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RAPD Markers are Associated with Self-incompatibility Characteristics as Related to the Number of Seeds per Fruit of Some Mandarin and Clementine Cultivars

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THE experiments were performed using (Nour, Fedela, Spinosa clementines, Kishu seedless, Balady, and Seedless mandarins) to determine the compatibility or incompatibility as related to the number of seeds per fruit and the genetic relation between them. For two consecutive seasons (2020 and 2021), the growth of the pollen tube in the styles following self-pollination has been analyzed using a fluorescence microscope to detect incompatibility properties. According to Nour and Fedela clementine cultivars are highly incompatible, while the other four cultivars are incompatible. After receiving pollen, Nour and Fedela clementines had very low pollen tubes that reached style. On the other hand, the highest rate was in the other four cultivars. The DNA of the six cultivars of mandarin and clementine investigated in this study was very similar. Balady mandarin and seedless mandarin appear to have very high similarities, while Fedela clementine and seedless mandarin appear to be very low. RAPD-PCR showed a specific molecule related to incompatibility such as (A-18) RAPD primer, at MW 361 bp. linked to the incompatibility of Nour and Fedela clementine *c.vs.*

Keywords: Self- incompatibility, Pollen tube growth, Fruit set, RAPD marker, Mandarins.

Introduction

Citrus self-incompatibility is important to fruit production trait because it results in seedless fruit (Mesejo, et al., 2014, Li, 1980, Yamamoto et al., 1995 and Yamamoto et al., 2006). Many factors can lead to seedless orange fruit, such as parthenocarpy (Talon et al., 1992 and Montalt., et al., 2021), male fertility (Hu et al., 2005, 2007, Zhang et al., 2012, Xiao et al., 2007 and Yu et al., 2011), inconsistencies (Yamamoto et al., 2006, Distefano et al., 2009, Wang & Lü, 2009, Wang et al., 2009, Yamasaki, et al., 2009 and Ngo et al., 2010) and maturity of pollen during fruit development (Wen & Cai, 2000). Seedless is desirable economic features in citrus fruit markets but as seedless seeds eliminate seed shortages, it makes use and processing faster and easier. Citrus growers around the world are committed to the development of seedless fruit varieties. If not, the incompatibility itself causes pollen resistance and inhibits seed growth. It is known

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that citrus gametophytes self-incompatibility is based on S-RNases, which act as S-gene receptors in suppressing pollen tube formation. Soost (1969) described citrus gametophytic incompatibility systems in a few self-incble cultivars, he discovered the incompatibility (S) allele, also discovered the presence of a single self-incompatible allele among self-compatible individuals. The S genotypes individuals were significant additions that are both incompatible with themselves and compatible with one another. The most important agricultural citrus families that are less susceptible to pummelos (*Citrus maxima* (Burm.) Clementines (*Citrus Clementina* Hort. Ex Tan.), and various natural hybrids or mandarin. Research, genetic map, genomic differentiation, and evaluation of intra and intergenomic variability, Dugo and Giacomo (2002).However, there is a deficit of information on the potential mechanisms of genetic alterations in citrus that result in seedlessness. It is important to research the orange seedless processes that are made due to the variety of shoots. Randomized polymorphic DNA (RAPD, Luro et al., 1995, Mabberley, 2008, Higashi et al., 2000, Moore, 2001, El-Adl et al., 2012, Mohamed et al., 2018) and simple sequence replication (ISSRs, Fang & Roose, 1997, Bornet & Branchard, 2001, Pradeep Reddy et al., 2002 and Lamyaa & Maklad, 2015, Abdein, 2018, Abdein et al., 2018, Osman & Abdein, 2019, Alqahtani et al., 2020, Abdein et al., 2020, Abdein et al., 2021 and El-Mansy et al., 2021) are widely used among various molecular techniques due to their analytical power and related simplicity. Using a single primer pair of negative nucleotide sequences, the Random Amplified Polymorphic DNA (RAPD) test detects polymorphisms in DNA (Welsh & McClelland 1990 and Wiliams et al., 1990). The procedure is straight forward and requires only a few nanograms of DNA. In hazelnut (*Corylus avellana*) (Pomper et al., 1998), *Medicago sativa* (Campbell, 2000), *Mango Mangifera* indica (Damodaran et al., 2009 and Maklad et al., 2011), as well as other Egyptian citrus plants, the RAPD method has been used to improve sexually transmitted symptoms (Lamyaa and Maklad, 2015). Inconsistencies in the six types of mandarin and clementine were investigated in this regard, and RAPD analysis was used to assess genetic variation in the similarity or inconsistency between them, which was related to the number of seeds per fruit.

Materials and Methods

Plant Material

The current study employed six mandarin and clementine cultivars. These cultivars were grown in plots at the Horticulture Research Station in Kassasin, Ismailia Governorate, and at the Laboratory of Molecular Genetics, Department of Genetics, Faculty of Agriculture, Ain Shams University, Cairo, Egypt. In this study, three mandarin cultivars (Kishu seedless, Balady, and seedless mandarins) and three clementine cultivars (Nour, Fedela, and Spinosa clementines) were used, the trees were eighteen years old.

Pollination experiments

To self-pollinate, all selected flowers were carefully packed with per gamin bags before a thesis to avoid any unwanted pollen, and pollen grains of each species (male parent) were collected for use by hand pollination. The chosen flowers were then bagged in per gamin bags after pollination. At hiscence, they separated the anthers from the stamens and placed them in Petri dishes on silica gel at room temperature until hiscence. Pollen was then used to pollinate the flowers. According to (Montalt, et al. (2021), fifteen pistils from each self-treatment from each cultivar were fixed in FPA solution and stored at 4˚C for seven days after pollination until histological observations were made. The other flowers were left to set fruit. Fruit setting data was taken into account.

Microscopic preparations

The pistils were prepared in the FPA seven days after the pollination treatment itself to determine histology parameters, including pollen tube growth, using the Leica fluorescence microscope (WILD LEITZ GMBH, Type 020-505-030., LEITZ WETZLAR GERMANY) according to Kho and Baer method (1970). Pistil samples were soaked in 8N NaOH for two hours and washed in distilled water for 24 hours before being contaminated with % aniline blue (W / S) dissolved in 0.1 N K3PO4.

DNA extraction and molecular markers based on PCR

The leaves are collected and frozen quickly until DNA is released. The modified acetyl trimethylammonium bromide (CTAB) method has been used to extract complete genomic DNA from the leaves of each genotype (Murray and Thompson, 1980) [54]. The extracted DNA was dissolved and purified with a 1X TE bath at a final concentration of 30 ng / L and stored at -20°C

until use. Table 1 summarizes ten RAPD primers (A-01, A-02, A-03, A-04, A-05, A-08, A-10, A-14, A-15, and A18). In a hot gradient cycle, a PCR reaction is performed (Eppendorf, Germany). 50 genomic DNA, 1 U Taq DNA polymerase, 2.5 l 10X PCR memory enhancement, 0.2 mM dNTP, 10 p per molecule first, and 1.5 mM $MgCl_2$ embedded in -25 l mixed reaction. The initial release of denaturation at 94°C for 4 minutes was followed by 30 cycles of denaturation rotation at 94°C for 45 seconds, the release of the primer's temperature at 37- 44°C, extension at 72°C for 2 minutes, final extension at 72°C for 10 minutes of RAPD-PCR.

Gel analysis and phylogenetic relationships

Each polymorphic RAPD band was considered as a locus, having two alleles that were scored as present (+) or absent (-). Only polymorphic, reproducible, and clearcut bands were kept for data analysis. The unweighted pair group method using arithmetic averages has been used to estimate phylogenetic relationships using NTSYS pc 2.01b software, UPGMA (Rohlf, 2000) [28]. As described by Sneath and Sokal (1973), the unweight pair group method using the arithmetic averages (UPGMA) algorithm has been used to generate dendrograms. The SPSS software has been used to calculate the similarity value.

Statistical analysis

The research used a randomized block design with 5 replications. Data's method has been used by Duncan's (1955) method to perform a data study.

Results

Many factors affect the speed at which pollen tubes grow in styles, such as soil fertility and fertilization methods (Williams, 1965), flowering dates (Church and Williams, 1983), temperature and relative humidity (Williams, et al., 1984) and sexual compatibility (Williams, et al., 1984). Spiegel-Roy and Alston, 1982, and Maklad et al., 2011).

Self-incompatibility and or/compatibility

Our results reveal that when Nour and Fedela clementine cultivars are self-pollinated, there's no growth of pollen tubes until 4 days after pollination, and after 5 days, they start to grow into the style tissue, indicating that it should be highly self-incompatible with microscopic investigations. After 5, 6, and 7 days from pollination, the average number of pollen tubes reaching all parts of the style for Nour and Fedela cultivars was 0.7, 4.3, 10.9 and 0.6, 1.4, 5.2, respectively. Furthermore, as shown in Fig.2, most pollen tubes from the other four Kishu seedless mandarin, Spinosa clementine, Balady mandarin, and seedless mandarin cultivars revealed normal rapidly growing development of pollen tubes through the styles (Fig 1), with pollen tubes beginning to grow the all parts of the style tissue after 4 days from pollination. Self-pollination of Kishu seedless mandarin, Spinosa clementine, Balady mandarin, and Seedless mandarin cultivars revealed an average ranging from 28 to 86 pollen tubes reaching the styles after 4, 5, 6, and 7 days from pollination.

Pollen growth was severely restricted to what is known as incompatibility (Table 2). Similar to hyuganatsu (Miwa, 1951) and hassaku (Nishiura no Iwa-saki, 1963). In addition, the pollen grains were unusually twisted and filled with heavy and unusual calose. However, in line with the common pollen tubes come in style (Table 2). Therefore, both the number of pollen tubes and the pollen tube behavior on the roads were used as indicators to judge the inconsistencies or compliance with this study. Test results for non-compliance or compatibility of 6 mandarin and clementine plants (Table 2) showed that they were all compatible, and relatives of Fedela and Nour clementines were incompatible.

TABLE 1. Codes, sequences, and G + C percentages for the RAPD-PCR technique's 10 random primers.

Primer	Sequence (5' - 3')	G+C%	Primer	Sequence (5' - 3')	G+C%
A-01	5′- CAG GCC CTT C -3′	70	A-08	5′- GTG ACG TAG G -3′	60
A-02	5′- TGC CGA GCT G -3′	70	A-10	5′- GTG ATC GCA G -3′	60
A-03	5′- AGT CAG CCA C -3′	60	A-14	5′- TCT GTG CTG G -3′	60
A-04	5′- AAT CGG GCT G -3′	60	A-15	5′- TTC CGA ACC C -3′	60
A-05	5′- AGG GGT CTT G -3′	60	A-18	5′- AGG TGA CCG T -3′	60

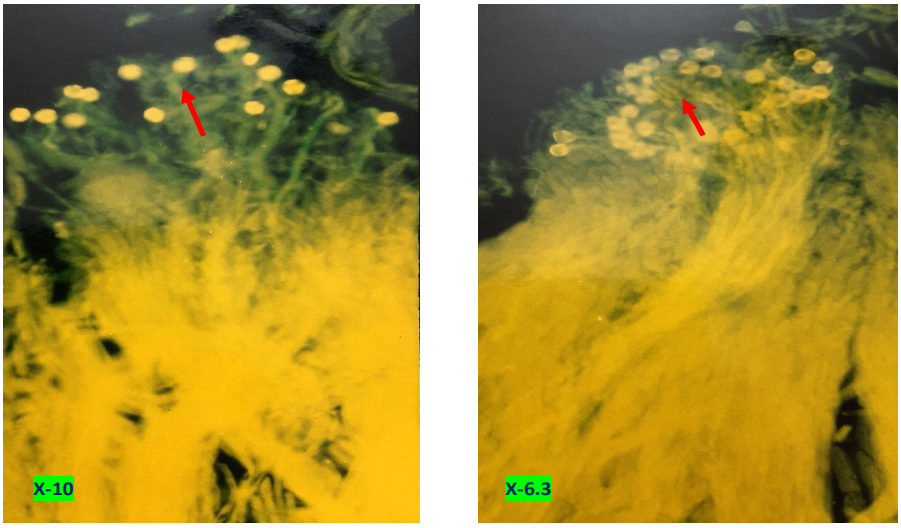

Fig.1. Compatible characterization, high number of germinated pollen grains, pollen tubes showed normal growth, pollen tubes grew to about 1/3 and 2/3 length of the style, pollen tubes reached the base of the style four days after pollination.

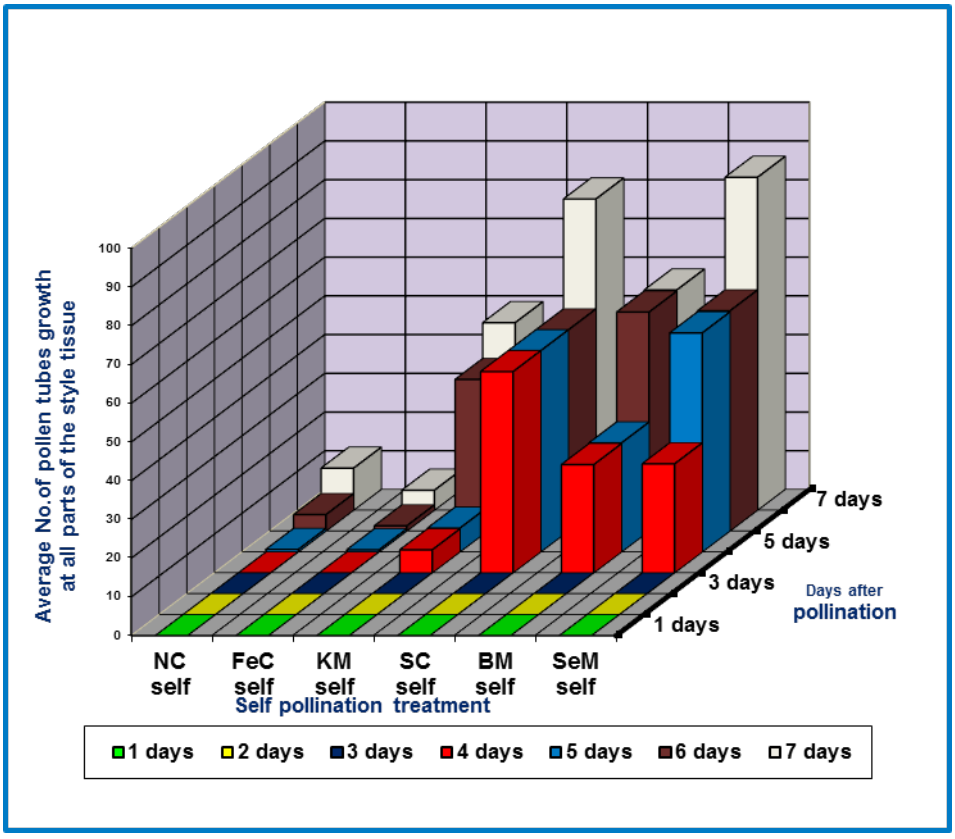

Fig.2. Pollen tube growth of Nour and Fedela clementines tubes (as a self-incompatibility) and Kishu seedless mandarin, Spinosa clementine, Balady and Seedless mandarin tubes (as a self-compatibility), in styles expressed as average no. of pollen tube growth reaching at all the parts of the style one till seven days after self-pollination.

TABLE 2. Self-compatibility and or incompatibility classification based on fruit set % and the number of seeds per fruit obtained from hand self-pollination treatments in each cultivar.

Female Parent	Pollination	Fruit setting (%)	No. of Seed per fruit	Self-incompatibility / compatibility
Nour clementine	Self	3	1	Self-incompatibility
Fedela clementine	Self	2	0	Self-incompatibility
Kishu seedless mandarin	Self	1	0	Self-compatibility
Spinosa clementine	Self	3.90	0	Self-compatibility
Balady mandarin and	Self	1	12	Self-compatibility
Seedless mandarin	Self	1	0	Self-compatibility

RAPD - PCR analysis

Identification based on DNA analysis

In a genetic analysis of both plant and animal species, a few powerful marker techniques are currently available. The most appropriate approach to the given research is not obvious and is strongly influenced by the research purpose and biological and genetic research of plant species. Therefore, comparisons are needed to determine which method is best suited to the existing problem (Biswas et al., 2010). Several methods have been used to evaluate their use in identifying and establishing genetic links among orange relatives. Schlotterer, 2004, Navarro, et al., 2015, Biswas et al., 2010, Tripolitsiotis et al., 2013) and Whitkus et al., 1994.

RAPD (randomly amplified polymorphic DNA) analysis

The RAPD-PCR (Random Amplified Polymorphic DNA) method is simple, fast, and sensitive. It can amplify a large number of DNA fragments to turn it around and does not require prior DNA sequence information, Karp, 1996 and Karp et al., 1997. Figure (3) and Table (3) showed the 151 amplified fragments from the 6 mandarin and clementine cultivars that were exhibited by the 10 random primers, as well as the 20 polymorphic bands. Primer A-04 had the lowest number of RAPD amplified fragments, with 6 fragments, while primer A-15 had the highest number, with 34 fragments. As shown in Table 2, primer A-03 produced a very low percentage of polymorphism (16.66%), while primers A-08 made a very high percentage of polymorphism (100%), Table 3. Ten primers showed 111 pieces as markers distinguishing between good and bad in each cultivar at the RAPD level for cell marking. Table 3 shows the total number of enlarged and polymorphic fragments produced by each plant, as well as the distinct characteristics of each of the six species of mandarin and clementine (3). As a result, many fruit and fruit trees, especially cultivars, have been successfully used using RAPD tags (Baig et al., 2009, Leng et al., 2012 and Sun et al., 2012).

The results of enlarged fragments using 10-mer arbitrary primers six cultivars of mandarin and clementine reveal success in enhancing DNA fragments. The levels of polymorphism vary from one primer to another. The number of complete amplified fragments (TAF), polymorphic bands (PB), amplified fragments (AF) and specific markers (SM) of each type using the 10 primers shown in Table (4). Certain fragments could be used to differentiate one species from the other, as each of these fragments was not present in all cultivars except one (Positive specific marker) or present in all cultivars except one (negative specific marker) Table (4). These markers were distributed in the six mandarin and clementine cultivars as follows, primer A-01 showed seven Positive specific markers, all two of which are Nour Clementine with molecular weight 829 and 545bp, one Fedela clementine with molecular weight 476bp, two Spinosa Clementine with molecular weight 792,460, one Balady mandarin with molecular weight 858bp and one Seedless mandarin with molecular weight 867bp. Primer A-02 showed eight Positive specific markers, all of which are two Nour clementine with molecular weight 474 and228bp, two Fedela Clementine with molecular weight 467 and 226bp, one Kishu seedless mandarin with molecular weight 326bp, one Spinosa Clementine with molecular weight 471bp, one Balady mandarin with molecular weight 323bp and one Seedless mandarin with molecular weight 320bp. Primer A-03 has shown just one Positive specific marker as a good symbol of Balady mandarin with a molecular weight 794bp. Primer A-04 featured three Positive specific

markers as fine markers one Nour Clementine with molecular weight 1198bp, one Fedela Clementine with molecular weight 1186bp and one Spinosa Clementine with molecular weight 315bp. Primer A-05 showed sixteen straight Positive specific markers, all four of which are Nour clementine with molecular weight 1262,1026,777 and 326 bp, three Fedela Clementine with molecular weight 1294,782 and 332bp, three Spinosa Clementine with molecular weight 1307,818 and 340bp and six Seedless mandarins with molecular weight 1353,118,914, 530,434 and 358bp. Primer A-08 has identified ten Positive specific markers as good marks one Nour Clementine with molecular weight 454bp, two Fedela Clementine with molecular weight 567 and 328bp, three Kishu mandarin seedless with molecular weight 537,380 and 229bp, two Balady mandarin with molecular weight 567and 357bp and two Seedless mandarins with molecular weight 572and 354bp. Primer A-10 featured seventeen positive specific markers of it as one good marker, one for Nour Clementine with molecular weight 193bp, four Fedela clementine with molecular weight 1390,922,649 and 206bp, two Kishu mandarin seedless with molecular weight 1399 and 907bp, three Spinosa clementine with molecular weight 1445,928 and 660bp, two Balady mandarin with molecular weight 1468 and 967bp and three Seedless mandarin with molecular weight 1590,1021 and 339bp. Primer A-14 featured sixteen positive specific markers all as four Nour clementine markers with molecular weight 815,695,434 and 210bp, two Fedela Clementine with molecular weight 706 and 440bp, three Kishu mandarin seedless with molecular weight 732,461 and 222bp, four Spinosa Clementine with molecular weight 853,754,478 and 238bp, two Balady mandarin with molecular weight 761 and 494bp and one for Seedless mandarin with molecular weight 784bp. Primer A-15 showed twenty-six straight positive specific markers, five of which are Nour Clementine with molecular weight 1522, 551, 444,368 and 291bp, four Fedela Clementine with molecular weight 1490,624,347 and 294bp, five for Kishu mandarin seedless with molecular weight 1420,1000,356,289 and 171bp, three Spinosa Clementine with molecular weight 1727,543 and 301bp, six Balady mandarin with molecular weight 649,568,471,370,307 and 127bp and three Seedless mandarins with molecular weight 565,478 and 313bp. Primer A-18 featured six positive specific markers, one Nour Clementine with a molecular weight of 852bp, one Spinosa Clementine with a molecular weight of 206 bp, two Balady mandarin with a molecular weight of 565 and 221bp and two Seedless mandarins with molecular weight 871 and 316bp and there was one negative specific marker one Fedela Clementine with molecular weight 437bp.

The highest level of similarity was 55% between Balady mandarin (BM) and Seedless mandarin (Se.M.), and the lowest level of similarity was between Fedela clementine (Fe.C.) and Seedless mandarin (seedless mandarin (Se. M.), Table 5. The phylogenetic relationship classified six types of mandarin and clementine into two major classes: Class I contains Balady mandarin and Seedless mandarin, and Class II, two sub-categories. Subclass I comprise Nour clementine and Fedela clementine, as well as the Kishu seedless mandarin and Spinosa clementine, found in Subclass II. As shown in Figure (4), the highest pollination rate in pollen is obtained after pollination has a very high genetic similarity (0.556), while very low pollen grains reach style after pollination.

Molecular genetic markers related to compatibility traits

As shown in Table (6), most RAPD markers (10-mer) may be linked to self-incompatibility and or compatibility traits after self-pollination such as self-incompatibility in both Nour clementine c.v. (nine RAPD primers A-01, A-02, A-04, A-05, A-08, A-10, A-14, A-15 and A-18 - with MW ranged from 193 bp to 1522 bp) and Fedela clementine c.v. (nine RAPD primers A-01, A-02, A-04, A-05, A-08, A-10, A-14, A-15 and A-18 - with MW ranging from 206 bp to 1490 bp) and self-compatibility in the other four Kishu seedless mandarin, Spinosa clementine, Balady mandarin and Seedless mandarin cultivars, five RAPD primers A-02, A-08, A-10, A-14, A-15 with MW ranged from 171 bp to 1420 bp for Kishu seedless mandarin to eight RAPD primers A-01, A-02, A-04, A-05, A-10, A-14, A-15 and A-18 with MW ranged from 206 bp to 1727 bp for Spinosa clementine, A-01, A-02, A-03, A-08, A-10, A-14, A-15 and A-18 with MW ranged from 177 bp to 1468 bp for Balady mandarin and A-01, A-02, A-05, A-08, A-10, A-14, A-15 and A-18 with MW ranged from 313 bp to 1590 bp for Seedless mandarin. Tanksley, et al. (1989),[33] Ismail (2003), [46]and Maklad (2012) [52] developed the DNA marker as a benefit, in which the genetic markers of mango species are expected to be useful in genetic identification and the discovery of links to important agricultural features. Table (7) shows that Nour and Fedela clementine c.vs. connected (A-18) RAPD primer, 361 bp, to self-incompatibility phenomena.

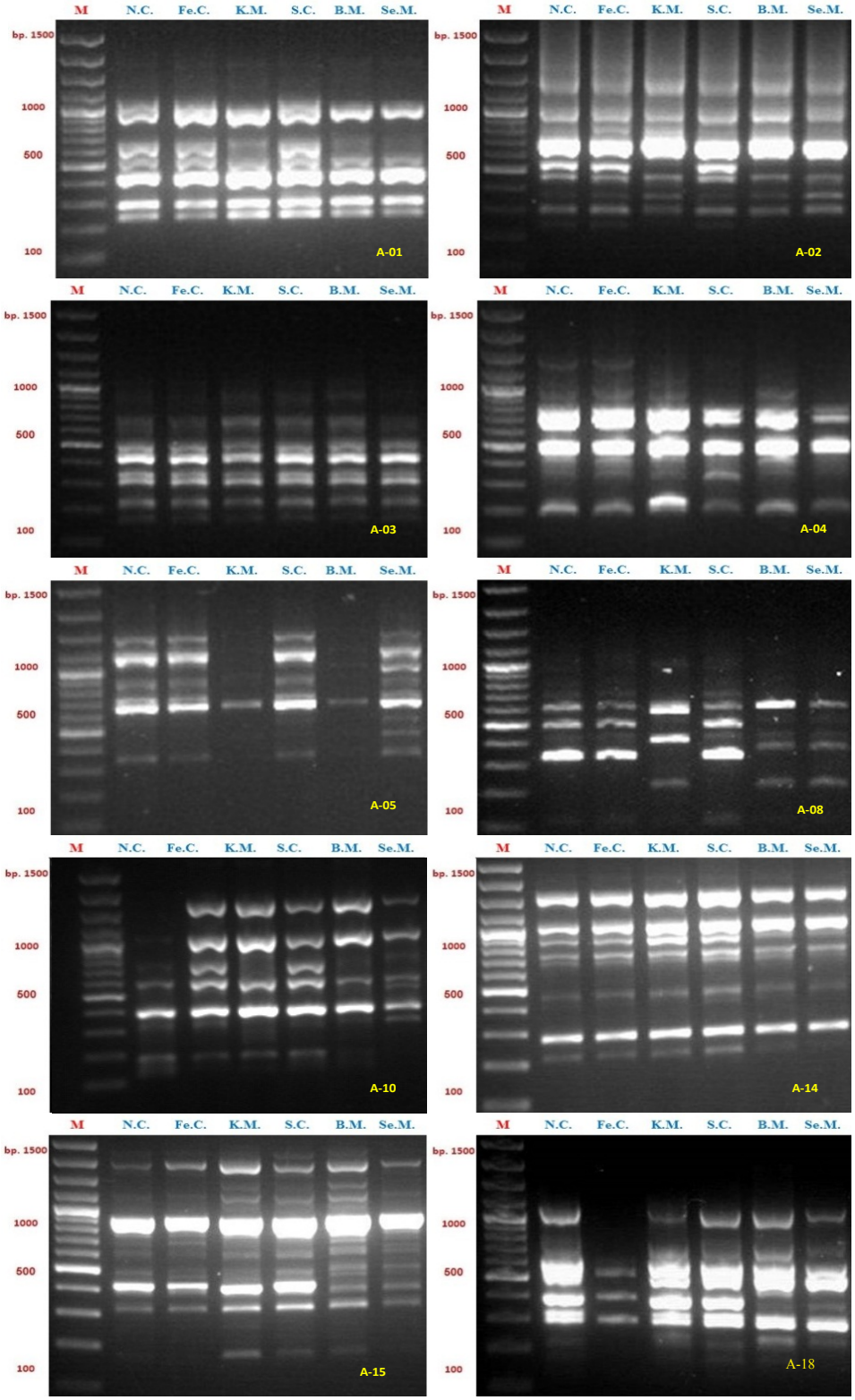

Fig. 3. DNA polymorphism of the six mandarin and clementine cultivars amplified with primers A-01, A-02, A-03, A-04, A-05, A-08, A-10, A-14, A-15 and A-18 using RAPD-PCR (M) DNA ladder marker (bp) (NC) Nour clementine, (FeC) Fedela clementine, (KM) Kishu seedless mandarin, (SC) Spinosa clementine, (BM) Balady mandarin and (SeM) Seedless mandarin.

TABLE 3. DNA amplified bands and polymorphism percentages were generated using 10 RAPD primers on 6 mandarin and Clementine Citrus cultivars.

Primer	Sequence (5' - 3')	TAF	MF	SM	PF	Polymorphic %
A-01	CAG GCC CTT C′	12	2	8	2	83.33 %
A-02	TGC CGA GCT G′	13	5	8	0	61.53 %
A-03	AGT CAG CCA C′	7	6	1	0	16.66 %
A-04	AAT CGG GCT G	6	3	3	0	50 %
A-05	AGG GGT CTT G	18	1	15	2	94.44 %
A-08	GTG ACG TAG G	14	0	10	4	100 %
A-10	GTG ATC GCA G′	18	2	15	1	88.88 %
A-14	TCT GTG CTG G	21	3	17	1	85.71 %
A-15	TTC CGA ACC C′	34	1	27	6	97.05 %
A-18	AGG TGA CCG T	12	1	7	4	91.66 %
Total		155	24	111	20	85.43 %

TAF = Total amplified fragments PF = Polymorphic fragments MF = Monomorphic fragments
SM = Specific markers

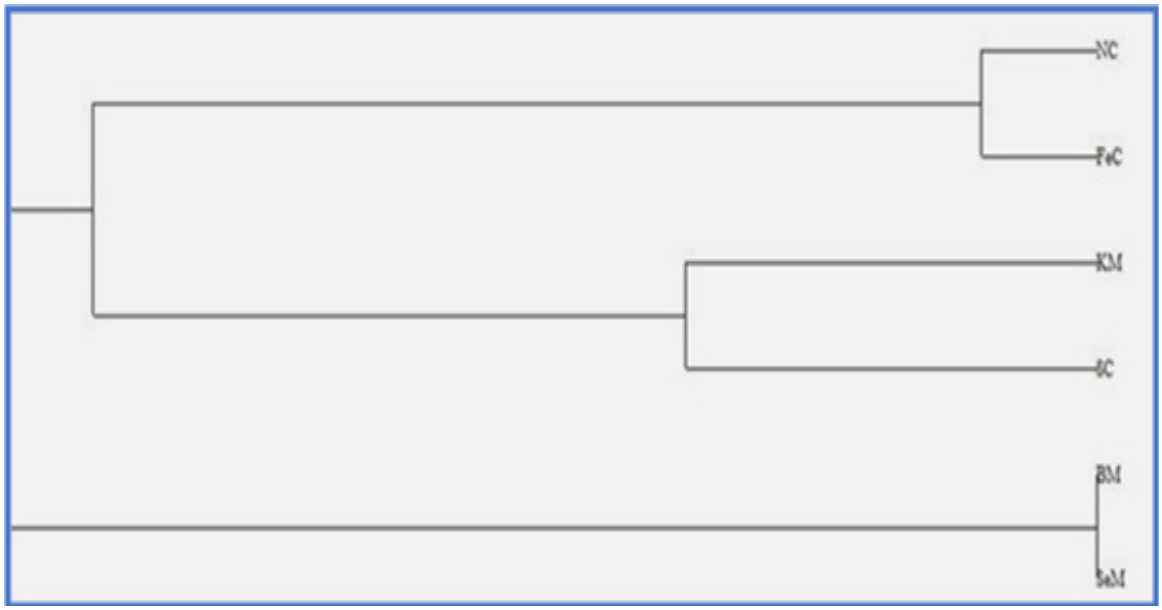

Fig. 4. Phylogenetic relationships between 6 mandarin and clementine cultivars according to RAPD-PCR technique using NTSYSpc 2.01b software.

TABLE 4. Number of amplified fragments and specific markers of the six mandarin and clementine cultivars based on RAPD-PCR analysis using 10 primers.

Primers	Mandarin and Clementine cultivars											
	Nour clementine		Fedela clementine		Kishu seedless mandarin		Spinosa clementine		Balady mandarin		Seedless mandarin	
	SM		SM		SM		SM		SM		SM	
	+	-	+	-	+	-	+	-	+	-	+	-
A-01	2 (829, 545) bp	-	1 (476bp)	-	-	-	2 (792,460) bp	-	1 (858bp)	-	1 (867bp)	-
A-02	2 (474,228) bp	-	2 (467,226) bp	-	1 (326bp)	-	1 (471bp)	-	1 (323bp)	-	1 (320bp)	-
A-03	-	-	-	-	-	-	-	-	1 (794bp)	-	-	-
A-04	1(1198bp)	-	1 (1186bp)	-	-	-	1 (315bp)	-	-	-	-	-
A-05	4 (1262,1026,777,326) bp	-	3 (1294,782,332) bp	-	-	-	3 (1307,818,340) bp	-	-	-	6 (1353,118,914, 530,434,358) bp	-
A-08	1 (454bp)	-	2 (567,328) bp	-	3 (537,380,229) bp	-	-	-	2 (567,357) bp	-	2 (572,354) bp	-
A-10	1 (193bp)	-	4 (1390,922,649,206) bp	-	2 (1399,907) bp	-	3 (1445,928,660) bp	-	2 (1468,967) bp	-	3 (1590,1021,339) bp	-
A-14	4 (815,695,434,210)bp	-	2 (706,440)bp	-	3 (732,461,222) bp	-	4 (853,754,478,238) bp	-	2 (761,494) bp	-	1 (784bp)	-
A-15	5 (1522, 551, 444,368,291)bp	-	4 (1490,624,347,294)bp	-	5 (1420,1000,356,289,171) bp	-	3 (1727,543,301) bp	-	6 (649,568,471,370,307,127) bp	-	3 (565,478,313) bp	-
A-18	1 (852bp)	-	-	1 (437bp)	-	-	1 (206 bp)	-	2 (565, 221)bp	-	2 (871, 316)bp	-
TSM	21	-	17	1	15	-	18	-	17	-	19	-

SM = Specific markers TSM = Total number of specific markers TAF = Total amplified fragments PF = Polymorphic fragments AF = Amplified fragments SM = Specific markers TSM = Total number of specific markers

TABLE 5. Similarity indices among the six mandarin and clementine cultivars based on RAPD-PCR using 10 primers..

	NC	FeC	KM	SC	BM	SeM
NC	1.00					
FeC	0.5433438	1.00				
KM	0.4653762	0.4285247	1.00			
SC	0.4678577	0.4352014	0.5112543	1.00		
BM	0.4785882	0.4098765	0.4565324	0.4342519	1.00	
SeM	0.4519129	0.4039080	0.4485219	0.4296544	0.5558530	1.00

TABLE 6. Performance of mandarin and clementine cultivars against self-incompatibility and or compatibility for each cultivar (after self pollination).

Female parent	Genotype	Pollen Parent						Marker linked	
		N.C	Fe.C	K.M	S.C	B.M	Se.M	Primer	MW
Nour Clementine (N.C)	**Self-incompatibility**	+	-	-	-	-	-	**A-01** **A-02** **A-04** **A-05** **A-08** **A-10** **A-14** **A-15** **A-18**	829, 545, 464 474, 228 1198 1262, 1026, 777, 326 454 193 815, 695, 434, 210 1522, 551, 444, 368, 291 852
Fedela Clementine (Fe.C)	**Self-incompatibility**	-	+	-	-	-	-	**A-01** **A-02** **A-04** **A-05** **A-10** **A-14** **A-15** **A-18**	476 467, 226 1186 1294, 782, 332 1390, 922, 649, 206 706, 440, 217 1490, 624, 374, 294 474
Kishu seedless Mandarin (K.M)	**Self-compatibility**	-	-	+	-	-	-	**A-02** **A-08** **A-10** **A-14** **A-15**	326 537, 380, 229 1399, 907 732, 461, 222 1420, 1001, 356, 289, 171
Spinosa Clementine (S.C)	**Self-compatibility**	-	-	-	+	-	-	**A-01** **A-02** **A-04** **A-05** **A-10** **A-14** **A-15** **A-18**	792, 460 471 315 1307, 818, 340 1445, 928, 660 853, 754, 478, 238 1727, 543, 301 206
Balady Mandarin (B.M)	**Self-compatibility**	-	-	-	-	+	-	**A-01** **A-02** **A-03** **A-08** **A-10** **A-14** **A-15** **A-18**	858 323 794 567, 357 1468, 967 761, 494 649, 568, 471, 370, 307, 177 565, 221
Seedless Mandarin (Se.M)	**Self-compatibility**	-	-	-	-	-	+	**A-01** **A-02** **A-05** **A-08** **A-10** **A-14** **A-15** **A-18**	867 320 1353, 1118, 914, 530, 434, 358 572, 354 1590, 1021, 339 784 1559, 565, 478, 313 871, 316

TABLE 7. Performance of mandarin and clementine cultivars against self-incompatibility phenomena (after self pollination).

Genotype	Cultivars						Marker linked	
	Nour clementine	Fedela clementine	Kishu seedless mandarin	Spinosa clementine	Balady mandarin	Seedless mandarin	Primer	MW
Self-incompatibility	+	+	-	-	-	-	A-18	361

Discussion

Seed lessness is a major orange element and much work has been done to develop seedless plants. Diploid and triploid breeding systems and interbreeding different methods are used to produce new seedless varieties. Increasing knowledge about your incompatibility with parthenocarpy is important in developing parental choices that will be used in sexual intercourse or mutagenesis. Clementine flowers can not only pollinate themselves or flowers of other clementine trees but can also pollinate other citrus species. Mandarins, including clementines, are among the few flowering plants that do not need pollen to produce fruit, but that produce poor yields when they do not need it. The eggs inside the uterus mature into seeds next to the fruit when the flower is watered with pollen. Fruits are seedless if the flowers are not polished. Incompatibility is one of the main causes of citrus seeds (Yamamoto et al., 2006, Ngo et al., 2001, Zhang et al., 2012) autoimmune disorders can be classified by sporophytic SI (SSI)) and gametophytic SI (GSI) for controlling pollen behavior. Citrus belongs to GSI and pollen grains are bound in the style of 'Comune' clementine (*C. clementina* Hort. Ex Tan.) (Distefano et al., 2012), (*C. grandis* var shatinyu Hort.) (Xue et al., 1995) and 'Xiangshui' lemon [*C. limon* (L.) (Zhang et al., 2012), in pistil of 'Guanxi' and 'Duwei' pomelo (*C. grandis*) (Wang & Lü, 2009) and eggs of 'Wuzishatangju' mandarin (*C. reticulata* Blanco) (Ye et al., 2009). In our study, pollen tubes were delayed until they reached the base of the pistil.

In terms of the method used in our study, the potential for non-compliance with each genotype is to produce seedless fruit. Additionally, we checked compliance by comparing the percentage of fruit between the remaining pollen flowers (Table 2). To ensure the stability of the results presented in Table 2, a large number of cooked flowers were also required and we used five trees per species, indicating the number of flowers and the percentage of fruit set for all five medicinal plants used in each species. . The details shown in the pollination drugs suggest that it can be seen in many cultivars by making treatment of 50 flowers. Concerning compliance level assessments, comparisons between all in-fusion treatments showed similar differences in each recurrence separately and the combined data (Table 2). showed the same percentage of fruit set in the corresponding treatment five times in the combined data (Table 2). In all cultivars with concomitant treatment, they produce a set of lower fruits than non-conventional treatment in all five varieties.

Nour and Fedela clementines are incompatible and produce seedless fruit in pollination treatment (Table 2), which produces facultative parthenocarpy. In both cultivars, the percentage of fruit set is relatively small and, as a result, vegetative parthenocarpy. Therefore, we have divided Nour and Fedela as facultative and vegetative parthenocarpy, this effect is similar to Clemenules clementine in Polli independent independence proposed by Mesejo et al., 2013 but challenges the separation of the stimulating parthenocarpy, as proposed by Vardi et al., 2008. Moreover, our results generally agree with those reported by Nagai and Tanikawa (1928), Miwa (1951), Mustard (1956), Soost (1956), Nishiura & Iwasaki (1963) and Hearn (1969).

Conclusions

In general, previous results have shown that it is possible to study the genetic relationship between the six species of mandarin and clementine studied about the horticultural undergoing different pollination treatments between them and as related to the seeds per fruit, which may be linked. near or far in this case, and the possibility of using RAPD-PCR analysis to identify specific features of the six study types and can be used to differentiate between plants, and determine the level of genetic interaction between these plants. In addition to determining the nature of the incompatibility, we have developed a very effective law to differentiate between plant species. Based on exit, hand turning, and histological observation of pollen tube growth in style tissue. and was written in the order of the fruits. In the two described interactions of mandarin and clementine plants, they found that Nour and Fedela clementine plants are incompatible, Kishu seedless mandarin, Spinosa clementine, Balady mandarin and Seedless mandarin species are compatible. The results obtained showed that the six tested plants of mandarin and clementine were very similar in DNA level, and were demonstrated using 10 random primers, 107 unique bands for RAPD markers. The highest level of similarity was between Balady mandarin and Seedless mandarin (55%) and the lowest similarity (40%) between Fedela clementine and seedless mandarin.

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The authors declare no conflict of interest.

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واسمات RAPD المرتبطة بخصائص عدم التوافق الذاتي المرتبطة بعدد البذور لكل ثمرة لبعض أصناف اليوسفي والكلمينتين

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أجريت هذه الدراسة باستخدام (أصناف الكلمنتين: نور، فيديلا، سبينوزا) ، (أصناف اليوسفي: كيشوعديم البذور واليوسفي البلدي ، واليوسفي عديم البذور) لتحديد مدى التوافق أو عدم التوافق الذاتي الجنسي في كل صنف وربطها بعدد البذور لكل ثمرة والعلاقة الوراثية فيما بينهما وذلك خلال موسمين متتاليين (٢٠٢٠- ٢٠٢١)، تم تحديد مدى نمو أنابيب حبوب اللقاح بعد التلقيح الذاتي باستخدام الميكروسكوب الفلوريسينسى لمعرفة خصائص عدم التوافق الذاتي. وجد أن صنفي الكلمنتين نور وفيديلا إنها أصناف غير متوافقة ذاتيا بدرجة كاملة، في حين أن الأصناف الأربعة الأخرى كانت غير متوافقة ذاتياً بدرجة جزئية. كانت أنابيب حبوب اللقاح لصنفي الكلمنتين نور وفيديلا بطيئة جدًا في الوصول إلى نهاية قلم الزهرة. بينما كان أعلى معدل لنمو أنابيب اللقاح في الأصناف الأربعة الأخرى. وأظهرت النتائج التي تم الحصول عليها أن الستة أصناف من اليوسفي والكلمنتين تحت الدراسة كانت متشابهة إلى حد كبيرعلى مستوى الحمض النووي DNA. وأن هناك تشابهًا كبيرًا بين صنف اليوسفي البلدي واليوسفي عديم البذور، بينما يبدو أن صنف الكلمنتين فيديلا واليوسفي عديم البذور أظهروا درجة أقل جدًا من التشابه فيما بينهما. أظهرأختبار RAPD-PCR بادئات عديدة محددة ومرتبطة بعدم التوافق مثل البادئ (A-18) عند الوزن الجزيئي ٣٦١ MW مرتبطة بعدم التوافق لصنفين كلمنتين نور وفيديلا.

الكلمات المفتاحية: عدم التوافق الذاتي، نمو أنابيب حبوب اللقاح، عقد الثمار، بادئات RAPD، اليوسفي والكلمنتين.