Assessment of genetic variability and genotyping of some Citrus accessions using molecular markers

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ABSTRACT

Genotyping of 14 Citrus accessions was carried out using RAPD and SCAR markers.The genetic variability among the 14 Citrus accessions was estimated using forty decamer RAPD primers. The total number of amplicons detected was 531, including 349 polymorphic amplicons. This represents a level of polymorphism of 65.7% and an average number of 8.7 polymorphic bands per primer. RAPD markers detected genetic similarity ranging from 10.8% to 87.7% between TC and each of WM, AWM and BSO and between MSG and RRG, respectively. The similarity matrices were employed in the cluster analysis to generate a dendrogram using the UPGMA method. The dendrogram separated Troyer citrange from the other Citrus genotypes. Moreover, accessions belonging to the same species always clustered together. Thirty-four out of the forty RAPD primers identified 13 out of the 14 Citrus accessions by unique positive and or negative markers.Each of the three primers OPC04, OPK16 and OPO13 revealed unique markers characterizing five different accessions. While, the other primers identified 1 to 4 accessions. Seven out of the eight RAPD primers and one out of three SCAR primers detected markers known to be linked to Citrus tristeza virus resistance in four and one Citrus accessions, respectively. On the other hand, three RAPD primers and one SCAR primer were used to detect markers linked to low fruit acidity with the 14 Citrus accessions.

Key words: *Citrus, RAPD, SCAR, genotyping, Citrus tristeza virus (ctv), acitric fruits.*

INTRODUCTION

Citrus is one of the major fruit crops all over the world. The coventional methods in *Citrus* cultivars identification relied on morphological features and isozymes (Protopapadadis, 1988). Using morphological traits, it is difficult to distinguish between many *Citrus* cultivars because some cultivars are distinguishable only by fruit traits and *Citrus* trees usually do not bear fruits until 3-4 years after planting. Moreover, isozyme markers can be mediated by secondary processes so that the normal patterns of expression are suppressed. Phenotypic diversity, polyembryony, hybridization and mutations have prevented cosensus on systematic classification of *Citrus* (Coletta *et al.*, 1998) and hampered *Citrus* improvement programs.

The development of molecular markers based on DNA sequences has provided an

ideal means for identifying genotypes, estimation of relatedness between different accessions and following inheritance of economically important characters. A wide variety of DNA-based markers have been developed in the past few years. RFLPs (Restriction fragment length polymorphism) were the first molecular markers (Botstein *et al.*, 1980) generated for DNA fingerprinting and genome mapping.However, the development of PCR-based markers has revolutionized the repertoire of genotype identification. Randomly amplified polymorphic DNA (RAPDs) or genetic markers resulting from PCR amplification of genomic DNA sequences recognized by ten-mer primers of arbitrary nucleotide sequence (Williams *et al.*, 1990) have already proved to be valuable in *Citrus* genotype identification (Deng *et al.*, 1995) and estimation of relationships (Machado *et al.*, 1996). RAPDs have been extensively used in assessing relationships amongst various accessions of different plant species (Ahmed, 1999; Nebauer *et al.*, 2000; Besnard *et al.*, 2001; Iruela *et al.*, 2002). Moreover, in *Citrus* several traits of horticultural importance, including resistance to *Citrus* tristeza virus (*Ctv*) (Gmitter *et al.*, 1996), nematode resistance (Ling *et al.*, 1994) and dwarfing (Cheng and Roose, 1995) have been tagged with RAPD markers. In addition, most of these markers could be converted into reliable sequence specific PCR-based markers or sequence characterized amplified region (SCARs) (Deng *et al.*, 1997; Fang *et al.*, 1997). The converted SCARs are highly reliable and can be easily manipulated. Thus, they are valuable in marker-assisted selection (MAS) and map-based gene cloning.

The objectives of the present study are to determine the genetic variability among 14 Citrus genotypes using RAPD markers and to assess the genetic relationships among these genotypes. Additionally, we are aiming to identify unique RAPD markers characterizing the different accessions and attempting to identify accessions resistant to Citrus tristeza virus and/ or containing low fruit acidity using specific RAPD and SCAR primers.

MATERIALS AND METHODS

Plant material

Fourteen *Citrus* accessions collected from the *Citrus* orchard of the Horticulture Research Institute, ARC,Giza, Egypt were included in this study. These accessions comprised 7 rootstocks and 7 budded accessions belonging to different species. The rootstocks were: Balady, Spanish and Brazilian sour orange (*C. aurantium*), Rangpure lime (*C. limonia*), Volkamer lemon (*C. volkameriana*), Cleopatra mandarin (*C. reticulata*) and Troyer citrange (*C. sinensis* X *P. trifoliata*). The scion accessions were Washington navel orange, Robertson navel orange and Valencia orange (*C. sinensis*), Willowleaf and Abd El Razek willowleaf mandarin (*C. deliciosa*) and Marsh seedless and Ruby red grapefruit (*C. paradisi*).

Extraction and purification of genomic DNA

A modified CTAB (hexadecyl trimethyl ammonium bromide) procedure based on the protocol of Porebski *et al.* (1997) was the method of choice for obtaining good quality of DNA.

Randomly amplified polymorphic DNA (RAPD)

A set of forty-six random 10mer primers (Table 1) was used in the detection of polymorphism among the fourteen *Citrus* genotypes. RAPD-PCR was carried out according to the procedure given by Williams *et al.* (1990) with minor modifications. The amplification was carried out in 50 µl reaction

volume containing 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 1 μM primer, 1U *Taq* DNA polymerase and 25 ng template DNA. PCR amplification was performed in a Perkin-Elmer/ DNA Thermal Cycler 2400 (Norwalk,CT) programmed to fulfill 40 cycles after an initial denaturation cycle for 5 min at 94 °C. Each cycle consisted of a denaturation step at 94 °C for 1 min, an annealing step at 37°C for 2 min , and an elongation step at 72°C for 2 min. The primer extension segment was extended to 7 min at 72°C in the final cycle.

Table (1): Sequences of the 46 decamer arbitrary primers used in RAPD-PCR.

Primer	Sequence (5'-3')	Primer	Sequence (5'-3')
OPA01	CAGGCCCTTC	OPH01	GGTCGGACAA
OPA02	TGCCGAGCTC	OPK16	GAGCGTCGAA
OPA05	AGGGGTCTTG	OPO03	CTGTTGCTAC
OPA06	GGTCCCTGAC	OPO04	AAGTCCGCTC
OPA08	GTGACGTAGG	OPO05	CCCAGTCACT
OPA15	TTCCGAACCC	OPO07	CAGCACTGAC
OPB01	GTTTCGCTCC	OPO08	CCTCCAGTGT
OPB10	CTGCTGGGAC	OPO09	TCCCACGCAA
OPB11	GTAGACCCGT	OPO10	TCAGAGCGCC
OPB12	CCTTGACGCA	OPO13	GTCAGAGTCC
OPB14	TCCGCTCTGG	OPO16	TCGGCGGTTC
OPB17	AGGGAACGAG	OPO20	ACACACGCTG
OPC02	GTGAGGCGTC	OPW18	TTCAGGGCAC
OPC04	CCGCATCTAC	OPX18	GACTAGGTGG
OPC07	GTCCCGACGA	OPZ11	CTCAGTCGCA
OPC09	CTCACCGTCC	OPZ12	TCAACGGGAC
OPC10	TGTCTGGGTG	OPZ14	TCGGAGGTTC
OPC15	GACGGATCAG	OPZ15	CAGGGCTTTC
OPC17	TTCCCCCCAG	OPZ16	TCCCCATCAC
OPC18	TGAGTGGGTG	OPZ17	CCTTCCCACT
OPF06	GGGAATTCGG	OPZ18	AGGGTCTGTG
OPG17	TCCCAGAGAC	OPZ19	GTGCGAGCAA
OPG18	AGACCAGTTC	OPZ20	ACTTTGGCGG

The amplification products were resolved by electrophoresis in a 1.5% agarose gel containing ethidium bromide (0.5 μg/ml) in 1X TBE buffer at 95 volts for two hr. PCR products were visualized on UV light and photographed using a Polaroid camera.

Sequence characterized amplified regions (SCARs)

Four published sets of SCAR primers were used (Table 2) and tested with the 14 *Citrus* accessions. One of these sets (SCZ20) was reported to be linked to fruit acidity (Fang *et al.*, 1997), while the remaining three sets were linked to *Citrus* tristeza virus resistance gene (Deng *et al.*, 1997).

Table (2): A list of SCAR loci, their flanking sequences, the corresponding GC%, melting and annealing temperatures.

Locus	Primer sequence (5' 3')	GC%	Melting temperature (°C)	Annealing temperature (°C)
SCT08	F=AACGGCGACATATAATAACGA	38.1	58	70-50
	R=AACGGCGACAGTCTTGGGAAT	52.4	64	
SCAA10	F=TCGGGTGATGGCAGAGGAATT	52.4	64	70-59
	R=AAACCCCAATTACAGAAGACACA	39.1	64	
SCAD02	F=AACCGCTATATTGTTTTGAATTGA	29.2	62	70-57
	R=GAACCGCTGAAAATTTACTCGAC	43.5	66	
SCZ20	F=TTTGGCGGCCCCATAAATCCCTAAT	48.0	74	60-50
	R=GGCGGGAAATCATGACCCTAAACTA	48.0	74	

The PCR reaction was carried out in 25 µl reaction volume containing 1X PCR buffer, 2mM $MgCl_2$, 2 mM of dNTPs, 1 µM of each of the forward and reverse primers,1X of Q solution, 1.25 U hot start *Taq* polymerase (GIBCO,BRL) and 25 ng genomic DNA. Hot start and touch down temperature profile was used as follows: An initial hot start and denaturing step at 94 °C for 15 min, followed by forty cycles at 94 °C for 1 min, annealing temperature (Ta) (Table 2) for 1 min, and primer elongation at 72 °C for 2 min. A final extension step at 72 °C for 7 min was performed. The PCR products were separated on 1.8% - 2.0% agarose gel in 1X TBE buffer containing ethidium bromide and photographed with a Polaroid camera.

Data analysis

The banding patterns generated by RAPD-PCR analyses were compared to determine the genetic relatedness of the 14 Citrus genotypes. The amplified fragments were scored either as present (1) or absent (0). Bands of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal,1973) .Dice formula: GS(ij)=2a / (2a + b +c).Where GS (ij) is the measure of genetic similarity between individuals I and j, (a) is the number of bands shared by I and j, (b) is the number of bands present in I and absent in j, and (c) is the number of bands absent in I and present in j.The genetic similarity matrices based on the Dice coefficient were used in the cluster analysis to generate a dendrogram using the UPGMA analysis.

RESULTS AND DISCUSSION

Polymorphisms as detected by RAPD markers

To assess the genetic uniformity of the different trees belonging to each accession, a preliminary assay was performed. In this respect, five leaf samples of each *Citrus* variety and ten samples of each rootstock were collected from different trees and tested with 10 different RAPD primers. A lower number of samples were taken from the varieties since they are vegetatively propagated while, rootstock accessions are propagated through seeds. In *Citrus*, it is well known that seeds

contain a number of embryos (polyembryony), one of these embryos is usually of a zygotic origin, while, the remaining are of nucellar origin. The latter embryos are of special interest in *Citrus* propagation since they produce trees of genotype similar to the mother tree. Therefore, it was useful to assess the genotypic stability of the seedlings of the different rootstocks. The results revealed that all the examined varieties exhibited uniform patterns with the ten examined primers. However, with rootstock accessions only two samples of the Brazilian sour orange accession exhibited minor differences in their patterns with only one of the tested primers (OPO03). Therefore, five uniform DNA samples were pooled to represent each accession and all the subsequent analyses were performed on the pooled samples.

One of the most important features of the RAPD technique is detecting of high levels of polymorphism and this feature has been met in the present study (Fig.1). Forty-six primers were screened with the DNA of the 14 *Citrus* genotypes.Among the forty-six tested primers, forty primers generated reproducible and easily scorable RAPD profiles with a number of amplified DNA fragments ranging from 8 to 26 (Table3). The total number of fragments produced by the forty primers was 531 with an average of 13.3fragments / primer. While the number of polymorphic fragments ranged from 2 to 21. In this respect, Deng *et al* .(1995) used thirty-six 10-mer arbitrary primers to detect RAPD markers in mutants of lemon, and reported that twenty two of the tested primers generated polymorphic profiles.As shown in Table(3), a maximum number of 26 amplicons was amplified with primer OPA08, while the minimum number of fragments was amplified with primers OPO04, OPO09 and OPZ12. The highest number of polymorphic bands (21) was obtained with primer OPA08. However, the highest percentage (100%) of polymorphism was exhibited by OPO08. The average number of polymorhpic fragments / primer among the 14 *Citrus* accessions was 8.7.In this respect, Machado *et al.*(1996) and Coletta *et al.*(1998) found that in mandarins the average number of polymorphic bands/primer was 2.2 and 1.95, respectively. This discrepancy could be interpreted, as these authors used accessions belonging to one species, whereas, the present investigation included accessions representing 8 species. Moreover, the size of the amplified fragments varied with different primers, ranging from 150 to 5000bp. Different authors working on *Citrus* reported variable fragment sizes, e.g. 100-3200bp (Deng *et al.*, 1995), 274 to 3006bp (Machado *et al.,* 1996) and 400-3000bp (Coletta *et al.,* 1998). These differences could be attributed to the use of different primers and different reaction conditions.

Genetic relationships as revealed by RAPD markers

To determine the genetic relationships among the 14 Citrus genotypes, the scoring data were used to compute the similarity matrices according to Dice (Sneath and Sokal, 1973). These genetic similarity matrices were then used in the cluster analysis to generate a dendrogram using the UPGMA analysis. The genetic similarity ranged from 10.8% to 87.7%. The highest genetic similarity revealed by the RAPD analysis (87.7) was between Marsh seedless grapefruit (MSG) and Ruby red grapefruit (RRG), both belonging to the species C. paradisi. On the other hand, the genetic similarity between Troyer citrange (TC) and any of the other accessions ranged from 10.8% to 12.6%.The low percentage of similarity between Troyer citrange and the other accessions might be due to its origin as a hybrid between two different genus, i.e. (Citrus sinensis x Poncirus trifoliata).

Therefore, Poncirus trifoliata constitutes a substantial amount of its genetic background resulting in the genetic divergence from the studied Citrus genotypes. Consequently, the RAPD-based dendrogram (Fig.2) separated the Troyer citrange genotype from all the other Citrus accessions, thus demonstrating the distinctiveness of the genetic background of the Troyer citrange.

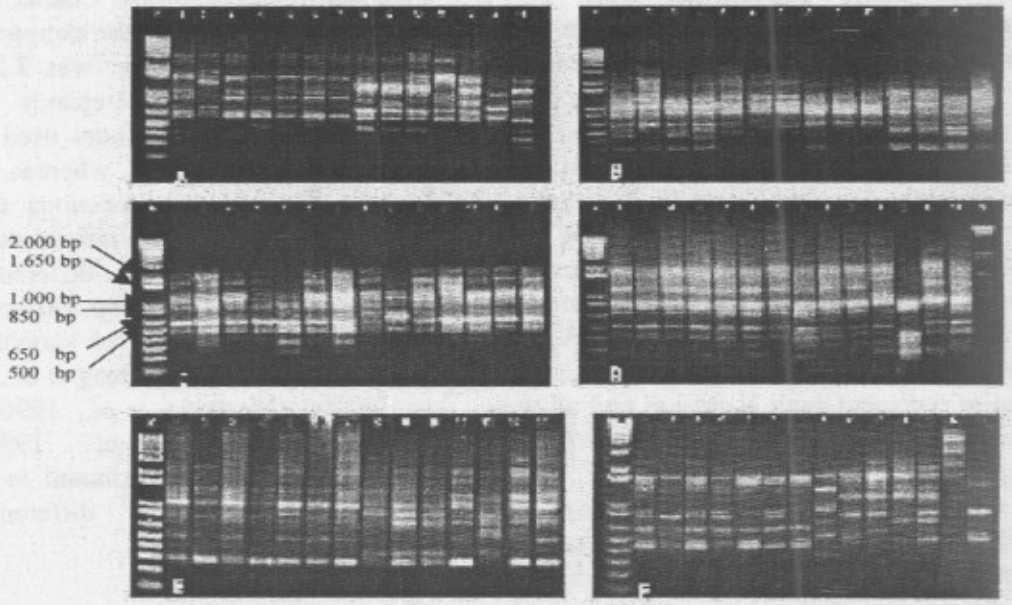

Fig. (1): RAPD profiles of the 14 Citrus genotypes amplified with RAPD primers OPB11(A), OPG17(B), OPO07(C), OPO13(D), OPO20(E) and OPF06 (F). Lanes 1 to 14 refer to Citrus genotypes, WNO, MW, VO, AWM, MSG, RNO, RRG, BSO, SSO, RSO, RL, VOL, TC and CM, respectively. M: molecular weight marker (1 Kb ladder plus).

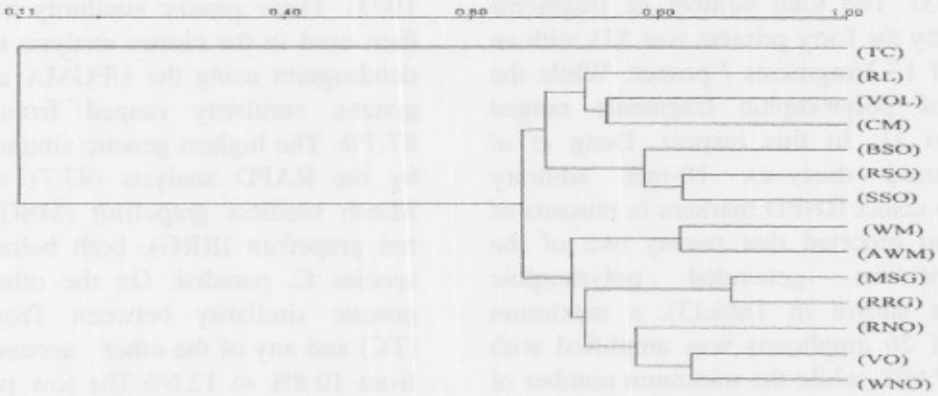

Fig. (2): Dendrogram for the 14 Citrus genotypes constructed from RAPDs data using Unweighted Pair-group Arithmetic Average (UPGMA) and similarity matrices computed according to Dice coefficients.

Table (3): Total number of amplicons, monomorphic amplicons, polymorphic amplicons and percentage of polymorphism as revealed by RAPD markers among the 14 Citrus accessions.

Primer	Total # of amplicons	Monomorphic amplicons	Polymorphic amplicons	% of polymorphism
OPA06	9	7	2	22.2
OPA08	26	5	21	80.8
OPA15	14	4	10	71.4
OPB01	9	2	7	77.8
OPB10	12	2	10	83.3
OPB11	22	3	19	86.4
OPB12	10	7	3	30.0
OPB14	16	7	9	56.3
OPC02	18	8	10	55.6
OPC04	14	6	8	57.1
OPC07	12	4	8	66.7
OPC09	10	4	6	60.0
OPC10	9	3	6	66.7
OPC15	14	2	12	85.7
OPC18	12	4	8	66.7
OPF06	18	1	17	94.4
OPG17	18	10	8	44.4
OPG18	11	4	7	63.6
OPH01	12	4	8	66.7
OPK16	19	7	12	63.2
OPO03	15	7	8	53.3
OPO04	8	3	5	62.5
OPO07	13	4	9	69.2
OPO08	14	0	14	100
OPO09	8	2	6	75.0
OPO10	18	9	9	50.0
OPO13	18	6	12	66.7
OPO16	13	10	3	23.1
OPO20	13	3	10	76.9
OPW18	16	1	15	93.8
OPX18	12	1	11	91.7
OPZ11	9	2	7	77.8
OPZ12	8	5	3	37.5
OPZ14	9	4	5	55.6
OPZ15	10	2	8	80.0
OPZ16	14	3	11	78.6
OPZ17	10	6	4	40.0
OPZ18	13	6	7	53.8
OPZ19	11	9	2	18.2
OPZ20	14	5	9	64.3
Total	531	182	349	65.7 %
Average	13.3	4.55	8.7	

The other *Citrus* accessions cluster comprised two subclusters . One subcluster was subdivided into two groups, the first included the two mandarin varieties [Willowleaf mandarin (WM) and Abd el-Razek mandarin (AWM)], while the other group was divided into two subgroups.The two accessions of grapefruit, Marsh seedless

grapefruit (MSG) and Ruby red grapefruit (RRG) clustered together in one group.While, the other subgroup included the three accessions of sweet orange [Washington navel orange (WNO),Valencia orange (VO) and Robertson navel orange (RNO)]. The second subcluster grouped the remaining accessions which represent all the rootstocks used in this study. This subcluster was divided into two groups. One group contained the accessions of sour orange [Balady sour orange (BSO), Brazilian sour orange (RSO) and Spanish sour orange(SSO)], while the other group contained the Rangpure lime (RL), Volkamer lemon (VOL) and Cleopatra mandarin (CM) rootstocks. In this respect, Cameron and Frost (1968) and Coletta *et al.* (1998) mentioned that genetic variability in *Citrus* is related to high number of taxonomic units (species and hybrids), as well as frequent mutations resulting in bud variation and limb sports.

The results obtained by the RAPD technique showed that RAPD assay clustered the accessions of each species together. This finding is in accordance with Coletta *et al.* (1998), they showed that the RAPD technique could be used to cluster mandarin into groups of similar accessions.

Genotype identification by unique RAPD markers

As shown in Tables (4 and 5), the RAPD assay permitted the identification of 13 *Citrus* accessions by unique positive and/or negative markers. Twelve accessions were characterized by 79 positive unique RAPD markers. Among these twelve accessions, seven were also characterized by negative RAPD markers. On the other hand, one accession (Spanish sour orange) was distinguished by negative RAPD markers only. Among the tested primers,17 exhibited negative markers and 31 revealed positive markers.These identified 107 unique markers across the 14 *Citrus* accessions. The RAPD primers generating the different markers and the markers approximate size are listed in Tables (4 and 5). Troyer citrange was characterized by the highest number of positive unique markers (14) and negative markers (7). On the other hand, the lowest number of unique markers was detected in Spanish sour orange and Valencia orange which were identified by only two unique negative and two positive markers, respectively.Certain primers were more informative than the others e.g., OPC04, OPK16 and OPO13 since they identified the highest number of genotypes (5), these primers together had the potential to identify 8 genotypes.In this respect, Koller *et al.* (1993) developed a key for 11 apple cultivars based on 14 RAPD markers amplified by 2 primers. Similarly, Wolf and Rijn (1993) demonstrated that, only two primers were necessary to distinguish 18 Chrysanthemum cultivars. Aruna *et al.* (1995) presented a key for 7 blueberry genotypes based on 11 markers amplified by four primers. The origin of these unique markers may be attributed to mutation at the priming site of the primers or to insertion/deletion mutation in the distance between the reverse and forward priming sites of the primers. The presence of unique RAPD markers among the various *Citrus* genotypes confirms the utility of the approach for fingerprinting purposes. RAPD fingerprinting has a number of potential applications including the determination of cultivar purity, efficient use and management of genetic resources collection, particularly in identification of mislabeled accessions (Ahmed, 1999).

Identification of *Citrus* accessions resistant to *Ctv* and/ or with acitric fruits as revealed by RAPDs and SCARs

Table (4): The seven Citrus rootstock genotypes characterized by unique positive and / or negative RAPD markers, marker size and total number of markers identifying each rootstock.

Genotype	Unique positive markers			Unique negative markers			Grand Total
	Size of the marker band (bp)	Primer	Total # of markers/ accession	Size of the marker band (bp)	Primer	Total # of markers/ accession	
Troyer citrange	400, 650	OPA15		2500	OPA15		
	750	OPB11		550	OPC04		
	1200	OPC04	14	400	OPG18	7	21
	1000	OPC10		1650	OPK16		
	1853	OPF06		650	OPO04		
	400, 1450	OPG18		850	OPZ12		
	1300	OPO08		590	OPZ20		
	603	OPO13					
	1450	OPX18					
	1200	OPZ12					
	650	OPZ18					
	500	OPZ20					
Balady sour orange	650	OPB10					
	195, 745	OPF06					
	1050, 1430	OPF06	12				12
	450	OPO13					
	430, 640, 850	OPW1					
	1690, 1755	8					
	1500	OPW1					
		8					
		OPZ12					
Spanish sour orange				935	OPB12		
				2390	OPO16	2	2
Brazilian sour orange	1520,1830,	OPH01					
	3000, 1000	OPK16	4				4
Cleopatra mandarin	300	OPB01		450, 500	OPB14		
	750	OPB10	7	900	OPC04	5	12
	950	OPC04		850, 1650	OPZ16		
	1455	OPC10					
	1650	OPO13					
	350, 1353	OPZ16					
Rangpure lime	1950	OPA15					
	1000	OPC07					
	1350, 1650,	OPC10					
	3000	OPK16	10				10
	1600,3000	OPO13					
	1000	OPO20					
	1680,1590	OPZ20					
Volkamer lemon	590	OPA15		700	OPO13		
	255	OPB12		800	OPO13		
	1000	OPB14	12	850	OPO13	5	17
	700	OPC04		1078	OPO13		
	150	OPK16		930	OPO20		
	700	OPO07					
	750	OPO13					
	620, 650, 900	OPO20					
7 rootstocks			59			19	78

Table (5): The budded Citrus genotypes characterized by unique positive and/ or negative RAPD markers, marker size and total number of markers identifying each genotype.

Genotype	Unique positive markers			Unique negative markers			
	Size of the marker band (bp)	Primer	Total # of markers/ accession	Size of the marker band (bp)	Primer	Total # of markers/ accession	Grand Total
Valencia orange	150 1950	OPA08 OPG18	2				2
Washington navel orange	1550	OPG18	1	1250 400	OPB01 OPB10	2	3
Robertson navel orange	1700 3000	OPG18 OPO03	2	1650 1353 1200 2027 1850	OPB10 OPB10 OPB14 OPZ11 OPZ11	5	7
Willowleaf mandarin	2000 400 250 200 2255	OPB11 OPO07 OPC04 OPC04 OPZ15	5	200	OPO16	1	6
Abdel- Razek willowleaf mandarin	150 850 450	OPC02 OPK16 OPZ20	3	250	OPZ19	1	4
Marsh seedless grapefruit	300 3000 1432 2150 2000 1600 1078	OPA06 OPC02 OPC04 OPC09 OPX18 OPZ12 OPZ14	7				7
6 Budded accessions			20			9	29

Among several marker systems currently available, randomly amplified polymorphic DNA (RAPDs)represents a type of genetic markers that can be generated efficiently and simply, thus, enabling quick tagging of traits of agricultural importance. In *Citrus*, genome maps containing RAPD loci have been constructed (Cai *et al.*, 1994) and several traits of horticultural importance including nematode resistance (Ling *et al.*, 1994), dwarfing (Cheng and Roose, 1995) and *Ctv* resistance (Gmitter *et al.*, 1996) have been tagged with RAPD markers. To enhance their usefulness in marker-assisted selection and map based cloning, most of these markers were converted into SCAR markers by cloning of the RAPD/PCR- product and sequencing.

In the present study, eight 10 mer RAPD primers and three SCAR primer pairs previously published as markers linked to *Citrus* tristeza virus, were used in an attempt to identify accessions resistant to this virus. Fig. (3 A) illustrates the RAPD marker linked to *Ctv* as revealed by primer OPB11. As shown in Table (6), all the examined primers amplified fragments with the exact size as

reported by Gmitter *et al.* (1996) except OPH01 which failed to amplify the expected marker with any of the examined accessions. This may be due to the absence of the target sequence in the examined accessions, or a mismatching at the priming site in the resistant accessions. Troyer citrange revealed the highest number of RAPD markers known to be linked to resistance to *Ctv* (six out of eight tested primers). Moreover, only one of the SCAR primer pairs amplified the expected fragment (650pb) only in Troyer citrange; this might be due to the gene flow from its parent (*Poncirus trifoliata*) which is known to be resistant to *Ctv*. Volkamer lemon revealed 3 markers identified by RAPD primers OPF06, OPO07 and OPX18.While, Rangpure lime and Spanish sour orange exhibited only one marker each with primers OPF06 and OPO07, respectively.

Concerning the absence of the SCAR markers, Deng *et al.* (1997) stated that no amplification by SCAD02 was observed in several *Citrus* varieties including Foster grapefruit (*Citrus paradisi* Macf), Valencia and Hamlin orange (*Citrus sinensis* L.osb), and Nova tanglo [(*Citrus reticulata*. Blanco) x (*Citrus paradisi*) x (*Citrus reticulata*)]. On the other hand, three RAPD primers and one SCAR primer pairs were used to screen *Citrus* accessions for markers linked to the acitric character. The tested primers were OPC10, OPB12, OPZ20 and SCZ20. The primer, OPC10 amplified a marker at the expected size as reported by Fang *et al.* (1997). This marker was detected in Willowleaf mandarin and Abdel Razek willowleaf mandarin, however, these two accessions are known to have a moderate fruit acidity.In this respect, Fang *et al.* (1997) stated that, the intermediate acidity of acitric heterozyotes (*Acac*) could be interpreted as indicating that acitric is not fully recessive. They also demonstrated that these three markers (OPB12, OPC10 and OPZ20) did not appear in Pummelo 2240, Red mandarin-lime, yellow sweet lime, Kinnow mandarin, or Rubidoux trifoliate orange. On the other hand, these markers appeared in Chandler and Pummelo 2241, and they stated that it is evident that the amplified sequences must originate from Pummelo 2241. Therefore, they concluded that, these RAPD markers are only informative in certain crosses involving Pummelo 2241 or its hybrids such as Chandler.

In the present study, 7 RAPD and one SCAR markers linked to *Ctv* were useful in detecting resistant accessions. Identification of trait linked markers that allow selection at the seedling stage is highly advantageous to *Citrus* breeder.

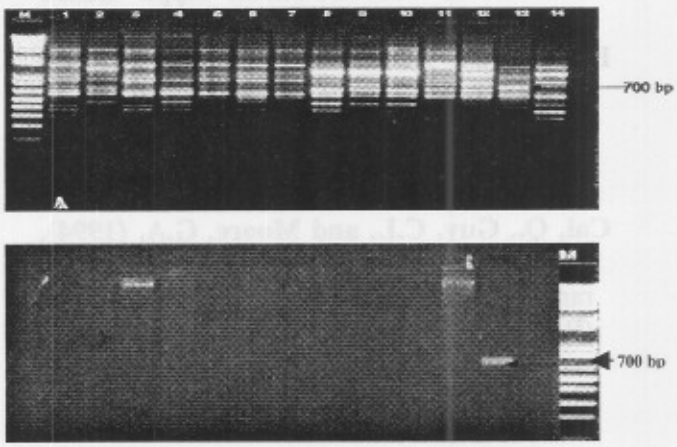

Fig. (3): RAPD (A) and SCAR (B) profiles illustrating markers linked to Citrus tristeza virus resistance in Troyer citrange as detected by primers OPB11 and SCT08, respectively.

Table (6): RAPD and SCAR markers linked to Ctv resistance, size of the corresponding band and Citrus accessions revealing the markers.

Marker type	Primer	Band size (bp)	Genotype
RAPD	OPA15	650	Troyer citrange (TC)
	OPB11	700	Troyer citrange(TC)
	OPF06	750	Rangpure lime, Volkamer lemon
	OPH01	—	–
	OPO07	650	Spanish sour orange, Volkamer lemon, TC
	OPO16	650	Troyer citrange (TC)
	OPW18	450	Troyer citrange (TC)
	OPX18	550	Volkamer lemon, Troyer citrange (TC)
SCAR	SCT08	650	Troyer citrange (TC)

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الملخص العربي

دراسة التباين الوراثي وتحديد البصمة الوراثية لبعض أصناف الموالح باستخدام الواسمات الجزيئية

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تم دراسة التباين الوراثي وتحديد البصمة الوراثية لـ١٤ صنفا من الموالح بواسطة تقنيات ال RAPD وال SCAR المعتمدة على تفاعل البلمرة المتسلسل ، حيث استخدم ٤٠ بادئ من نوع RAPD لدراسة التباين مما أظهر ٥٣١ شظية من شظايا الDNA، منها ٣٤٩ شظية أوضحت تباينا بين الأصناف المختلفة وبذلك كانت نسبة التباين ٦٥,٧% وكان متوسط عدد الشظايا المتباينة للبادئ الواحد ٨,٧ شظية. كشفت تقنية ال RAPDأيضا عن نسبة تشابه وراثي تتراوح بين ١٠,٨% ، ٨٧,٧١% بين الترويرسيترانج وكل من اليوسفي البلدي ،يوسفي عبد الرازق والنارنج البلدي وبين كل من الجريب فروت مارش سيدلس والروبى رد على التوالي.استخدمت درجات التشابه الناتجة من هذه التقنية لرسم الدندر وجرام الذي يوضح درجات القرابة بين الأصناف تحت الدراسة وأدت إلى فصل الترويرسيترانج فى مجموعة منفردة، بالإضافة إلى أن الأصناف التي تنتمي لنفس النوع كانت دائما تقع فى مجموعة واحدة .وقد أمكن تمييز ١٣ صنفا من مجموعة الأصناف تحت الدراسة (١٤ صنف) بواسطة ٣٤ بادئ من نوع ال RAPD حيث تميزت هذه الأصناف بواسمات فريدة موجبة أو سالبة أو كليهما وقد أمكن باستخدام كل من بادئات ال OPCO4,OPK16,OPO13 تمييز خمس أصناف مختلفة بينما باقي البادئات ميزت من ١-٤ أصناف.

أظهرت ٧ بادئات RAPD ($OPA15_{650}$, $OPB11_{700}$,$OPFO6_{750}$,$OPOO7_{650}$,$OPO16_{650}$,$OPW18_{450}$,$OPX18_{550}$) معروفة بارتباطها بصفة المقاومة للتريستيزا الواسمات الخاصة بهذه الصفة فى أصناف الترويرسيترانج وليمون رانجبور وليمون الفولكاماريانا والنارنج الأسباني. كما أظهر زوج من بادئات الSCAR الثلاث المستخدمة فى الدراسة والمعرفة أيضا بارتباطها بصفة المقاومة لفيروس التريستيزا الواسم الخاص بهذه الصفة فى صنف الترويرسيترانج. من ناحية أخرى تم استخدام ٣ بادئات RAPD (OPC10, OPZ20, OPB12) وزوج من بادئات ال SCAR (SCZ20) للكشف عن واسم مرتبط بانخفاض الحموضة.