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Phenotypic evaluation of putative zygotic seedlings in different Inter-specific crosses between sweet orange (*Citrus sinensis* L. Osbeck) and mandarin (*Citrus reticulata* Blanco)

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Abstract

An investigation was carried out to examine the morphological features of molecularly confirmed putative hybrids derived from different cross combinations of sweet orange and mandarin varieties. The results revealed that phenotypic leaf characters of F1 zygotic hybrid seedlings in all the crosses had a simple type of leaf division. The shape of leaves in all the hybrids was either elliptic or lanceolate, without any clear-cut differentiation. Results indicated a significant variation in leaf lamina shape in hybrids, elliptical and lanceolate form was observed in Mosambi ×Mukaku Kishu and Jaffa × Mukaku Kishu, elliptical and obovate leaf lamina type was observed in Mosambi × W. Murcott and Jaffa × W. Murcott cross. Leaf lamina shape in hybrids of Mosambi × Clementine and Jaffa × Clementine was lanceolate and elliptical type. Leaf apex shape that dominates in Mosambi × W. Murcott hybrids were acute and obtuse types. In Mosambi × Clementine hybrids, acuminate and acute forms were more dominant. Leaf apex that dominates in hybrids of Jaffa × Mukaku Kishu cross was acute and acuminate type. Hybrids derived from Jaffa × W. Murcott cross had acute and obtuse leaf apex. While in Jaffa × Clementine cross, hybrids had acuminate and acute as the most dominant forms. Hybrid progenies derived from all the crosses showed variation for the presence and absence of a spine. Results indicated that spiny hybrids ranged from 0 to 20.00 % whereas spineless zygotic hybrids ranged from 80.00 to 100 % in all the crosses. These potential hybrids may be recommended for commercial citriculture after thorough horticultural evaluation. The information obtained is expected to contribute to the early identification of zygotic hybrid seedlings derived from different cross combinations involving polyembryonic parents like sweet oranges. The valuable information gained from this study may help in shortening the breeding cycle and reduce the costs of the scion breeding programme in sweet oranges.

Key words: F1 hybrids, zygotic, nucellar, sweet orange, mandarins, inter-specific cross, morphological, characters

Introduction

Citrus is one of the most important fruit crops in the world due to its nutritional importance. Citrus spp. (2n=18) belongs to the Rutaceae family and encompasses globally commercial fruits like mandarins, sweet oranges, lemons, limes, grapefruits, and pummelos. Sweet orange (*Citrus sinensis* (L.) Osbeck) is the most important species among those belonging to the Citrus genus, representing about 50 % of global citrus production. Despite numerous citrus varieties available worldwide, only a few exhibited desirable quality traits, prompting a need for new varieties as per market demands. Conventional hybridization has been the most important method for genetic improvement in sweet oranges, but it is costly as well as challenging. However, polyembryony parents involved in citrus cross pose a great obstacle in the early identification of hybrids, which lengthen the variety development. Therefore, the integration of molecular markers into the hybridization-based breeding program can expedite the pace of varietal development in citrus (Gmitter *et al.,* 2007). Developing citrus hybrids with improved pomological qualitative traits such as fruit size and shape, rind and pulp color, flavor, seedlessness and round-the-year availability of fruit is the main objective in scion breeding programs by utilizing Citrus spp. genetic diversity. Citrus breeding aims to identify optimal allele combinations for desired traits (Ollitrault and Navarro, 2012), yet breeders face the challenge of creating genotypes that meet market demands, exhibit quality characters, and ensure grower profits (Caruso *et al.,* 2020; Gaikwad *et al.,* 2023). Citrus spp. has limited sexual crossing due to extended juvenile phases, needing 25- 30 years from hybrid seed to commercial release *viz.*, Orri (Spiegel-Roy *et al.,* 2007; Vardi *et al.,* 2008) and Nadorcott cultivar (Nadori, 2004). Early identification of zygotic seedlings is crucial to conserve resources by eliminating nucellar ones. Molecular markers, such as SSRs, were successfully employed for early confirmation of the zygotic seedlings obtained from different inter-specific crosses to overcome the drawbacks of morphological classification (Xu *et al.,* 2006; Kumar *et al.,* 2019). Carlos de Oliveira *et al.* (2002), and Singh *et al.* (2020b).

Furthermore, Gaikwad *et al.* (2024), performed an interspecific cross between Volkamer lemon (♀)×Kinkoji (♂), Volkamer lemon (♀) × Swingle citrumelo (♂), Volkamer lemon (♀)×Cleopatra mandarin (♂), the F1 hybrid seedlings were confirmed by using 25 SSR markers and hybrids were phenotypically evaluated. Fifteen qualitative and six quantitative characters were found to have significant variability in leaf, spine, branch, and stem in hybrids obtained from different crosses. In this study, an evaluation of morphological characters of molecularly confirmed putative zygotic hybrids of different interspecific crosses between sweet orange and mandarin varieties was carried out. The newly developed F1 hybrids exhibited phenotypic and genetic diversity. These potential hybrids may be recommended for commercial citriculture after thorough horticultural evaluation. The information on morphological features of hybrids derived from Jaffa and Mosambi as seed parent and Mukaku Kishu, W. Murcott and Clementine varieties as pollen parent is scarce. The information obtained in this study will not only aid in the early confirmation of zygotic seedlings but also aid in the selection of parents in citrus crop improvement programs. Therefore, the experiment was designed to evaluate hybrids on phenotypic basis to gain information on their morphological features.

Materials and methods

Parent material: The study was conducted at Dr. J. C. Bakhshi, Punjab Agricultural University, Regional Research Station Abohar, India during 2021-2022. A total of 71 confirmed zygotic hybrids in Mosambi × Mukaku Kishu cross was evaluated, followed by 27 in Mosambi × Clementine cross, a total of 25 in Mosambi × W. Murcott, 2 in Jaffa × Mukaku Kishu cross, 10 in Jaffa × W. Murcott cross and 15 in Jaffa × Clementine cross were evaluated for morphological characters.

Hybrids derived from different cross combinations were morphologically evaluated for characters like leaf characters, spine characters, and seedling height (IPGRI, 1999). Data obtained on seedling height was statistically analysed using one-way analysis of variance (ANOVA) with a significant difference ($P \leq 0.05$) in Microsoft Excel version 10. A range was recorded for each parameter in all the hybrids from different cross combinations. The overall mean of the parameters, standard error of mean and coefficient of variation were calculated in each cross. Hybrid progeny were categorized as medium, low and high depending on the resemblance of their character to each other and the range under which these hybrids fall.

Results and discussion

Phenotypic leaf characters: In this study, both qualitative and quantitative characters of confirmed putative zygotic hybrids showed a significant variation in all the hybrids. Morphological characters like leaf lamina shape, leaf lamina margin, leaf apex, division of leaf, the intensity of green colour, variegation in leaf colour, leaf lamina attachment, absence/presence of petiole wings, the junction between petiole and lamina were studied.

Leaf division: In all the F1 hybrids, simple types of leaf division were recorded (Table 1).

Leaf lamina shape: Leaf morphology is one of the most important phenotype markers that assist in the taxonomic classification of citrus. Observations of leaves in different hybrids showed that they have intermediary morphology compared with the leaves of their parental varieties (Table 1). The shape of leaves in all the hybrids was either elliptic or lanceolate, without any distinctive differentiation. Results showed that the most dominant type of leaf lamina shape in all the F1 hybrids of the Mosambi × Mukaku Kishu cross was elliptical and lanceolate type over ovate type. Three types of leaf lamina shape, *viz.,* elliptical, lanceolate and obovate were recorded in hybrids of Mosambi × W. Murcott; however, most dominant type was elliptical and obovate forms. Leaf lamina shape in hybrids of Mosambi × Clementine was recorded as elliptical, lanceolate and ovate type and the most dominant shape was lanceolate and elliptical type. While in hybrids of Jaffa × Mukaku, Kishu elliptical and ovate were dominant form. Leaf lamina shape in hybrids of Jaffa × W. Murcott cross was elliptical, lanceolate and obovate types, and the most dominant type was elliptical and obovate shape. Results revealed that leaf lamina shape in hybrids of Jaffa × Clementine cross varied from lanceolate, elliptical and ovate shape and the most dominant was lanceolate and elliptical form. Intermediary vegetative morphology of allotetraploid plants in relation to their parents was earlier reported in different citrus cultivars (Grosser *et al.,* 1992 and Tusa *et al.,* 1992). Similarly, Louzada *et al.* (1992) reported that the leaf morphology of all recovered somatic hybrid plants was intermediate to that of the parents (sour orange + Volkamer lemon). Somatic hybrids and polyploids possess bigger petiolar wings and thicker leaf blades over both the parents. Furthermore, inter-specific F1 hybrids are usually sterile and intermediate types, due to repulsion between the chromosomes as a result, these occupy opposite sides of the F1 zygote nuclei, thus exerting equal influence in the ontogeny of F1 organisms. Consequently, it exhibits little variation and thus remains sterile and synapsis is often impossible (Miranda *et al.,* 1997). Significant variations in leaf apex was also recorded in all three crosses of citrus *i.e.*, acuminate and acute type of leaf apex was observed in Volkamer lemon (♀) × Kinkoji (♂) cross, in Volkamer lemon (♀) × Swingle citrumelo (♂) cross, acuminate, acute, obtuse, and rounded leaf apex was observed whereas, in Volkamer lemon (♀)×Cleopatra mandarin (♂) cross, acuminate, acute, obtuse, and emarginate type of leaf apex was observed (Gaikwad *et al.,* 2024).

Leaf lamina margin: The result revealed a significant variation in leaf lamina margin among all the F1 hybrids obtained from six different inter-specific crosses (Table 1). Leaf lamina margin in

Table 1. Variation in vegetative characters in F1 hybrid zygotic seedlings

Crosses (♀×♂)	Leaf division	Leaf shape	Leaf margin	Leaf apex	Intensity of green colour of leaf blade	Leaf colour Variegation	Leaf lamina attachment	Junction between petiole and lamina	Shoot tip colour
Mosambi × Mukaku Kishu	Simple	Elliptical-lanceolate	Sinuate-denate	Acute-Acuminate	Medium-Dark	Absent and Present	Brevipetiolate	Articulate	Green
Mosambi × W.Murcott	Simple	Elliptical-obovate	Sinuate-denate	Acute-obtuse	Light-medium	Absent and Present	Brevipetiolate	Articulate	Green
Mosambi × Clementine	Simple	Lanceolate-elliptical	Denate – entire	Acuminate – acute	Light - medium	Absent and Present	Brevipetiolate	Articulate	Green
Jaffa × Mukaku Kishu	Simple	Elliptical-lanceolate	Sinuate-Crenate	Acute-Acuminate	Light-medium	Absent and Present	Brevipetiolate	Articulate	Green
Jaffa × W. Murcott	Simple	Elliptical-obovate	Sinuate-denate	Acute-obtuse	Medium-dark	Absent and Present	Brevipetiolate	Articulate	Green
Jaffa × Clementine	Simple	Lanceolate-elliptical	Denate – entire	Acuminate – acute	Light -medium	Absent and Present	Brevipetiolate	Articulate	Green

F1 hybrids varied from sinuate to crenate, sinuate to denate and denate to entire type. Results revealed that the most dominant type of leaf lamina margin in F1 hybrids of Mosambi × Mukaku Kishu was sinuate to denate type. The most dominant leaf lamina margin in F1 hybrids of Mosambi × W. Murcott was sinuate to denate type. In Mosambi × Clementine hybrids, the leaf margin was recorded as denate to the entire type. The leaf lamina margin in Jaffa × Mukaku Kishu cross was sinuate and crenate type. In Jaffa × W. Murcott hybrids had sinuate to denate forms as the most dominant forms. While Jaffa × Clementine hybrids exhibited denate to entire type leaf lamina margin.

Leaf apex: The result revealed different types of leaf apex, *viz.* acute, acuminate and obtuse type in all the hybrids. The most dominant type of leaf apex in hybrids of Mosambi × Mukaku Kishu was the acute and acuminate type. The leaf apex type that dominate in Mosambi × W. Murcott hybrids was the acute and obtuse type (Table 1). In Mosambi × Clementine hybrids, acuminate and acute forms were most dominant. Leaf apex dominate in Jaffa × Mukaku Kishu cross was acute and acuminate type. F1 hybrids of Jaffa × W. Murcott cross had acute and obtuse as dominant forms. While in Jaffa × Clementine hybrids, acuminate and acute forms were recorded as dominant.

Intensity of green colour of leaf blade: Hybrids obtained from six different inter-specific crosses exhibited varied intensities of green colour of leaf blade (Table 1). Medium to dark green colour intensity was recorded in Mosambi × Mukaku Kishu and Jaffa × W. Murcott. Light to the medium green colour of leaf lamina blade was noticed in Mosambi × W. Murcott, Mosambi × Clementine, Jaffa × Mukaku Kishu and Jaffa × Clementine crosses. Singh and Singh (2006) reported similar variation in leaf colour intensity in F1 hybrids obtained from inter-specific cross between trifoliate orange and Red fleshed pummelo, where medium to dark leaf colour intensities was observed.

Leaf colour variegation: Variegation was observed in leaf colour in all the F1 hybrids of different crosses (Table 1).

Leaf lamina attachment: The result showed no differences in leaf lamina attachment among zygotic hybrids obtained from six different inter-specific crosses (Table 1). All the F1 hybrids had bravipetiolate leaf lamina attachment.

Junction between petiole and lamina: No significant variation was recorded among F1 hybrids for the junction between petiole and lamina (Table 1). Articulate type of junction between petiole was recorded in all the F1 hybrids obtained from six different inter-specific crosses.

Shoot tip colour: Shoot tip colour showed no variation among all the F1 hybrids obtained from six different inter-specific crosses. Result showed the green colour of shoot tip in all the F1 hybrids of all six different crosses (Table 1).

Absence or presence of petiole wings: Results revealed the presence of petiole wing in all the F1 hybrids of six different inter-specific crosses (As no variations were observed among the crosses, the table is omitted).

Petiole wing shape: The result showed no variation in petiole wing shape among all the F1 hybrids. The obovate shape of the petiole wing was noted in all the hybrids obtained from six different inter-specific crosses (As no variations were observed among the crosses, the table is omitted). The broadness of leaf petiole wing can be used as a morphological marker for screening hybrids. Leaf petiole wing was used as a marker in narrow-winged species *Citrus limonia* and *C. sunki* with broad-winged species *C. aurantium* and *C. sinensis* (Ballve *et al.,*1997).

Leaf morphology is another most important reliable morphological marker, which is a dominant phenotypic trait in certain species (Soares-Filho *et al.,* 2000). For example, in trifoliate orange, the expression of the trifoliate leaf is a dominant trait over the recessive unifoliate leaf trait and identification of F1 hybrid seedlings in crosses between unifoliate citrus and trifoliate orange male parents is very easy (Sykes *et al.,* 2011). However, when neither of the crossing parents possess any dominant traits, then the reliability of these markers in identifying hybrids is quite difficult. Not all morphological markers are reliable in distinguishing zygotic from nucellar seedlings (Caruso *et al.,* 2014). However, a combination of molecular and morphological markers could be more reliable to distinguish between zygotic and nucellar seedlings obtained from different cross combinations. It is noteworthy that phenotypic leaf characters in F1 hybrid progenies of all the six crosses showed considerable variation compared to their parents. This variation could be due to quantitative inheritance of the leaf characters (Iwata *et al.,* 2002). Furthermore, this variation in phenotypic leaf characters might be due to the genetic recombination during crossing over between homologous chromosomal segments during meiosis (Jiguang *et al.,* 1995). The genetic background of the parents involved in this cross might also have attributed to the variation.

Seedling height (cm): A significant difference was observed for F1 zygotic seedling height obtained in all crosses. The result revealed that F1 zygotic hybrid seedling's height ranged between 21.56 to 47.34 cm in Mosambi × Mukaku Kishu cross with a mean height of 31.71 cm (Table 2 and Fig. 1). More than half (54.54%) of the seedlings were categorized under medium height followed by high (27.27%) and low (18.19%) category, respectively. In comparison to zygotic seedlings, nucellar seedlings had more plant height of 27.56 to 58.6 cm with an average height of 34.82 cm. 50.15 % of seedlings were grouped under a high category, followed by 28.21 and 20.00 % plants in medium and low category, respectively.

Furthermore, the result revealed that the height of the F1 zygotic hybrid progeny of Mosambi × W. Murcott cross ranged from 12.66 to 38.78 cm with an average seedling height of 25.82 cm. More than half of the seedlings (63.63 %) possessed medium height, followed by 29.00 to 7.37 low and high, respectively. In comparison to zygotic seedlings, nucellar seedlings possessed more height, and their height varied from 23.56 to 48.23 cm, with an average height of 29.83 cm. Most of the hybrid progeny (45.83 %) were grouped under medium, followed by 28.17 and 26 % seedlings in high and low category, respectively. F1 seedling height for hybrid progeny of Mosambi × Clementine cross ranged from 22.34 to 44.35 cm with an average seedling height of 31.75 cm. More than half of the hybrids (55.64 %) possess medium height, followed by 30 and 14.36 low and high, respectively.

In contrast, nucellar seedling height ranged from 25.88 to 56.60 cm with an average of 31.75 cm height. Most of the hybrid progeny (45.64 %) were grouped in the high category, 36.36 % were in the medium category and 18 % of seedlings were in the low category, respectively. Plant height in F1 zygotic hybrid seedlings of Jaffa × Mukaku Kishu cross ranged from 23.17 to

Fig. 1. Variation in seedling height (cm) of F1 hybrid individuals obtained from different inter-specific crosses. A) Mosambi x Mukaku B) Kishu Mosambi x W. Murcott C) Mosambi x Clementine, D) Jaffa x Mukaku Kishu E) Jaffa x W. Murcott F) Jaffa x Clementine

Table 2. Variation in F1 zygotic hybrid and nucellar seedling of different inter-specific crosses for seedling height

Crosses (♀×♂)	Range (cm)	Mean	S.E. (Mean)	C.V. (%)	Category (%)		
					Low	Medium	High
Mosambi x Mukaku Kishu							
Zygotic	21.56-47.34	31.71	1.29	4.97	18.19	54.54	27.27
Nucellar	27.56-58.60	34.82	1.11	3.72	21.85	28.00	50.15
Mosambi x W. Murcott							
Zygotic	20.66-38.78	25.82	1.22	4.44	29.00	63.63	7.37
Nucellar	23.56-48.23	29.83	1.19	4.24	26.00	45.83	28.17
Mosambi x Clementine							
Zygotic	22.34-44.35	31.38	1.48	6.60	30.00	55.64	14.36
Nucellar	25.88-56.60	31.75	1.26	4.80	18.00	36.36	45.64
Jaffa x Mukaku Kishu							
Zygotic	23.17-43.55	29.29	1.31	5.15	33.33	33.33	33.34
Nucellar	28.25-45.18	32.43	1.33	5.29	4.93	32.54	62.53
Jaffa x W. Murcott							
Zygotic	11.24-26.52	18.6	1.38	5.71	71.11	21.98	6.91
Nucellar	24.04-33.11	28.11	1.39	5.76	36.29	60.37	3.34
Jaffa x Clementine							
Zygotic	20.26-32.03	24.39	1.43	6.15	21.11	67.54	11.35
Nucellar	27.13-38.33	29.67	1.44	6.20	24.10	58.38	17.51

43.55 cm with an average value of 29.29 cm. Results showed that 33.33 % of hybrids were grouped in low, medium and high categories. Meanwhile, nucellar seedlings had heights ranging from 28.25 to 45.18 cm with an average of 32.43 cm height. Most of the hybrid progeny were grouped under a high category (62.53 %). Hybrid progeny in Jaffa × W. Murcott cross possess seedling heights ranging between 11.24 to 26.52 cm with a mean 18.6 cm height. More than half of the hybrid seedlings (71.11 %) exhibited low height and 21.98 and 6.91 % as medium and low category, respectively. The height of the nucellar seedlings ranged between 24.04 to 33.11 cm, with an average height of 28.11cm. More than half of seedlings (60.37 %) were grouped in the medium category, followed by 36.29 and 3.34 % of seedlings in the low and high category, respectively.

F1 zygotic hybrid seedlings' height of Jaffa × Clementine cross ranged between 16.26 to 32.03 cm with a mean height of 24.39 cm. More than half of the hybrid seedlings (67.54 %) possess medium height, followed by 21.11 and 11.35 % grouped in low and medium category, respectively. On the other hand, nucellar seedling height varied between 27.13 to 38.33 cm, with the mean value of 29.67cm as the average height. More than half of seedlings (58.38 %) were grouped in the medium category, followed by 24.11 and 12.51 percent plants in the low and high category, respectively. Our results are in accord with the findings of Kaur *et al.* (2020), who reported a varying range between 0.74m to 0.62m plant height of hybrids derived from an intra-specific cross between Kinnow × Mukaku Kishu mandarin. The inheritance pattern of dwarfness character was studied in Poncirus trifoliate cv. Flying Dragon and the dwarfness character were governed by a single dominant gene (Cheng and Roose, 1995). Singh *et al.* (2020a) reported a hybrid plant height of 22.2 cm in Rough lemon × X-639 Citrandarin cross and 24.7 cm seedling height was recorded for hybrid seedlings obtained from Rough lemon × Swingle citrumelo cross. Furthermore, the results indicated that zygotic hybrid progeny showed lesser growth in terms of plant height over the nucellar seedlings obtained from different inter-specific crosses. Similarly, Cameron and Jonhston (1949) observed vigorous growth in nucellar seedlings and the presence of thorns. Villegas-Monter *et al.* (2022) also reported that vigorous growth of nucellar seedlings is the first phenotypic visible morphological character used to differentiate nucellar plants from zygotic ones.

Spine characters in hybrids derived from different inter-specific crosses

Absence or presence of spine: The result revealed that both spiny and spineless types of hybrids were obtained in different proportions in all the cross combinations. Similarly, Kaur *et al.* (2020) also obtained both spiny and spineless F1 hybrids in an intra-specifc cross between mandarins, *i.e.*, Kinnow (♀) and Mukaku Kishu (♂) (Table 3).

Spine density: The data given in Table 3 indicated no variation in spine density among F1 hybrids. Results also indicated that spine density was low in all the F1 hybrids obtained from six different inter-specific crosses.

Spine shape: F1 hybrids showed no variation in spine shape (Table 2). Straight spine shape was recorded in all the F1 hybrids obtained from different inter-specific crosses.

Hybrid progeny evaluated for spine traits: Both spiny and spineless hybrid progenies were obtained in varying numbers in all the cross combinations (Table 3). In Mosambi × Mukaku Kishu cross 8 (11.27 %) hybrids were spiny type and 63 (88.73 %) were spineless out of 71 hybrids. In Mosambi × W. Murcott cross, 3 (12.00%) hybrids were spiny type and 22 (88.00 %) were spineless out of 25 hybrids. Furthermore, in Mosambi × Clementine, 4 (14.81 %) hybrids were recorded as spiny and 23 (85.19 %) hybrids were spineless out of 27 hybrids. In Jaffa × Mukaku Kishu cross, none of the hybrid progeny possessed spines and both the hybrids obtained were spineless type. Similarly, none of the hybrids possessed spines in Jaffa× W. Murcott cross; all were spineless. In Jaffa × Clementine cross, 3 (20.00 %) hybrids were recorded as spiny type and 12 (80.00 %) hybrids were spineless out of 15 hybrids. Similar results of both spiny and spineless hybrids were reported in interspecific cross between Volkamer lemon (♀) × Kinkoji (♂) cross, Volkamer lemon (♀) × Swingle citrumelo (♂) cross, Volkamer lemon (♀)×Cleopatra mandarin (♂) cross (Gaikwad *et al.,* 2024). Most of the hybrids showed straight spines on every node; this character was inherited from the female parent, but some hybrids had not shown due to segregation (Bowman, 1998).

Results indicated that spiny hybrids ranged from 0 to 20.00 % and spinelessness ranged from 80.00 to 100 % (Table 3). The variation obtained in a number of F1 hybrids with or without spines indicates the genetic contribution of two parents. Parents involved in this cross also varied from each other for spine characters, the mature trees of Mosambi and Jaffa sweet orange (seed parents) possess spines whereas, mature trees of Mukaku Kishu, W. Murcott and Clementine mandarins (pollen parents) had no spines. Therefore, the variation in the thorniness traits within the hybrid progeny may be due to the segregation and genetic contribution of both the parents involved in this cross. Citrus cultivars differ greatly with respect to the expression of spiny characters, indicating that thorniness has a genetic base. According to Spiegel-Roy and Teich (1972), two histogenic layers, L1 and L2, which are a few dominant genes, may govern the thorniness characteristics in citrus cultivars. The results of this study agree with those of the previous studies, Kaur *et al.* (2020) identified varying numbers of spiny and spineless hybrids from the intra-specific cross between Kinnow × Mukaku Kishu mandarin using SSR markers. Spiny hybrids had spine size less than 4mm in length, while 25 percent of hybrids were spineless. These new hybrid progenies obtained are expected to contribute significantly to the future scion breeding program in sweet oranges as a source of high fruit quality in terms of seedlessness and easy-peel characteristics. Collectively, the results from this study suggest that the analysis of molecular markers may contribute significantly to citrus breeding programs.

Table 3. Variation in number of spiny and spineless F1 zygotic hybrid progeny obtained from different inter-specific crosses

Crosses (♀×♂)	F1 zygotic hybrid seedling				
	Total No.	Spiny (No)	Spineless (No)	Spiny (%)	Spineless (%)
Mosambi × Mukaku Kishu	71	8	63	11.27	88.73
Mosambi × W. Murcott	25	3	22	12.00	88.00
Mosambi × Clementine	27	4	23	14.81	85.19
Jaffa × Mukaku Kishu	2	-	2	-	100
Jaffa × W. Murcott	10	-	10	-	100
Jaffa × Clementine	15	3	12	20.00	80.00

The study helped in finding the morphological variations in hybrids derived from different cross combinations between sweet orange and mandarin varieties. The results of the study are expected to be useful in the verification of zygotic hybrids at an early seedling stage. The dominant leaf morphological characters observed in different hybrids can be used as an index along with molecular markers to identify zygotic hybrids derived from different cross combinations involving polyembryonic citrus parents. The combination methods of selection could be used as a new approach that saves time and reduces the costs involved in maintaining large numbers of seedlings in citrus breeding programs. The results may help the breeders make an efficient early selection of zygotic hybrids and help in the selection of best-combining parents. It also helps in designing a breeding programme with sweet oranges for proper utilization of genetic resources. However, the performance of hybrids obtained from different cross combinations needs to be evaluated for quality and quantitative characteristics by comparing them with their parents through further field trials.

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