specific hybridization between varieties of Sweet orange (*Citrus sinensis*Osbeck) and Mandarin (*Citrus reticulata* Blanco)

Kunzang lamo[1], Anil Kumar Sangwan[2], Nav Prem Singh[3], Manveen Kaur Batth[3]

[1]National Institute of Sowa Rigpa Leh, UT-Ladakh, India.
[2]Dr J. C. Bakhshi Regional Research Station, Abohar 152116, India.
[3]Department of Fruit Science, Punjab Agricultural University, Ludhiana, India.

ABSTRACT

To determine the best pollen source in sweet orange varieties, viz., Mosambi and Jaffa, flowers were hand pollinated with the pollen grains of mandarin varieties, viz., Mukaku Kishu, W. Murcott and Clementine. The results of the controlled cross-pollination indicated that in Moambi, flowers pollinated with Mukaku Kishu exhibited the highest fruit set (49.75%), lowest fruit drop (77.70%), and highest fruit retention percentage (9.20%). While in Jaffa, the highest fruit set (35.37%) was recorded when crossed with pollen grains of W. Murcott, the highest fruit retention (4.02%), and the lowest fruit drop (85.63%) were recorded when crossed with Clementine mandarin. Results further indicated that the highest fruit retention per cross in Mosambi was recorded when crossed with pollens of Mukaku Kishu (9.20%), and the lowest fruit retention (3.96%) was recorded when crossed with Clementine. Whereas in Jaffa, fruit retention per cross was highest (4.02%) when crossed with W. Murcott, and the lowest fruit retention (2.08%) was recorded when Mukaku Kishu was used as a pollen parent. Based on the results, pollen grain sources had a significant effect on fruit set, fruit retention, and the number of seeds per fruit in Mosambi and Jaffa sweet oranges. Therefore, MukakuKishu and W. Murcott mandarins could be used as suitable pollenizer for Mosambi and Jaffa, respectively. However, W. Murcott resulted in a higher number of seeds per fruit, thus reducing the quality of fruits. Results also indicated that Mukaku Kishu was sexually incompatible with Jaffa and had very low fruit set and fruit retention percent.

Keywords: *Citrus, Inter-specific hybridization, fruit set, fruit retention,fruit drop, Mosambi, Jaffa, MukakuKishu, W.Murcott, Clementine*

Introduction

Citrus is one of the most significant and predominant fruit crops grown worldwide, it belongs to the family Rutaceae. Citrus fruits are a good source of macro, micronutrients, and antioxidant compounds, it has anticancer and anti-inflammatory properties [22,15,16]. In India, citrus covers an area of 10.5 lakh ha and is the third most important fruit crop with an annual production of about 14.0 million tons [2]. In Punjab, citrus is the main leading fruit among other fruit crops and is mainly grown in arid irrigated and sub-mountainous regions on an area of 57289 ha with an annual production of 1.28 million tons [3]. Among citrus, the most commercially important species grown in India are the mandarins (*Citrus reticulata* Blanco) followed by, sweet orange (*Citrus sinensis* Osbeck) and acid lime (*Citrus aurantifolia* Swingle). However, citriculture in North India is mainly confined to a single variety i.e. Kinnow mandarin with major commercial significance because no other recommended variety is at par in yield and quality for the region. Thus, there is a need to increase the availability of new varieties with higher yield and improved quality traits in the citrus industry. Till now several commercial varieties have been developed through traditional breeding methods such as inter and intra-specific hybridization, mutagenesis, and clonal selection in citrus [6]. However, sexual breeding in citrus is not always feasible due to several obstacles such as sexual incompatibility, polyembryony, and sterility[21]. In citrus, cross incompatibility between the species pose serious constraints in inter-specific hybridization because of pre and post-zygotic barriers. Besides, post-zygotic barriers such as excess fruit drop, low and non-viable seed yield, low recovery of hybrids, high mortality rate of hybrid seedlings and poor field survival results in low cross-pollination success Barrett [5]. Therefore, high cross-compatibility between parents involved in crossing is important and it determines the success of a controlled hybridization program. It is of utmost importance to verify the compatibility between the species involved in crossing. Inter-specific hybridization provides an opportunity to introgress traits from both the parents and recombine their genomes that broaden the genetic base of the population. However, several reports on citrus breeding revealed different intra- and inter-specific cross-compatibility rates.Despite these past efforts, an attempt was made to determine the inter-specific cross-compatibility of sweet orange varieties as seed parent and mandarin varieties as pollen parents.This study, therefore, explored the success of citrus inter-specific cross using different pollen parents and their compatibility rates.

Corresponding Author:* ***Kunzang Lamo*
Email Address: ***kunzanglamospadumpa@gmail.com***

DOI: https://doi.org/10.58321/AATCCReview.2023.11.04.201

Materials and Methods

Parent material and hybridization

Three mandarin varieties viz., Mukaku Kishu, W.Murcott, and Clementine (male parent) and two sweet orange varieties viz., Mosambi and Jaffa (female parents) were used to generate hybrids. This investigation was carried out at Punjab Agricultural University, Regional Research Station, Abohar, Punjab India during 2019 and 2020. Varieties were planted at 6 x 6 m spacing. Anthers from unopened flowers from male parent were collected, allowed to dehisce on silica gel, and stored at 4^0C until required. The emasculation of flower on the female parent was done before the opening of the petal and immediately dusted with the camel hairbrush. Controlled cross-pollination was done on 17th to 29th March 2019 and 15th to 30th March 2020. In each cross, data were recorded on the initial fruit set (%), fruit drop (%), fruit retention (%) seed content per fruit, fruit qualities in terms of Total soluble solids, acidity, and juice content, and were expressed in percentage.

Statistical Analysis: The data were subjected to an analysis of variance (ANOVA) and SAS (Statistical Analysis of Variance) version 9.4 software. All data were performed in triplicates and presented as means. Statistical significance was determined at $p \leq 0.05$.

Results and Discussion

Fruit set and fruit drop percent

Fruit set percent in both the years (2019 and 2020) followed a similar trend and a significant maximum mean fruit set (49.75 %) was observed in Mosambi × Mukaku Kishu cross and minimum in W.Murcott (31.56 %) fruit set in Mosambi was maximum when Mukaku Kishu used as pollen parent

Table 1. Variation in fruit set and fruit drop percent among different inter-specific crosses

F1 Parentage (F × M)	Fruit Set %			June Drop %			Pre-Harvest Drop (%)		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Mosambi × MukakuKishu	55.16 a	44.34 a	49.75 a	78.05 e	77.35 d	77.70 d	7.14 e	25.91a	16.52c
Mosambi × W. Murcott	48.00 c	30.30 c	39.15 c	80.13 d	81.16 c	80.64 c	18.65 d	27.77 a	23.21a
Mosambi × Clementine	53.57 b	42.00 b	47.78 b	93.08 a	86.47 b	89.78 a	20.00cd	13.02 e	16.51 c
Jaffa × MukakuKishu	41.81 d	25.50 e	33.66 e	92.99 a	90.30 a	91.64 a	24.82 a	22.50 c	23.66 a
Jaffa × W.Murcott	42.00 d	28.73 cd	35.37 d	83.38 c	89.25 a	86.32 b	21.34bc	18.21 d	19.77 b
Jaffa × Clementine	35.77e	27.34 d	31.56 f	90.95 b	80.31 c	85.63 b	23.21ab	24.00bc	23.61 a
Mean	46.05	33.03	39.54	86.43	84.14	85.28	19.19	21.90	20.55
LSD ($p<0.05$)	1.51	1.76	1.42	1.87	1.94	1.90	3.99	3.43	2.69
C.V	1.85	3.00	2.02	1.21	1.30	1.25	2.05	2.12	2.08
S.E.M.	0.49	0.57	0.46	0.61	0.63	0.62	0.66	0.69	0.68

Data with different letter in each column are significantly different at $p \leq 0.05$.

(49.75 %) and minimum fruit set (39.15 %) was observed when crossed with W. Murcott. However, fruit set in Jaffa possess the maximum fruit set (35.37 %) when W. Murcott was used as the pollen parent and exhibited a minimum fruit set when Mukaku Kishu is used as a pollen parent. The result indicates that the fruit set was influenced by different pollinators for both sweet orange varieties (Table 1). Overall, Jaffa × Clementine differed significantly from all other crosses. Similar variations in fruit set percentage were reported by Hossein and Rabbani [12] in lemon genotypes which ranged between (20 to 56%) and also influenced the seed setting in different crosses. Our results are in accord with the findings of Chao [7] investigated the effect of different pollinators on fruit set in mandarins and reported that cross combination between Nules × Afourer mandarins observed a maximum of 39.62 % fruit set in 2002 and in similar cross very low fruit set of 4.92 % was recorded in 2003. It was reported that due to low temperatures in 2003, the flowers in Nules Clementine and Afourer could not open properly for crossing therefore resulting in a low fruit set. Atawia*et al* [4] showed that Shaddock (*Citrus maxima* L.) pummelo, had a maximum fruit set of 51.17 % when Balady orange was used as the pollen parent followed by 48.30 % when Succary orange was used as the pollen parent and 47.00 % fruit set was observed when Balady mandarin was used as a pollen parent. While a minimum fruit set of 37.00 % was observed when Marsh grapefruit was used as pollen parent. Singh *et al* [18] reported maximum mean fruit set percentage (15.49) in W. Murcott x Kinnow cross. Jahromi*et al*[13] also obtained variation and recorded highest percentage of fruit set (73.75 %) in Clementine when crossed with sweet lime and fruit set was minimum (69.25%) when Lisbon lemon used as a pollen parent. All these reports indicated to suggest that the percent fruit set depends on the genotype and environment.

In citrus, two successive waves of fruit drop occur viz., June drop and Pre-harvest drop. The results revealed that the overall maximum (91.64 %) average fruit drop in June was recorded in Jaffa × Mukaku Kishu, and minimum in Mosambi × Mukaku Kishu (77.70%) (Table 1). A similar trend was followed for both the years of investigation in Mosambi. However, in case of Jaffa, a different trend was observed during both the years. In 2019, June drop was recorded maximum (of 92.94 %) particularly in Jaffa × Kishu and minimum in Jaffa × W.Murcott (83.38 %) and in 2020 minimum (80.35 %) was observed in Jaffa × Clementine. Mosambi had a maximum June drop when Clementine was used as the pollen parent and had a minimum fruit drop when Mukaku Kishu was used as a pollen parent. Whereas, Jaffa had a maximum fruit drop when Kishu was used as a pollen parent and minimum fruit drop was observed when Clementine used as a pollen parent. This may be due to the fact that citrus possess an internal self-regulatory adjusting mechanism that adjusts fruit loads to enable supply of metabolites to the trees Goldschmidt and Monselise[10]. June drop in citrus may also occurs due to depletion of carbohydrates caused by growing fruitlets leading to rise in ABA and ethylene content ultimately leading to the abscission of fruit Domingo *et al* [9] and Gomez-Cadenas *et al* [11]. Present results were in concord with the findings of Singh *et al* [18], who also observed variation and maximum June drop percent of 40.21 % in Daisy x Kinnow cross

and a minimum fruit drop of 31.87 % in Kinnow x W.Murcott.
The effect of pollinators on the pre-harvest fruit drop percent in different inter-specific crosses are shown in (Table 1). The result revealed that maximum pre-harvest fruit drop was recorded in Mosambi when W. Murcott pollen was used, and minimum fruit drop percentage was observed when Clementine was used as pollen parent. Whereas, in Jaffa maximum fruit drop was observed when Mukaku Kishu was used as the pollen parent, and minimum fruit drop was observed when W. Murcott was used as the pollen parent, and minimum fruit drop was observed when W. Murcott was used as a pollen parent. The mean pre-harvest drop was maximum (23.66 %) in Jaffa × Mukaku Kishu and minimum in Mosambi × Clementine (16.52 %). However, the maximum fruit drop percentage pattern was different for both years of investigation. In 2019, the maximum (24.82 %) pre harvest fruit drop percentage was recorded in Jaffa ×MukakuKishu cross and in 2020 pre harvest fruit drop was maximum (27.77 %) in Mosambi × W. Murcott cross. Jawanda*et al* [14] also reported varying percentage of fruit drop in Mosambi (57.2), Pineapple (47.6), Hamlin (48.5), Jaffa, Blood Red (48.6) and Valencia (34.5). The result of present investigation coincides with the findings of Singh *et al*[18], who recorded minimum fruit drop (31.87%) in Kinnow x W. Murcott cross and maximum in Daisy x Kinnow cross (40.21) fruit drop.

Table 2. Variation in fruit retention and fruit retention per cross among different inter-specific crosses

F1 Parentage (F × M)	Fruit retention (%)			Fruit retention per cross (%)		
	2019	2020	Mean	2019	2020	Mean
Mosambi × MukakuKishu	27.53a	16.77a	22.15a	11.04a	7.36a	9.20a
Mosambi × W. Murcott	25.57	13.61c	19.59b	7.80b	4.02bc	5.91b
Mosambi × Clementine	5.54d	11.77d	8.66e	3.04cd	4.88b	3.96c
Jaffa × MukakuKishu	5.27d	7.75f	6.51f	2.20d	1.97d	2.08d
Jaffa × W.Murcott	27.23a	8.99e	18.11c	5.60bc	2.43cd	4.02c
Jaffa × Clementine	6.95c	15.20b	11.08d	2.51d	4.28bc	3.40cd
Mean	16.35	12.35	14.35	5.36	4.16	4.76
LSD (p<0.05)	3.56	2.97	1.72	1.21	0.31	0.62
C.V.	1.06	0.67	0.45	12.38	4.09	7.12
S.E.M	0.34	0.21	0.14	1.44	0.79	1.02

Data with different letters in each column are significantly different at p≤0.05.

Fruit retention percent

Fruit retention percentage on fruit set basis was significantly influenced by pollinators (Table 2). Fruit retention in Mosambi and Jaffa was influenced differently by different pollen parents and maximum retention (22.15 %) was recorded with Mukaku Kishu and minimum (8.66 %) with Clementine pollens in the case of Mosambi whereas, in the case of Jaffa maximum fruit set (18.11 %) was recorded with W. Murcott pollens and minimum fruit set (6.51 %) was recorded with Mukaku Kishu same trend was followed during both the years (2019 and 2020). Overall fruit retention was recorded higher in Mosambi as compared to Jaffa.

Fruit retention per cross

On the basis of the number of crosses made, overall percent fruit retention was ranged in between 2.08 to 9.20 and individually in Mosambi it ranged in between 3.96 to 9.20 and in Jaffa it ranged in between 2.08 to 4.02 (Table 2). Similar trend was followed during both the year of investigation (2019 and 2020). Results also indicated that highest fruit retention per cross in Mosambi was observed when crossed with Mukaku Kishu pollens and the lowest was recorded with Clementine pollens. Whereas, in Jaffa fruit retention per cross was highest when cross with Clementine, and lowest fruit retention was observed when Mukaku Kishu was used as a pollen parent. The less fruit retention may be since, generally in sweet orange cultivars only less than 1 % of fruits reach maturity from 2.5 lakh flowers per plant Domingo *et al* [9]. And fruit retention percentage depends on the compatibility between parents involved in crossing as well as on environmental conditions. Similarly, Hossein and Rabbani [12] also reported higher fruit set percent (20 to 56 %), retention, and seed setting in a cross-pollination of lemon genotypes.

Seed characters

In general, average number of normal seeds was recorded higher in Mosambi as compared to Jaffa with all three male parents and maximum mean number of normal seeds per fruit (18.08) were observed in Mosambi × W. Murcott and minimum in Mosambi × Mukaku Kishu (14.58) (Table 3). Similarly, Jaffa also exhibit significant maximum normal seed with W.Murcott and minimum 7.13 with Mukaku Kishu. The trend in the year followed the same pattern. Similarly, Singh *et al* [18] evaluated number of seeds per fruit obtained from different intra-specific crosses in Mandarin and reported variation among different crosses, and the maximum number of normal seeds per fruit (21.21) were recorded in fruits from Kinnow x Daisy cross which was at par with mean normal seed number fruit (18.01) in Kinnow x W. Murcott cross. And significantly lower mean normal seed number per fruit (12.48) was recorded in Daisy x Kinnow cross. The data on the number of rudimentary seeds per fruit clearly indicated significant differences in different inter-specific cross. The maximum mean rudimentary seeds per fruit (7.05) were recorded in the cross involving × Mukaku Kishu and the minimum in Jaffa × W.Murcott (3.11) cross. Similar trend was followed for two consecutive year of investigation (2019 and 2020).

Table 3. Variation in the average number of seeds per fruit obtained from different inter-specific crosses.

F1 Parentage (F × M)	Average normal seed per fruit (No)			Average rudimentary seed per fruit (No)			Total seeds per fruit (No)		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Mosambi × MukakuKishu	15.28 b	13.88 b	14.58 c	6.44 a	7.65a	7.05 a	21.72 b	21.53b	21.63b
Mosambi × W. Murcott	18.74 a	17.42 a	18.08 a	5.96ab	6.43 a	6.20 a	24.70 a	23.85a	24.20a
Mosambi × Clementine	16.60 b	15.68 a	16.14 b	6.38 a	6.99 a	6.68 a	22.98ab	22.67ab	22.83 ab
Jaffa × MukakuKishu	7.20 d	7.04 d	7.13 f	3.9 b c	3.27 b	3.62 b	11.18 d	10.31d	10.74d
Jaffa × W.Murcott	10.95 c	9.75 c	10.35 d	3.00 c	3.25 b	3.11 b	13.95 c	13.00 c	13.48c
Jaffa × Clementine	8.43d	9.11 c	8.77 e	3.65c	3.19 b	3.42 b	12.08cd	12.30c	12.19cd
Mean	12.87	12.15	12.51	4.90	5.13	5.01	17.77	17.28	17.52
LSD (p<0.05)	1.96	1.76	0.10	2.08	2.21	1.86	2.29	1.87	2.08
C.V.	8.55	8.15	0.44	23.88	24.17	20.81	7.26	6.08	6.68
S.E.M.	0.64	0.57	0.03	0.68	0.72	0.60	0.74	0.61	0.68

Data with different letter in each column are significantly different at p≤0.05.

Studies showed different degrees of compatibility among different crosses, and some combinations recorded significantly higher seed numbers (Table 3). Results showed a significant difference in total seed per fruit. The maximum mean number of total seed per fruit were recorded (24.20) in Mosambi × W. Murcott cross and the minimum in Jaffa × Mukaku Kishu (10.79). A similar trend for the total number of seeds per fruit was observed in both the years of investigation (2019 and 2020). Results also revealed that maximum seeds per fruit in Mosambi and Jaffa were observed when crossed with W. Murcott as male parent. Results suggest the high compatibility of Mosambi and Jaffa with W. Murcott. Similarly, Ellendale tangor gave the largest and multiseeded fruits when cross-pollinated with Murcott and Emperor Vithanage [23]. Similarly, Abknar[1]also proved the differential effect of different pollinators and observed that Moro blood orange is the best pollinator in Clementine mandarin for reducing the number of seeds, whereas clustered lemon is the best pollinator for increasing the percent fruit set in Clementine mandarin. Likewise, Chao [7] also observed different numbers of seeds per fruit with different pollen parents in Mandarins crosses. And reported that cross combination between Nules × Afourer mandarins observed a maximum of 25.36 % seeds per fruit and a cross between Nules × Taho Gold recorded a minimum number of seeds per fruit (1.71). While the Afourer × Nules cross observed maximum seeds per fruit (12.00) and minimum seeds per fruit (10.77) was observed in the Afourer × Nules cross. Our results are in agreement with the findings of Atawia *et al* (2016), who obtained different numbers of seeds per fruit with different pollinizers in Shaddock (*Citrus maxima* L.) pummelo. Maximum seeds per fruit (110.33) were observed when crossed with Balady mandarin followed by (97.85) with Balady orange, (79.50) and minimum seeds per fruit was obtained with Marsh grapefruit (62.34). Yildiz and Kaplankiran[25] also reported different effects of pollinators on number of seeds per fruit in Robinson mandarins, and maximum seed per fruit (27.81) was reported in Robinson mandarin × Valencia Late orange cross and minimum seeds per fruit (8.18) wasrecorded in Robinson mandarin × Midknight Valencia cross.

Table 4. Variation in physical parameters of fruits obtained from different inter-specific crosses.

F1 Parentage	Fruit length (mm)			Fruit diameter (mm)			Fruit weight (g)			Number of segments per fruit		
(F × M)	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Mosambi × MukakuKishu	62.92ab	66.63 a	64.78ab	65.50abc	64.47 ab	64.99 c	158.65 bc	164.52bcd	161.59 bc	10.72	10.69	10.71
Mosambi × W. Murcott	65.98a	71.76 a	68.87a	67.91a	67.19a	67.55a	164.52 a	170.49 a	167.50 a	11.35	11.96	11.66
Mosambi × Clementine	64.26 ab	69.75 a	67.00a	67.16ab	66.9a	67.04b	161.29 ab	168.40ab	164.85ab	11.16	11.31	11.24
Jaffa × MukakuKishu	57.03c	57.34 b	57.19c	60.58c	59.45c	60.02f	152.42 d	159.55d	155.99 d	10.4	10.65	10.53
Jaffa × W.Murcott	60.12 bc	59.06 b	59.59bc	61.93bc	61.21bc	61.57d	158.55bc	165.38abc	161.97bc	10.85	11.09	10.97
Jaffa × Clementine	59.53bc	58.32 b	58.92c	60.72c	60.41bc	60.56 e	155.13cd	161.84 cd	158.49cd	10.31	10.92	10.62
Mean	61.64	63.81	62.72	63.97	63.27	63.62	158.43	165.03	161.73	10.80	11.10	10.95
L.S.D. (p≤0.05)	3.12	3.41	3.27	3.44	3.96	3.24	5.07	5.53	5.3	NS	NS	NS
C.V.	4.67	4.76	4.72	4.78	4.41	4.21	1.80	1.88	1.84	5.50	4.70	4.93
S.E.M.	1.66	1.76	1.71	1.77	1.61	1.38	1.65	1.80	1.72	1.99	1.80	1.85

Data with different letter in each column are significantly different at p≤0.05.

Physical characteristics of fruit

Maximum mean fruit length (68.87 mm, diameter (67.55 mm), and weight (167.50 g) was observed in Mosambi when crossed with W.Murcott at par with Clementine, and the minimum was noticed when crossed with Mukaku Kishu during both year. Similar, trend was also observed in the case of Jaffa. In general fruit size and weight of the Jaffa was lesser than the Mosambi with all the crosses. Our results are in agreement with the findings of Atawia *et al* [4], who obtained different fruit sizes with different pollinizers in Shaddock (*Citrus maxima* L.) pummelo. Maximum fruit length (13.47cm) was recorded when crossed with Balady mandarin followed by (12.93cm) with Marsh grapefruit and minimum fruit length (11.30 cm) was obtained with Balady orange. Yildiz and Kaplankiran[25] also found different effects of pollinators on fruit weight in Robinson mandarins, and maximum fruit weight (137.49g) was recorded in Robinson mandarin × Rhode investigation (Table 4 and Fig 1).

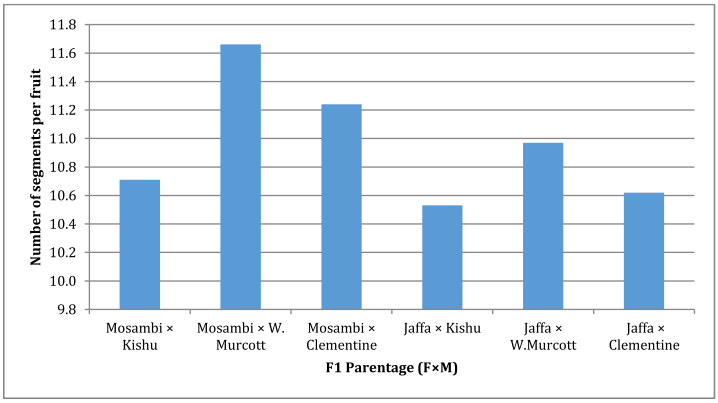

Fig 1. Variation in the number of segments per fruit obtained from different inter-specific crosses.

A significant result was recorded in different crosses and maximum mean peel weight (42.72 g) and peel thickness (3.00mm) and peel percent (22.20 %) was recorded in Mosambi when crossing with W. Murcott which was statistically at par with Clementine and minimum was recorded when crossed with Mukaku Kishu (Table 5). A similar, trend was also observed in case of Jaffa. The variation in peel weight and peel thickness in all the crosses for two seasons could be due to environment and genotype interaction.

Table 5. Variation in physical parameter of fruits obtained from different inter-specific crosses.

F1 Parentage(F×M)	Peel weight (g)			Peel thickness (mm)			Peel (%)			Rag (%)		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Mosambi × MukakuKishu	38.52b	39.03a	38.77 a	2.98b	2.67b	2.83b	20.11ab	19.17ab	19.64b	33.96b	32.74c	33.35c
Mosambi × W. Murcott	42.77a	42.67a	42.72a	3.18b	2.81b	3.00b	21.84a	22.56a	22.20a	36.31b	35.44bc	35.88bc
Mosambi × Clementine	41.91a	40.20a	41.05a	3.05b	2.77b	2.91b	20.63ab	21.21ab	20.92ab	37.01ab	35.79bc	36.40bc
Jaffa × MukakuKishu	27.01d	29.05b	28.03b	3.54a	3.41a	3.48a	17.32b	16.05c	16.69c	38.42ab	37.67ab	38.05b
Jaffa × W.Murcott	30.82c	33.21b	32.01b	3.60a	3.63 a	3.58 a	19.49ab	21.63ab	20.56ab	38.35ab	36.95ab	37.65b
Jaffa × Clementine	29.81cd	31.24b	30.52b	3.16ab	3.50 a	3.44 a	16.08c	18.32b	17.20c	42.13a	40.77a	41.45a
Mean	35.14	35.9	35.52	3.26	3.13	3.19	19.25	19.82	19.53	37.7	36.56	37.13
L.S.D (p≤0.05)	3.20	3.16	3.15	0.36	0.34	0.35	0.79	0.45	0.52	0.77	0.79	0.85
C.V	5.12	8.08	6.57	6.00	6.05	6.02	2.27	1.26	1.48	1.13	1.19	1.11
S.E.M	1.04	1.67	1.35	0.12	0.12	0.11	0.78	0.41	0.58	0.13	0.11	0.12

Data with different letter in each column are significantly different at p≤0.05.

Quality characters

A non-significant difference in Mosambi and Jaffa was observed due to pollinators on fruit quality characteristics (Table 6). However, the maximum mean TSS (9.65 ^{0}B) was recorded in the Mosambi × Clementine cross and the minimum in Jaffa × W.Murcott (8.65^{0}B). A similar trend was followed in both the years of investigation (2019 and 2020). The results discussed above is supported by the findings of Wallace *et al*[24]. Maximum mean acidity percent (0.60 %) was recorded in Jaffa × Mukaku Kishu and minimum in Mosambi × Mukaku Kishu (0.53 %). The result indicated that the type of pollen parent did not affect the acidity percent of fruits in both varieties. Likewise, Papadakis *et al* [17] reported that cross-pollination of Nova with either SRA63 or Marisol did not affect fruit quality characters such as total acids in the juice.

Table 6. Variation in a quality parameter of fruits obtained from different inter-specific crosses.

F1 Parentage (F × M)	TSS ^{0} B			Acidity (%)			TSS: Acid ratio			Juice (%)		
	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean	2019	2020	Mean
Mosambi × MukakuKishu	9.21	9.51	9.36	0.54	0.56	0.53	16.88	16.11	16.49	45.51a	48.09a	46.80a
Mosambi × W. Murcott	9.03	9.16	9.10	0.58	0.60	0.58	15.31	14.89	15.10	41.85c	42.00c	41.93b
Mosambi × Clementine	9.49	9.80	9.65	0.52	0.54	0.50	18.54	18.09	18.31	42.36bc	43.00bc	42.68ab

Jaffa × MukakuKishu	9.08	8.96	9.03	0.62	0.65	0.60	15.12	13.49	14.30	44.27ab	46.28ab	45.28a
Jaffa × W.Murcott	8.76	8.53	8.65	0.63	0.66	0.60	14.97	12.45	13.71	40.67c	41.06d	40.87bc
Jaffa × Clementine	9.42	9.38	9.40	0.60	0.63	0.56	19.34	14.44	16.89	41.79bc	43.54b	42.40ab
Mean	9.17	9.22	9.20	0.58	0.61	0.56	16.69	14.91	15.80	42.74	43.91	43.32
L.S.D (p≤0.05)	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.33	0.32	0.16
C.V	1.44	1.80	1.92	2.41	2.22	2.89	4.01	3.95	1.99	5.53	5.12	5.00
S.E.M	0.08	0.11	0.13	0.06	0.05	0.04	0.34	0.35	0.17	0.11	0.18	0.16

A significant variation in juice percent was recorded in fruits obtained from different inter-specific crosses. Maximum mean juice (46.80 %) was noticed in Mosambi × Mukaku Kishu which was statistically at par with Jaffa × MukakuKishu (45.28 %), followed by Mosambi × Clementine (42.68 %) and Jaffa × Clementine (42.40 %) followed by Mosambi × W.Murcott (41.93 %) and minimum in Jaffa × W.Murcott (40.87 %). Whereas the maximum mean rag (41.45 %) was recorded in Jaffa × Clementine followed by Jaffa × Mukaku Kishu (38.05%) and minimum in Mosambi × Mukaku Kishu (33.35 %). A similar trend in rag percent was recorded in all the crosses during both the years. Similarly, Yildiz and Kaplankiran[25] also found different effects of pollinators on number of juice content in fruits of Fremont mandarins, and maximum juice percent (51.60 %) was observed in Fremont × Rhode Red Valencia and minimum juice percent (48.01 %) was observed when crossing with Midknight Valencia.

Conclusions

High sexual compatibility in inter-specific crosses of sweet orange and mandarin was observed using the former as the maternal parent. Wide variation in terms of fruit set and seed content was observed in all the crosses. In all the cross combinations, the highest fruit set was recorded in Mosambi when crossed with Mukaku Kishu, whereas in Jaffa, the highest fruit set was recorded when crossed with W. Murcott. June drop was recorded minimum in Mosambi with Mukaku Kishu, whereas in Jaffa, fruit drop was minimum with Clementine used as a pollen parent. This studyoffers comprehensive information on pollination success rates involving inter-specific cross among sweet orange and mandarin varieties. The valuable information gained from this study may help the citrus breeders select suitable cross combinations and will contribute to the enhancement of the genetic base, which may play a major role in the improvement and scion breeding program of sweet orange. We presented a perspective for the future on the selection of suitable varieties in sweet orange and mandarin for the inter-specific hybridization program.

Acknowledgments: The authors are grateful to Dr J. C. Bakhshi, Regional Research Station, Punjab Agricultural University, Abohar, India for providing material to carry out this research study.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abknar, A. 1996. Effect of pollen of different cultivars of Citrus on somequantitative and qualitative of Clementine mandarin. Master's Degree Thesis inHorticulture. School of Agriculture. University of Tehran, Iran.

2. Anonymous, 2020a. FAOSTAT. Food and agriculture organization corporate statistical database. http://faostat.fao.org/site/567/default.

3. Anonymous.2020b. Indian Horticulture Database. National Horticulture Board, Gurgaon (Haryana), New Delhi. http://www.nhb.gov.in.

4. Atawia, A., R.,Abd, EL-L.,Badawy, H., Abo-Aziz, A., B., AbouRayya, M., S., M., Baiea, M., H., M. andAbdelkhalek, A. Effect of various pollination treatments on yield characteristics and fruit quality of shaddock fruits. *Int J SciEng Res* 7:1–9.

5. Barrett, H. C. 1985. Hybridization of Citrus and related genera. *Fruit VarJ* 39: 11-16.

6. Caruso, M., Smith, M. W., Froelicher, Y., Russo, G. and Gmitter, F. G. 2020. Traditional breeding, in The Genus Citrus, 1st edition. Eds. M. Talon, M. Caruso, and F. G. Gmitter (Cambridge, UK: Elsevier), 129–148.

7. Chao, C., C., T. 2005. Pollination study of the mandarins and the effect on seediness and fruit size: implications for seedless mandarin production. *HortSci,*40:362-365

8. Domingo, J. Iglesias, Manuel, Cercos, J. M. 2007.Colmenero-Flores, Miguel A. Naranjo, Gabino Ríos, Esther Carrera, Omar Ruiz-Rivero, Ignacio Lliso, Raphael Morillon, Francisco R. Tadeo and Manue Physiology of citrus fruiting. *Talon. Braz. J. Plant Physiol.*, 19: 333-362.

9. Domingues, E. and Tulmann, N. A. 1999. Influence of pollination and floral morphology on fruit setting in sweet orange varieties. *Scientia Agricola*, 56: 163-170.

10. Goldschmidt, E. E. and Monselise, S., P. 1977. Physiological assumptions toward the development of a citrus fruiting model. Proc. Int. Soc. Citrus 2, 668-672.

11. Gomez-Cadenas, A., Mehouachi, J, Tadeo, F. R, Primo-Millo, E, Talon, M. 2000. Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus. *Planta,* 210: 636-643.

12. Hossein, M, I. and Rabbani, M., G. 2011. Study on the cross compatibility of some lemon genotypes (Citrus limon L.). *Bangladesh Journal of Agricultural Resources*, 36:241-246.

13. Jahromia, H. A., Zareib, A. and Mohammadkhania, A. 2019. Analysis the effects of pollen grain sources on the fruits set and their characteristics of 'Clementine' mandarin using microscopic and molecular approaches *ScientiaHorticulturae,* 249: 347–354.

14. Jawanda J., S, Sinha, M., K. and Uppal, D., K. 1972. Studies on nature and periodicity of pre-harvest fruit drop in sweet orange. *Indian J Hort,* 29:269-76.

15. Liu, Y., Heying, E. and Tanumihardjo, S. A. 2012. History, global distribution, and nutritional importance of citrus fruits. *Compr Rev Food SciFoodSaf,* 11: 530–545.

16. Ma, G., Zhang, L., Sugiura, M., and Kato, M. 2020. Citrus and health, in The Genus Citrus, 1st edition. Eds. M., Talon, M. Caruso and F. G. Gmitter (Cambridge, UK: Elsevier), 495–511.

17. Papadakis, I. E., Protopapadakis, E. E., Therios, I. N. 2009. Yield and fruit quality of 'Nova' hybrid [Citrus clementina hort. Ex Tanaka (Citrus reticulata Blanco C. paradisiMacfad)] and two Clementine varieties (C. clementina hort. Ex Tanaka) as affected by self- and cross-pollination. *ScientiaHorticulturae*, 121: 38–41.

18. Singh, G., Aulakh, P. S. and Rattanpal, H. S. 2017. Fruit setting patterns and germination of F1 seeds in some intra-specific crosses of mandarin (*Citrus reticulate* Blanco). *Applied Biological Research,* 19: 264-269.

19. Soost, R. K. and Cameron, J. W. 1975. Citrus. In Advances in Fruit Breeding (J. Janick and J.N. Moore, eds.). Purdue Univ. Press. West Lafayette, Indiana. pp. 507-540.

20. Sweety, G. S., Rana.and Reddy, G., C. 2018. Effect of growth regulators on yield and quality of sweet orange (*Citrus sinensis*L. Osbeck.) cv. Jaffa. *Int J CurrMicrobiol App Sci,* 2963-2967.

21. Talon, M, and Gmitter, F. G. 2008. Citrus genomics. *IntJ Plant Genomics* 1– 17.

22. Ting, S., V. 1980. Nutrients and Nutrition of Citrus Fruits, in Citrus nutrition and quality. Eds. S. NagyJohn and A. Attaway (Washington, DC. USA: American chemical society), 3-24.

23. Vithanage, V. 1991. Effect of different pollen parents on seediness and quality of 'Ellendale' tangor. *SciHortic,* 48:253-60.

24. Wallace, H., M., King, B., J. and Lee, H., S. 2004. Pollen flow and the effect on fruit size in an imperial mandarin orchard. *HorticSci,* 37:84-86.