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Hybridization Studies on some Clementine (*Citrus clementina*) Cultivars By

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ABSTRACT

The present study was conducted using three Clementine cultivars, namely, Fedela, Fina, and Spinosa as female parents, and four mandarin cultivars, namely, Kishu, Avana aprieno, Sunburst, and Willow leaf as male parents, to select the pollen source that has high cross-compatibility with the Clementine cultivars. The results showed that 'Kishu' has the highest viability in comparison with other cultivars. Both 'Fedela' with 'Kishu', 'Spinosa' and 'Sunburst' had the maximum initial fruit set. The highest percentages of retained fruit were registered when pollen of 'Kishu' was used to pollinate both 'Fina' and 'Fedela'. When all pollens of tested Mandarin cultivars were used for 'Spinosa' Clementine, they did not obtain any retained fruit, and these hybridization combinations revealed the highest percentages of fruit drop. 'Fedela' fruits recorded a maximum value of the average number of developed and undeveloped seeds when they were cross-pollinated with 'Kishu' and 'Sunburst' pollens. Fluorescence microscopy examination showed that 'Kishu' and 'Avana' as pollen sources had a high level of full cross-compatibility with 'Fedela', while 'Sunburst' and 'Willow leaf' have a high level of partial cross-compatibility with 'Fedela'. 'Fina' showed a low degree of partial cross-compatibility, while 'Spinosa' showed some degree of cross-incompatibility with all studied pollen sources. It can be concluded that all studied Mandarin cultivars could be promising pollenizers for the self-incompatible 'Fedela' Clementine cultivar, thus it is recommended to culture these cultivars together in the same orchard.

Key words: Citrus, compatibility, cross-pollination, pollen tube, breeding

1. INTRODUCTION

Clementine (*Citrus clementina* Hort.) is a tangor, a hybrid between a willow leaf Mandarin (*Citrus deliciosa* Ten.) and a sweet orange (*Citrus sinensis* (L.)). This hybridization produced an easy-peeling fruit with a very sweet flavor, and the exterior is a deep orange color with a smooth, glossy appearance. It also ripens much faster than a Mandarin. Clementine is an important citrus group because they produce high-quality, nearly seedless fruits when grown alone (without cross-pollination) because of their sexual self-incompatibility and give seeds when they are cross-pollinated withother citrus varieties. Clementine is one of the most significant mono-embryonic cultivars of Mandarin

that consistently experiences issues with fertilization and fruit set if cross-pollination does not occur because of its self-incompatibility. Selfincompatibility is a system that inhibits selffertilization. Most citrus hybrids appear to be affected by a gametophytic self-incompatibility (Distefano et al., 2009). However, a variable level incompatibility has been observed for of Clementine cultivars (Ton and Krezdorn, 1966), suggesting that in citrus, self-incompatibility interactions between style and pollen tubes could be categorized by the level of incompatibility of each part of the style (Yamamoto and Tominaga, 2002). Therefore. cross-pollination with appropriate pollen is one way of overcoming self-

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incompatibility. Excellent pollen sources for Clementine ability to produce seeds include Valencia orange, grapefruit, and mandarin (Barry, 1995). Numerous countries are creating new cultivars more appropriate to their environment by applying various breeding techniques (Ollitrault and Navarro, 2012). Cross-pollination is one of these techniques, which has been applied for the creation of new cultivars for a long time.

The present study was conducted to characterize cross-compatibility and incompatibility reactions between Clementine cultivars as a seed source and Mandarin cultivars as a pollen source and to find out the best pollen source of high crosscompatibility with Clementine cultivars under study, as well as the creation of new varieties from progenies of these hybridization combinations that may have desirable traits derived from both parents, could be introduced to citrus breeding programs later.

2. MATERIAL AND METHODS

2.1. Plant material

The study was conducted over two successive seasons (2020 and 2021) on three Clementine cultivars, namely, Fedela, Fina, and Spinosa (*Citrus clementina*) as female parents (seed source) and four Mandarin cultivars namely, Kishu, Avana Aprieno, Sunburst, and Willow leaf (*Citrus deliciosa*) as male parents (pollen source). Trees of all the cultivars under study were 12-years-old and grown in the citrus orchard of Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

2.2. Pollen viability assessment

Unopened flower buds of the four Mandarin cultivars were collected at balloon stage (before petals opening) from mature field-grown trees and kept in paper bags until use. Afterwards, the anthers were excised from the stamens using forceps and placed in a clean Petri dish that was kept at room temperature for 2-3 days. A great amount of pollen dispersed, which increased by hand shaking the Petri dish. The anthers were divided into delicate fragments with a razor blade until the anthers and pollen combination showed a powdery appearance. Finally, the pollen was kept in penicillin vials closed with cotton and stored at 4°C until use. The viability percentage of pollen grains of all Mandarin cultivars was estimated using the TTC (2,3,5-triphenyl tetrazolium chloride) stain test (Norton, 1966). Pollen grains were incubated in 1% TTC solution (0.2 g TTC and 12 g sucrose, dissolved in 20 ml distilled water). After two hours, a drop of the mixture was placed on a microscope slide and the pollen spread with a slim brush and covered with a coverslip. The redcolored pollen grains were recorded as viable at 100X magnification, using a light microscope. The pollen viability percentage of all the Mandarin cultivars under study was calculated as follows:

Pollen viability % = No. of stained pollen \times 100 / Total number of pollens.

2.3. Hybridization procedures

Five branches per tree of the three Clementine cultivars were selected and emasculated at balloon stage by removing stamens with forceps, then immediately hand pollinated by the Mandarin cultivar pollen with a soft hairbrush immersed in a pollen vial, and then touching the sticky stigma. To avoid open pollination with unwanted pollen grains, all flower buds that had been pollinated were covered with paper bags, and then labeled with the number of pollinated flowers per bag and pollination date. All hybridization combinations and the number of pollinated flowers is shown in Table (1). The experiment comprised 12 hybridization combinations (Clementine trees) with 5 replicates (branches). At 7-10 days after pollination, the stigmas of the pollinated flowers turned brown, and the styles began to dry and fall. After that, the paper bags were removed, and the initial fruit set percentage was recorded about 21 days after pollination. After the June drop period and pre-harvest date, retained fruit and total fruit drop percentages were calculated as follows:

Initial fruit set % = Number of fruitlets \times 100 / Total numbers of pollinated flowers.

Retained fruit % = Number of final remained fruits \times 100 / Total numbers of pollinated flowers.

Total fruit drop % = 100 - Retained fruit (%) At harvest time, all remained fruits were collected and then the seeds of mature fruits derived from different hybridization combinations were

Table	(1): Hybridizat	tion c	ombination	s between
	Clementine	and	Mandarin	cultivars
	and the num	ber of	f pollinated	flowers.
				No of

Hybridization combinations	No. of pollinated flowers
Fedela Clementine × Kishu Mandarin	78
Fedela Clementine× Avana Aprieno Mandarin	65
Fedela Clementine ×Sunburst Mandarin	73
Fedela Clementine × Willow leaf Mandarin	80
Fina Clementine × Kishu Mandarin	66
Fina Clementine × Avana Aprieno Mandarin	52
Fina Clementine × Sunburst Mandarin	73
Fina Clementine × Willow leaf Mandarin	58
Spinosa Clementine × Kishu Mandarin	38
Spinosa Clementine × Avana Aprieno	23
Mandarin	
Spinosa Clementine × Sunburst Mandarin	29
Spinosa Clementine× Willow leaf Mandarin	37
Total	672

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extracted. The number of developed and undeveloped seeds per fruit was recorded. The developed seeds were planted in boxes filled with peat-moss and sand (1:1) inside the greenhouse. After seed germination (Fig. 1a), the hybrid seedlings were transferred into plastic bags (Fig. 1b).





2.4. Histological analysis

Five pistil samples were collected from flowers of each pollinated hybridization combination, on 3, 5, 7, 9, 11, 13, 15, and 20 days after pollination (DAP). The samples were instantly transferred to the FAA solution (5 ml formalin, 5 ml glacial acetic acid, and 90 ml ethanol 70%) for fixation and kept at 4°C until use. The fixed samples were washed in running tap water for 24 hrs. to eliminate any trace of FAA, and then softened in 8 N NaOH for 5 hrs. in order to facilitate penetration of stain solution into sample tissues. All samples were washed overnight in running tap water to be free of NaOH. Finally, samples were dyed with 0.1% of aniline blue dissolved in 0.1 N K₃PO₄ and then stored at 4°C for 24 hours before microscopic examinations as described in Kho and Baer (1968).

2.5. Fluorescence microscopy examination of pollen grain germination and pollen tube growth

The fluorescence in ultraviolet light allows the pollen tube growth to be traced through the style tissue and identifies the rejection site. This technique has already been described by Martin (1958). The pistils were chopped along the length of the style, squashed, and then studied under a Leica fluorescence microscope to determine the pollen tube's growth rate. The stigma was examined, and pollen grains on the surface and the appearance, development, and anomalies of pollen tubes growing in the fleshy bulbous region were evaluated. The top, center, and base parts of the style as well as those that reached the ovary were examined in samples showing pollen tube penetration through the stigma.

2.6. Statistical analysis

The analysis of variance (ANOVA) test was conducted on the data from the current study using a randomized complete block design.

At the 5% level of probability, the least significant differences (L.S.D.) were calculated using a computer program Costat according to Snedecor and Cochran (1980).

3. RESULTS AND DISCUSSION

3.1. Pollen viability

Data in Table (2) and (Fig. 2 a-d) show the pollen viability percentage of Mandarin cultivars under study. 'Kishu' Mandarin exhibited the highest value of viability (82.8 %) in comparison to the other cultivars. On the other hand, the 'Avana Aprieno' Mandarin recorded the least value (71.6%) with significant differences between them.

Several studies were reported on the assessment of pollen viability in citrus. Baswal et al. (2015) found that the maximum pollen viability was recorded in 'Mosambi' variety of sweet orange (80%), while the minimum pollen viability was recorded in 'New Hall Navel' (5%). Likewise, Demir et al. (2015) also found that the highest percentage of viable pollen was recorded in the lemon 'Mayer' variety (86.74), while the minimum pollen viability was found in Batem 'Sarisi' lemon (40.62). The highest pollen viability was obtained from the blood orange cultivars 'Sanguinello' (22.15%) and genotype H3 (43.38%), whereas 'Moro'' exhibited the least pollen viability (7.43%) (Oruç and Dalkiliç, 2017). Generally, assessment of pollen viability is an important tool in fruit trees

 Table (2): Pollen viability percentage of Mandarin cultivars.

cultivals	
Mandarin cultivars	Pollen viability (%)
Kishu	82.8 a
Avana Aprieno	71.6 c
Sunburst	74.2 bc
Willow leaf	77.8 ab

Means followed by the same letter(s) in each column are not significantly different at (P < 0.05) using Duncan's multiple range test.

and is essential to improve the effectiveness of breeding programs and the selection of a suitable pollinator for varietal crop improvement.

3.2. Effect of different pollen sources on initial fruit set, retained fruit, and fruit drop percentages of Clementine cultivars



Fig. (2): Some viable pollen of Mandarin cultivars.

- a) Avana Aprieno, b) Sunburst, c) Willowleaf and d) Kishu
- *The arrows indicate non-viable pollen.

Clementine initial fruit set, retained fruit, and fruit drop are shown in Table (3). Crosspollinating of 'Fedela' Clementine with 'Kishu' Mandarin pollen, recorded the highest initial fruit set (38.5%). A marked decrease in initial fruit set values was noticed when both of 'Sunburst' and 'Willow leaf' (16.4 and 15.2%), respectively, were used without significant differences between them. On the other hand, the least percentage of initial fruit set was obtained with 'Avana Aprieno' pollen (1.5%). Concerning 'Spinosa' Clementine, data showed the highest percentage of initial fruit set (37.9%) when it was cross-pollinated with 'Sunburst' Mandarin pollen and was followed in descending order by 'Kishu' (26.3%), 'Avana Aprieno' (21.7%), and 'Willowleaf' (21.6%). Regarding 'Fina' Clementine, data showed that using both of 'Willowleaf' and 'Kishu' pollens gave the highest percentage of initial fruit set (22.4 and 21.2%), respectively, and with insignificant differences between them, while using 'Avana Aprieno' pollen recorded the lowest value of initial fruit set (11.5%).

The data emphasized that the retained fruit percentage of hybridization combinations was affected by different pollen source. However, the highest percentages of retained fruit were registered when pollen of 'Kishu' Mandarin was used to pollinate both of 'Fina' and 'Fedela' Clementines (19.7 and 19.2%), respectively, followed by 'Willowleaf' Mandarin with 'Fina' Clementine (17.2%) and with insignificant differences between them. On the other hand, the lowest percentages of retained fruit were recorded when all pollens of Mandarin cultivars were used for 'Spinosa' Clementine flowers, which did not record any retained fruit, and these hybridization combinations revealed the highest percentages of fruit drop (100.0%). Combinations of 'Fina' \times 'Kishu' and 'Fedela' × 'Kishu' showed the lowest percentages of fruit drop (80.3 and 80.8%), respectively.

Atawia et al. (2016), reported a marked increase in fruit set when Clementine flowers were cross-pollinated with March grapefruit pollen, followed in descending order by cross-pollination with Balady orange pollen. The highest values of fruit retained (%) were recorded when crosspollination with March grapefruit pollens was used, followed in descending order by crosspollination with Succary orange pollens, with Balady Mandarin pollens, with Balady orange pollens and then by open pollination. Another study revealed that Clementine flowers crosspollinated with sweet lemon pollen gave the highest percentage of fruit set (73.75%), followed by Lisbon lemon pollen (69.25%) (Jahromi et al., 2019). Pollination studies were conducted by Chao (2005) on 'Nules', 'Fina Sodea', 'Marisol', 'Fina' Clementine, 'Afourer', 'Tahoe Gold', and 'Gold Nugget' Mandarin who reported that the highest fruit set (20 to 40%) was obtained using cross-pollination between two Clementines ('Nules' and 'Fina Sodea'), and a Mandarin ('Afourer').

Hybridization combinations		Percentage (%)		
Clementine cultivars (Female parent)	Mandarin cultivars (Male parent)	Initial fruit set	Retained fruit	Total fruit drop
	Kishu	38.5a	19.2a	80.8 ef
Fedela	Avana Aprieno	1.5f	1.5ef	98.5a
	Sunburst	16.4cde	4.1de	95.9b
	Willow leaf	15.2cde	5.1cd	94.9bc
	Kishu	21.2bcd	19.7 a	80.3 f
Fina	Avana Aprieno	11.5e	11.5b	88.5d
	Sunburst	13.7de	6.9c	93.1c
	Willow leaf	22.4bc	17.2a	82.8e
	Kishu	26.3b	0.0f	100.0a
Spinosa	Avana Aprieno	21.7bc	0.0f	100.0a
	Sunburst	37.9a	0.0f	100.0a
	Willow leaf	21.6bc	0.0f	100.0a

 Table (3): Effect of different pollen sources on initial fruit set, retained fruit and fruit drop percentages of Clementine cultivars.

Means followed by the same letter(s) in each column are not significantly different at (P < 0.05) using Duncan's multiple range test.

3.3. Effect of different pollen source on number of developed and undeveloped seeds per fruit of clementine cultivars

As shown in Table (4), there was a significant variation between the different hybridization combinations in the number of developed and

undeveloped seeds per fruit. It is clear that 'Fedela' Clementine fruits recorded the maximum value of the average number of developed and undeveloped seeds when they were cross-pollinated with 'Kishu' and 'Sunburst' Mandarin pollens (22.1 and 7.7 seeds/fruit), respectively. The lowest values of the average number of developed and undeveloped

 Table (4): Effect of different pollen sources on number of developed and undeveloped seed per fruit of Clementine cultivars.

Hybridization combinations		Number of seed/ Fruit		
Clementine cultivars (Female parent)	Mandarin cultivars (Male parent)	Developed	Undeveloped	
	Kishu	22.1a	3.8c	
Fedela	Avana Aprieno	18.0b	0.0f	
	Sunburst	13.0c	7.7 a	
	Willow leaf	11.5 c	4.5 b	
	Kishu	8.3d	0.5ef	
Fina	Avana Aprieno	8.2d	1.0 de	
	Sunburst	7.8d	0.6e	
	Willow leaf	7.0d	1.3d	
	Kishu	0.0e	0.0f	
Spinosa	Avana Aprieno	0.0e	0.0f	
	Sunburst	0.0e	0.0f	
	Willow leaf	0.0e	0.0f	

Means followed by the same letter(s) in each column are not significantly different at (P < 0.05) using Duncan's multiple range test

seeds were obtained from 'Fina' Clementine fruits with all tested Mandarin cultivars.

Similar results were obtained by Wallace (2004), who stated that 'Oroval' Clementine crosspollinated with 'Imperial' Mandarin contained approximately 15 to 20 seeds per fruit. Clementine flowers pollinated by Lisbon lemon pollen gave the highest hale seeds (12.22%) (Jahromi *et al.*, 2019). Also, Chao (2005) obtained about 23 to 32 seeds per fruit in Clementine flowers pollinated by 'Afourer' Mandarin.

3.4. Fluorescence microscopy examination of pollen grain germination and pollen tube growth pollen tube characteristics in 'Fedela' style combination of 'Fedela' Clementine × 'Kishu' Mandarin

After cross-pollination, it was possible to observe pollen grains germination on the stigma surface and pollen tubes growing through the style in 'Fedela' Clementine. In the case of pollination with 'Kishu' Mandarin pollen, the results showed that numerous pollen grains normally germinated on the stigma surface followed by the formation of pollen tubes two days after pollination (Fig. 3a, b). Four days after pollination, the pollen tubes grew across the stigma surface and passed down into the style (Fig. 3c, d). After that, pollen tubes reached the lower part of the style ten days after pollination (Fig. 3e). The ovules were penetrated within 10-13 days after pollination (Fig. 3f). It is obvious that the combination of 'Fedela' Clementine \times 'Kishu' Mandarin showed a high level of full cross-compatibility.

3.5. Combination of 'Fedela' Clementine × 'Avana Aprieno' Mandarin

Microscopic examination clarified that 'Avana Aprieno' pollen grains germinated on the stigma surface of the 'Fedela' Clementine three days after pollination. All the pollen tubes were visible in the top portion of the style four days after pollination, and they reached the lower part ten days after pollination. It is obvious that the combination of 'Fedela' Clementine × 'Avana Aprieno' Mandarin showed a high level of full cross-compatibility.

3.6. Combination of 'Fedela' Clementine × 'Sunburst' Mandarin

In the case of pollination of 'Fedela' flowers with 'Sunburst' pollen, the pollen grains germinated on the stigma surface, and their development was then tracked through the style to determine the extent of pollen tube penetration into



Fig. (3): Pollen tube characteristics of full compatible crosses.

- a) Pollen tubes were visible on the stigma.
- **b**) Pollen tubes were visible in the upper part of the style.
- c) Pollen tubes grew about half the length of the style.
- d) Pollen tubes reached the lower part of the style.
- e) Pollen tubes reached the end of the style ten days after pollination.
- **f**) Pollen tubes penetrated into the micropyle and then traversed the nucleus and fertilized the ovules.

the various tissues of the style and the ovary. Pollen tube morphologies were similar to those of the combination of 'Fedela' \times 'Kishu'; however, the growth speed of some pollen tubes was slower and the other tubes' growth stopped before they reached the end of the style. It is obvious that the combination of 'Fedela' Clementine \times 'Sunburst' Mandarin showed a high level of partial cross-compatibility.

3.7. Combination of 'Fedela' Clementine × 'Willow leaf' Mandarin

As previously mentioned, the same results were obtained by the microscopic examination, which showed that the combination of 'Fedela' Clementine \times 'Willow leaf" Mandarin had a high level of partial cross-compatibility also.

3.8. Pollen Tube Characteristics in 'Fina' Style

Microscopic examination showed a low degree of partial cross-compatibility in 'Fina' flowers when pollinated with all studied pollen sources. Several pollen grains germinated on the stigma surface (Fig. 4a), and as their growing pollen tubes moved toward the ovary, their number was reduced until eventually only seven or eight (Fig. 4b, c) reached the lower part of the style and penetrated the ovules thirteen days after pollination. There was no variation in the growth characteristics of pollen tubes along the longitudinal axis of the style.

3.9. Pollen tube characteristics in 'Spinosa' style

All the pollen grains on the stigma surface germinated, demonstrating that the pollen grains

used in the study were all viable and capable of sprouting (Fig. 5a, b). However, the pollen tubes stopped growing in the first half of the style four days after pollination (Fig. 5c). Moreover, the pollen tubes were short and spiral with irregular callose sedimentation (Fig. 5d, e). Microscopic examination showed some degree of crossincompatibility trait in 'Spinosa' flowers when pollinated with all studied pollen sources. These previous reasons may explain why 'Spinosa' Clementine flowers did not give any retained fruit when they pollinated with all pollen of Mandarin cultivars and the percentage of fruit drop was 100% as above-mentioned.

Generally, the stigmas of the compatible and incompatible crosses didn't show any noticeable differences (Fig. 3a, b and Fig. 5a, b). However, it was noticed that the styles of the two types of crosses had clear differences. The compatible combinations revealed thin-walled pollen tubes that grew straight, untwisted, and produced regularsized callose plugs at periodic intervals (Fig. 3a-c), reaching the base of the style (Fig. 3e). But in incompatible combinations in the style, fewer pollen tubes grew (Fig. 5 c), and they stopped, displaying changed morphologies with irregular callose depositions in the tube walls (Fig. 5 f, g) and thick callose deposition at the ends of the pollen tubes (Fig. 5 h), which led to a null number of pollen tubes at the style base (Fig. 5i). The lack of pollen tube growth along the style and ovary indicated the cross-incompatibility in 'Spinosa' Clementine with all studied pollen sources may be due to gametophytic nature.



Fig. (4): Pollen tube characteristics of partial compatible crosses.

- **a**) Germination of numerous pollen grains on the stigma and growth of pollen tubes across the stigma surface and passed down into the style.
- b) Pollen tubes reached half the length of the style ten days after pollination.
- c) A few pollen tubes reached the lower part of the style thirteen days after pollination.



Fig. (5): Pollen tube characteristics of incompatible crosses.

a) Germination of all pollen grains on the stigma surface.

b) Pollen tubes pass down into the style.

c) Less pollen tube grows along the style which was arrested.

d and e) Pollen tubes are short and spiral with irregular callose sedimentation.

f and **g**) Irregular callose deposition in the tube walls.

h) There is thick callose deposition at the ends of the pollen tubes.

i) There were no pollen tubes at the style base.

The present results are in accordance with previous studies by Chao (2005), who stated that crosscompatibility among 'Nules', 'Fina Sodea', 'Marisol', and 'Fina' Clementine and 'Afourer' Mandarin was very high. Findings from microscopic examination of pollen tubes obtained by Jahromi et al. (2019) revealed that the style base and ovary of Clementine were most successfully penetrated by sour orange pollen. Wallace and Lee (1999) observed that in pollination combinations of the 'Eleanor' and 'Murcott' Mandarins, pollen tubes were found in the upper part of the style and at the style base. Pollen tube growth of 'Ellendale', 'Ellenor', and 'Murcott' through the style of 'Imperial' was not inhibited. On the other hand, in some citrus cultivars, such as Clementine, varying levels of incompatibility have been recorded (Distefano *et al.*, 2012). Ton and Krezdorn (1966) observed various levels of incompatibility in Clementine cultivars, and it has been suggested that reactions of incompatibility between the pollen tubes and the style can be categorized by the level of self-incompatibility of each part of the style (Yamamoto and Tominaga, 2002). Kacar *et al.* (2015) stated that none of the 40 Clementine tested cultivars had any pollen tubes that had reached the style base; all were stopped in growth at the top or mid-portion of the style. Additionally, it was stated that this behavior may be affected by the maturation of pistil and style through pollination and being operative no longer before flowering (Ngo *et al.*, 2001).

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Conclusion

According to the obtained results, the study concludes that 'Kishu' and 'Avana' Mandarin as a male parent pollen source considered highly crosscompatible with 'Fedela' Clementine as a female parent, while 'Sunburst' and 'Willow leaf' proved highly partial cross- compatible with 'Fedela'. Among all studied pollen sources, 'Fina' Clementine showed a low degree of partial crosscompatibility and 'Spinosa' showed some degree of cross-incompatibility. According to the previous results, all studied Mandarin cultivars as pollen sources could be good, promising pollenizers for the self-incompatible 'Fedela' Clementine cultivar, and then these cultivars can be cultured together in the same orchard.

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Author agreement

The author has read and agreed to the published version of the manuscript.

Competing interests

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this manuscript.

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ملخص

أجريت هذه الدراسة على ثلاث أصناف من الكليمانتين (فينا، سبينوزا وفيديلا) كمصدر للبذرة (مؤنث) مع أربعة أصناف من اليوسفي (أفانا أبريانو، ويلوليف، سان بيرست وكيشو) كمصدر لحبوب اللقاح (مذكر) بهدف تحديد أفضل صنف من اليوسفي (مصدر اللقاح) له درجة عالية من التوافق الخلطي مع أصناف الكليمانتين محل الدراسة. تم تقدير النسبة المئوية لحيوية حبوب اللقاح لكل أصناف اليوسفي اتحت الدراسة. أجريت التهجينات المختلفة بين أصناف الكليمانتين واليوسفي وتقدير النسبة المنوية لكل من نسبة العُقد المبدئي، للثمار المتبقية، إجمالي تساقط الثمار ، البذور المتطورة (الكاملة) وغير المتطورة (غير الكاملة) لكل ثمرة من أصناف الكليمانتين. أجرى أيضا الفحص الميكروسكوبي الفلوروسينتي لإنبات حبوب أللقاح علي سطح الميسم وكذلك نمو الأنابيب اللقاحية خلال القلم. أظهرت النتائج أن صنف يوسفي "كيشو" أعطى أعلى نسبة حيوية حبوب اللقاح مقارنة بالأصناف الأخري. وكانت أعلى نسبة من عقد الثمار المبدئي في صنف كليمانتين "فيديلا" عند تلقيحه بلقاح "كيشو". تم الحصول على أعلى نسبة من الثمار المتبقية عند استعمال صَنف يوسفي "كيشو" في تلقيح كلا من "فينا" و "فيديلا" عند تهجين صنف كليمانتين "سبينوزا" مع كل أصناف اليوسفي فلم يتم الحصول على أي ثمار وسجلت هذه التهجينات أعلى نسبة تساقط ثمار. وسجلت ثمار صنف "فيديلا" أعلى متوسط لعدد البذور المتطورة وغير المتطورة عند تلقيحها بلقاح كلا من "كيشو" و"سان بيرست". تبين من الفحص الميكروسكوبي الفلوروسينتي أن صنفي اليوسفي "كيشو" و "أفانا أبريانو" كمصادر للقاح أظهرا مستوي عالى من التوافق الخلطي الكلي (التام) مع صنف الكليمانتين "فيديلا"، بينما أظهر أيضا صنفي اليوسفي "ويلوليف" و "سان بيرست" مستوي عاَّلي منّ التوافق الخلطي الجزئي مع صنف الكليمانتين "فيديلا". أما صنف الكليمانتين "فينا" فأظهر مستوي منخفض من التوافق الخلطى الجزئي. كما أظهر صنف "سبينوزا" بعض درجات عدم التوافق الخلطي مع كل مصادر اللقاح محل الدراسة. نستخلص من النتائج أن كل أصناف اليوسفي تحت الدراسة يمكن استعمالها كملقحات واعدة لصنف الكليمانتين "فيديلا" عديم التوافق الذاتي، ومن ثم يوصى بزراعة هذه الأصناف معا في نفس البستان.

المجلة المصرية للعلوم الزراعية المجلد (73) العدد (2) (أبريل 2022): 19-28.