ASSESSMENT OF GENETIC DIVERSITY OF TOMATO (*Lycopersicon esculentum* L.) GERMPLASM USING MOLECULAR MARKERS (RAPD AND ISSR)

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Tomato (*Lycopersicon esculentum* L.), is an important and widespread vegetable in the world for fresh consumption and processed products. However, the reduction of genetic variation in tomato (*Solanum lycopersicum* L.) through domestication and breeding (Tanksley and McCouch, 1997) has resulted in the need for conservation and utilization of all existing genetic resources. Heterogeneous landrace populations are among the most important genetic resources (Zeven, 1998) and have been, and will continue to be used in plant breeding schemes. In Egypt, tomato landraces were widely cultivated till the introduction of hybrids. Currently, very few farmers still grow them only for their own or local consumption. Therefore, it is important to collect, characterize and conserve tomato landraces. The efficient conservation and exploitation of landraces require the study of their genetic diversity structure (van Hintum, 1995). The genetic diversity in any local population does not occur at random, but is structured based on various biological and environmental factors. The high level of within landrace heterogeneity is related to its adaptability (Cooper *et al*., 2000). Landraces are mixtures of phenotypes and represent diverse and dynamic gene pools that evolve over time under both farmer and natural selection pressures (Hawtin *et al*., 1997). The role that farmers play is both direct and/or indirect as happens through farming system changes or due to social reasons (Zeven, 2002). However, narrow genetic bases have become a bottleneck in tomato breeding. Therefore, it is essential to know the genetic relationships among the tomato species. Molecular markers are generally recognized as a reliable means for the genetic identification among plant genotypes (Bretó *et al.*, 2003; Gupta and Rustgi, 2004; Claudio *et al.*, 2004; Omrani *et al*., 2007). In the past, all kinds of molecular markers such as restriction fragment length polymorphism (RFLP) (Williams and Clair, 1993; Messeguer *et al.*, 1991), inter-simple sequence repeat (ISSR) (Tikunov *et al*., 2003), randomly amplified polymorphic DNA (RAPD) (Claudio *et al.*, 2004; Bernardette *et al.*, 2006), simple sequence repeat SSR (Powell *et al*., 1996; He *et al*., 2003; Jin *et al.*, 2004; Cooke *et al.*, 2003) and amplified length polymorphic (AFLP) (Claudio *et al*., 2004) have been used to analyze the genetic relationships among the cultivated tomato varieties. RAPD and ISSR are two powerful DNA fingerprinting techniques. RAPD was applied to as-

sess genetic diversity in tomato varieties (Saavedra *et al*., 2001; Li-Wang *et al*., 2007; Elham *et al.*, 2010; Juan *et al*., 2010; Ezekiel *et al*., 2011). Also, recently there are few reports regarding the utilization of ISSR to study the genetic relationships among the tomato varieties (Kochieva *et al*., 2002; Tikunov *et al*., 2003; Terzopoulos and Bebeli, 2008; Bojinov, 2009; Terzopoulos and Bebeli, 2008). The objective of the present study was to analyze the genetic diversity of some tomato varieties in Egypt and to compare the effectiveness of two techniques: RAPD and ISSR.

MATERIALS AND METHODS

Molecular characterization of nine tomato cultivars (*Lycopersicon esculentum* L.) and one tomatille (*Physalis philadelphica*) and one cherry tomato (*Solanum lycopersicum* var. cerasiforme) accessions were used in the present investigation and were collected from different regions in Egypt.

Molecular marker

Molecular fingerprinting of tomato accessions based on (Randomly Amplified Polymorphic DNA (RAPD

DNA extraction

Several preliminary experiments were performed to optimize the RAPD protocol. Based on the results of these preliminary experiments, a standard protocol was developed and used for subsequent experiments. DNA extraction was carried out using leaf materials collected from different tomato accessions seedling. Genomic DNA was extracted and purified using a modified CTAB method.

Estimation of DNA concentration

The DNA concentration of the different samples was estimated on agarose gel (0.8%) to estimate the purity, and measured according to Biophotometer (Eppendorf) and diluted to 100 ng/µl. The DNA stored at -20°C to be used in subsequent analysis.

Amplification of RAPD markers

Ten-mer oligonucleotide primers (Bioron Co.) were used. The base sequences of the primers are given in (Table 1). PCR reactions were performed in 25 µl reaction volume containing 1 U of Taq DNA polymerase (Thermo), 2 mM dNTPs (Thermo), 1.5 µl $MgCl_2$ (25 mM), 2 µl primer (100 pm, Bioron Co.) and 1.0 µl DNA (100 ng). The reaction mixture was vortexed and centrifuged briefly

PCR was initiated by an initial denaturation step for 4 min at 94°C followed by 35 cycles (94°C/45 sec, 40°C/30 sec, 72°C/2 min), and then a final extension cycle at 72°C for 5 min. The PCR products were separated on 2.5% agarose gel in 1X TBE buffer containing ethidium bromide and visualized on a UV transilluminator and photographed using a Gel Documentation System (Alpha Innotech). The sequences of the ten RAPD primers (B-03, B-06, B-07, B-13, B-15, B-20, B-05, B-11 and B-18) are presented in Table (1).

Molecular fingerprinting of tomatoes accessions based on (Inter Simple Sequence Repeats (ISSRs)

Isolation of plant genomic DNA

DNA extraction was carried out using leaf materials collected from each accession. ISSR analysis was performed using the diluted DNA extracted from the samples. The PCR was performed in 25 µl reaction volume containing 1 U Taq (Thermo), 2 mM dNTPs (Thermo), 1.5 µl $MgCl_2$ (25 mM), 0.5 µl primer (50 pm, Bioron Co.) and 1.0 µl DNA (100 ng). A high stringency touchdown thermocycling profile was used as follows: an initial denaturation step for 4 min at 94°C followed by 10 touchdown cycles (94°C/45 sec, (Ta)°C/30 sec, 72°C/ 2 min). This was followed by 25 cycles (94°C/45 sec, (Ta)°C/30 sec, 72°C/2 min) and then a final extension cycle at 72°C for 5 min. The PCR products were separated on 2.5% agarose gel in 1X TBE buffer containing ethidium bromide and photographed with a Gel Documentation System (Alpha Innotech). The sequences of the 18 ISSR primers are presented in Table (2).

Scoring of the data

Scoring of ISSR data was performed using 1% agarose gel electrophoresis profile, as clear and distinct fragment were scored as (1) for presence and (0) for absence.

RESULTS AND DISCUSSION

Molecular marker

Molecular fingerprinting of tomatoes accessions by RAPD

Nine RAPD primers were screened for polymorphism and based on the clear scorable band pattern; primers were selected for DNA analysis of the accessions and were of good quality (Fig. 1). The size of the amplification products ranged from 196 to 1790 bp. The total numbers of scored bands were 180, the number of bands per primer varied from 13 to 29 with an average of 13 bands /template. The highest number of polymorphic bands was obtained with primers, B-03, B-07, B-13, B-15 and D-11 (Table 3).

The total number of polymorphic bands was 158 with an average of 17.5 polymorphic fragments/primer. This represents an average of polymorphism 87.77% (Table 3). The number of polymorphic markers varied among the different primers. Primers D-11 generated 24 polymorphic bands with 100% polymorphism. While both of primers B-20 and D-06 showed low level of polymorphism, 61.5 and 84%, respectively (Table 3).

Twenty two out of one hundred and eighty RAPD (about 12%) were found to be useful as cultivar-specific markers (Table 4 and Fig. 1) with some of them present in some accession and absent in the others cultivars in this study.

Molecular fingerprinting of tomato accessions by ISSRs

Eighteen ISSR primers were tested for DNA analysis of the cultivars and were of good quality and all of them yielding polymorphic amplification products and based on the clear scorable band pattern, (Fig. 2). The 18 primers chosen for the present study yielded a total of 293 amplified bands, 248 (84.6%) of which were polymorphic (Table 5 and 6). The number of polymorphic bands per primer ranged from 9 to 29 with an average of 16.2 and the percentage of polymorphism per primer ranged from 69% to 100% (Table 5). Taking into consideration all of the 18 primers, all accessions had positive and negative unique bands for each one except Pakmore accession had four negative bands. The size of the amplification products ranged from 32 to 1550 bp. The total numbers of scored bands were 293. The number of bands per primer varied from 9 to 25 with an average of 13.2 bands/template. The highest number of polymorphic bands was obtained with primers, 842, 834, 3 and 807 (Table 6).

The total number of polymorphic bands was 390 with an average of 21.6 polymorphic fragments/ primer. This represents an average of polymorphism 83.44% (Table 5). The number of polymorphic markers varied among the different primers. Primers 842 generated 24 polymorphic bands with 96% polymorphism. While primer 811 showed low level of polymorphism (70%) (Table 5). Forty five out of two hundred ninety three ISSRs (about 15.3%) were found to be useful as cultivar-specific markers (Table 5 and Fig. 2) which some of them present in one cultivar and absent in the others cultivars in this study.

The number of ISSR-PCR fragments generated by using the eighteen primers, and could be used as cultivar-specific markers, were arranged descending as primer 842 (eleven markers), primer 16 (nine markers), primer ISSR 2 (eight markers), primer 3 (seven markers), primers ISSR 1 and 834 (six markers), primer 890 (five markers), primers 891, 807, 1789A and 841 (four markers), primers 17899B, AW-3, 17 and ISSR 35 (three markers), primer 811 (two markers) and primers DAT and ISSR 34 (one marker). (Table 6 and Fig. 2).

Previous reports stated that the application of both RAPD and ISSR techniques have an important potential to provide new tools for the identification and characterization of species. It became possible through fingerprinting for each species since DNA is a source of informative polymorphism (El-Rabey, 2008), consequently, techniques of molecular genetic markers have an important potential for the detection of genetic differences among species. RAPD technique have been used for varietal identification of tomato (Rajput *et al.*, 2006; Singh *et al.*, 2007), using for genetic diversity among tomato varieties (Elham *et al*., 2010; Meng *et al*., 2010; Ezekiel *et al*., 2011) and application of RAPD in tomato hybrid genetic purity

testing (Rome *et al*., 1995; Liu *et al*., 2007).

Moreover, several authors reported on the usefulness of ISSR for cultivar identifications. The ISSR technique, isn't much more difficult for marker development than RAPD, and also, requiring a small amount of DNA for amplification, enables the detection of the genome. ISSRs are ideal as markers for genetic mapping and population studies because of their abundance, and the high degree of polymorphism between individuals within a population of closely related genotypes (Lanham and Brennan, 1998).

In tomato, ISSRs have been employed for the assessment of diversity among accessions of various *Lycopersicon* species and among tomato cultivars (Kochieva *et al.*, 2002; Tikunov *et al*., 2003; Bojinov, 2009) and in characterizing the diversity of Greek tomato landraces (Terzopoulos and Bebeli, 2008). The genetic diversity among different tomato cultivars were investigated by using RAPD-PCR based markers (Williams *et al*., 1990) and ISSR (Wang, 2004). Both methods provide quick, reliable and informative data for genotyping tomato cultivars (Nagoka and Ogihara, 1997; Levi and Rowland, 1997; Mansour *et al*., 2009).

RAPD and ISSR clustering analysis

The RAPD dendrogram obtained by UPGMA analysis grouped the 11 tomatoes accessions into one main robust group, six minor groups. The Jaccard's coefficient ranged from 0.38 to 0.92 (Fig. 3a). The lowest similarity coefficients were observed in Tomatillo and Floradade accessions (0.38) while the highest similarity coefficients were obtained between 'Edkawy' and 'Strain B' (0.92). VFNT and Castle Rock and Strain B and Edkawy were ranked in sub-groups and the other seven accessions were clustered into 7 nodes, one major and 6 minor separated robust group. Cluster I comprised one accession (Tomatillo), cluster II include one accession 'Floradade'. Cluster III contained one accession 'Super maramande'. Also, each of clusters V, IV and IIV contained Pakmore, Peto86 and Junhile, respectively, (Fig. 3a).

The ISSR dendrogram obtained by UPGMA analysis grouped the 11 tomatoes accessions into two main clusters. The Jaccard's coefficient ranged from 0.42 to 0.89 (Fig. 3b). The lowest similarity coefficients were observed in Tomatillo and Floradade accessions (0.42) but the highest similarity coefficients were obtained between 'Junhile and Edkawy' and 'Peto 86 and Strain B' (0.89). Two major clusters and three minor separated clusters were observed. The first major cluster contained Tomatillo and the second major cluster contained Floradade accession and three sub-clusters. Sub cluster I include one accession 'Cherry tomats'. Cluster II compresed VFNT and Super marmande. Also, each of clusters III contained Castle Rock accession alone, Pakemore, (Peto 86 and Stran B accessions) and (Junhilee and Edkawy accessions), (Fig. 3b).

Combined dendrograms analysis grouped the 11 tomatoes accessions also, into two main clusters, three sub-clusters. The Jaccard's coefficient ranged from 0.41 to 0.90 (Fig. 3c). The lowest similarity coefficients were observed in Tomatillo and Floradade accessions (0.41) as shown in both of RAPD and ISSR dendrogram, whereas the highest similarity coefficients were obtained between 'Edkawy' and Strain B' (0.90). Two major clusters and three sub clusters. The first consist of Tomatillo accession, the second major cluster contained of Floradade. Sub cluster I contained Pakmore and cherry tomats accessions, sub-cluster II composed of super marmande and (VFNT and Castle Rock) accessions. Sub-cluster III consists of Strain B and Edkawy, Junhilee and Peto 86 accessions, (Fig. 3c).

The results confirmed that the tomato accessions are highly variable species which reflect the agronomic diversity within tomato cultivars. The high diversity found between tomato accessions is probably due to a diverse germplasm origin. Also, showed a clear separation of the landraces from tomato and tomato cherry cultivars, which was also observed by (Carelli *et al*., 2006) in Brazilian landraces using RAPDs, in American heirloom varieties using SSRs (Labate and Robertson, 2002) and in Spanish local cultivars using SSRs and AFLPs (Garcia-Martinez *et al.*, 2006).

The phylogenetic analysis on the basis of RAPD derived a dendrogram revealed almost the same cluster pattern that obtained from the ISSR and confirm the phylogenetic relationship between the 11 tomatoes accessions studied indicating congruence between these two systems. It could be concluded that, both of ISSR and RAPD markers are equally valuable for genetic analysis and indicate a considerable amount of genetic diversity between the different studied accessions of *Lycopersicon esculentum* L. (Munazza *et al*., 2009) reported that the assessment of genetic diversity within and between landraces should have priority for varieties improvement. At the same time, it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm and to increase the utilization of plant genetic resources.

SUMMARY

Two DNA molecular marker systems, RAPD and ISSR were used to assess genetic diversity among nine tomatos (*Lycopersicon esculentum* L.), one tomatille (*Physalis philadelphica*) and one cherry tomato (*Solanum lycopersicum* var. cerasiforme) accessions, collected from different regions in Egypt. Accurate and unambiguous identification of these accessions is essential for germplasm preservation and use. Genomic DNA from the 11 accessions was screened with 18 ISSR primers and nine RAPD primers. A total of 293 and 180 clear fragments were amplified by ISSR and RAPD, respectively. On the other hand, unique positive markers were detected for 'Tomatillo' and

for 'Super marmande', by 4 and 3 RAPD primers, respectively. Moreover, 18 primers of ISSR produced unique positive markers for Tomatille and Castle Rock, respectively. The ISSR technology proved useful in describing genetic diversity among tomato accessions and studies the phylogenetic relationships between cultivars. Cluster analysis using the UPGMA method placed all tomato accessions and cultivars into a single group, while the Tomatille and cherry tomato accessions were placed in a second group.

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Table (1): Name and sequence of the primers used in RAPD analysis.

Primer code	Nucleotide sequences 5'→3'
B-03	CATCCCCCTG
B-06	TGCTCTGCCC
B-07	GGTACGCAG
B-13	TTCCCCCGCT
B-15	GGAGGGTGTT
B-20	GGACCCTTAC
B-05	TGAGCGGACA
B-11	AGCGCCATTG
B-18	GAGAGCCAAC

Table (2): Name and sequence of the primers used in ISSR analysis.

Primer code	Nucleotide sequences 5`→3`
17899-B	(CA)6 GG
17898-A	(CA)6 AC
ISSR-1	$CAC(TCC)_5$
ISSR-2	$AGA(TCC)_5$
890	$ACG(GT)_7$
891	$TCT(TG)_7$
807	(AG)8 T
811	$(GA)_8C$
AW-3	$(GT)_7$ AG
3	$(CA)_8AT$
16	$CGTC(AC)_7$
17	$CAGC(AC)_7$
DAT	$(GA)_7AC$
ISSR-34	$(AG)_8TG$
ISSR-35	$TCGA(CA)_7$
834	$(AG)_8CT$
841	$(GA)_8TC$
842	$(GA)_8TG$

Table (3): Statistics of the RAPD fragments for the eleven tomato accessions based on the nine RAPD primers.

RAPD primers	Total amplified fragments	Polymorphic fragments	Monomorphic fragments	Percentage of polymorphism (%)
B-03	20	17	3	85%
B-06	13	11	2	85%
B-07	29	27	2	93%
B-13	22	20	2	91%
B-15	21	18	3	86%
B-20	13	8	5	62%
D-05	19	17	2	89%
D-11	24	24	0	100%
D-18	19	16	3	84%
Total	180	158	22	86.11

Table (4): Unique RAPD markers showing polymorphic bands among the eleven tomato accessions.

Primer	Band size in bp	Super marmand	Floradade	Juhilee	Peto86	Edkawy	Strain B	Pakmore	Castle Rock	VFNT	Cherry tomato	Tomatillo
B-03	851.200	0	1	0	0	0	0	0	0	0	0	1
	622.400	1	1	1	1	1	1	0	1	1	1	0
	466.300	0	1	0	0	0	0	0	0	0	0	1
	365.900	0	1	0	0	0	0	0	0	0	0	1
	344.800	0	1	0	0	0	0	0	0	0	0	1
B-06	1123.077	0	0	0	0	0	0	0	0	0	0	1
	1038.400	0	0	0	0	0	0	0	0	0	0	1
	953.500	0	0	0	0	0	0	0	0	0	0	1
	819.800	0	0	0	0	0	0	0	0	0	0	1
	613.700	1	0	0	1	0	0	0	0	0	0	0
	540.100	0	0	0	0	0	0	1	0	0	0	1
	524.200	1	0	0	1	0	0	1	0	0	0	0
B-07	1530.000	0	0	0	0	0	0	0	0	0	0	1
	1400.000	0	0	0	0	0	0	0	0	0	0	1
	1270.000	0	1	0	0	0	0	0	0	0	0	0
	839.800	0	0	0	0	0	0	0	0	0	0	1
	623.037	0	0	0	0	0	0	0	0	0	1	0
	412.400	1	0	0	0	0	0	0	0	0	0	0
	384.400	0	0	0	0	0	0	0	0	1	0	0
	336.900	0	0	0	0	0	0	0	1	0	0	0
	319.600	0	0	0	0	0	0	0	0	0	0	1
	209.700	0	0	0	0	0	0	0	0	0	1	0

Table (4): Cont'

B-13	1550.000	0	0	0	1	0	0	0	0	0	0	0
	1500.000	0	0	0	0	0	0	0	1	0	0	0
	973.900	0	0	0	0	0	0	0	0	0	0	1
	911.500	0	0	0	0	0	0	0	0	0	1	0
	878.000	0	0	1	0	0	0	0	0	0	0	0
	511.500	0	0	0	0	0	0	0	0	0	0	1
	355.000	0	0	0	0	0	0	0	0	0	0	1
	338.500	0	0	0	0	0	0	1	0	0	0	0
	232.000	0	0	0	1	0	0	0	0	0	0	0
B-15	1066.700	0	0	0	1	0	0	0	0	0	0	0
	880.600	0	0	0	0	0	0	0	0	0	0	1
	784.000	0	0	0	0	0	0	0	0	0	0	1
	495.100	0	0	0	1	0	0	0	0	0	0	0
	476.300	1	0	0	0	0	0	0	0	0	0	0
	449.700	0	0	0	0	0	0	0	0	0	0	1
	293.400	0	0	0	0	0	0	0	0	0	0	1
D-05	1277.800	0	0	0	0	0	0	0	0	0	0	1
	1188.900	0	0	0	0	0	0	0	0	0	0	1
	1077.800	0	0	0	0	0	0	0	0	0	0	1
	809.000	0	0	0	0	0	0	0	0	0	0	1
	659.300	0	0	0	0	0	0	0	0	0	0	1
	613.100	0	0	0	0	0	0	0	0	0	0	1
	529.200	0	0	0	0	0	0	0	0	0	0	1
	432.700	0	0	0	0	0	0	0	0	0	0	1
	372.119	0	0	0	0	0	0	0	0	0	0	1
D-11	1790.900	0	0	0	0	0	0	0	0	0	0	1
	1572.700	0	0	0	0	0	0	0	0	0	0	1
	1481.800	0	0	0	0	0	0	0	0	0	0	1
	1209.000	0	0	0	0	0	0	0	0	0	0	1
	1136.300	0	0	0	0	0	0	1	0	0	0	0
	933.300	0	0	0	0	0	0	0	0	0	0	1
	586.000	0	0	0	0	0	0	0	0	0	0	1
	457.410	0	0	0	0	0	0	0	0	0	0	1
	346.300	0	0	0	0	0	0	0	0	0	0	1
	264.100	0	0	0	0	0	0	0	0	0	0	1
	196.100	0	0	0	0	0	0	0	0	0	0	1
D-18	1516.600	0	0	0	0	0	0	0	0	1	0	0
	457.200	0	0	0	0	0	0	0	0	0	0	1
B-20	1066.667	0	0	0	0	0	0	0	0	0	0	1
	316.876	0	0	0	0	0	0	0	0	0	0	1

Notes: (1) means presence band, (0) means absent band.

Table (5): Statistics of the RAPD fragments for the eleven tomato accessions based on the nine RAPD primers.

AF ISSR primers	Total amplified fragments	Polymorphic fragments	Percentage of polymorphism (%)
17899-B	12	6	50.0%
17898-A	7	3	42.9%
ISSR-1	13	12	92.0%
ISSR-2	17	14	82.0%
890	17	14	82.0%
891	13	9	69.0%
807	20	18	90.0%
811	10	7	70.0%
AW-3	12	9	75.0%
3	21	18	85.0%
16	18	12	67.0%
17	20	16	80.0%
DAT	13	12	92.0%
ISSR-34	9	7	78.0%
834	20	20	100.0%
841	16	15	94.0%
842	25	24	96.0%
Total	263	216	85.4%

Table (6): Unique ISSR markers showing polymorphic bands among the eleven tomato accessions.

Primer	Band size in bp	Super marmand	Floradade	Juhilee	Peto86	Edkawy	Strain B	Pakmore	Castle Rock	VFNT	Cherry tomato	Tomatillo
17899-B	1242.800	0	0	0	0	0	0	0	0	0	0	1
	442.900	0	0	0	0	0	0	0	0	0	0	1
	400.000	0	0	0	0	0	0	0	0	0	0	1
17898-A	779.269	0	0	0	0	0	0	0	0	0	0	1
	579.140	0	0	0	0	1	0	0	0	0	0	0
	517.968	0	0	0	0	0	0	0	0	0	0	1
	409.070	0	0	0	0	0	0	0	0	0	0	1

Table (6): Cont'

ISSR-1	1211.111	0	0	0	0	0	0	0	0	0	0	1
	969.436	0	0	0	0	0	0	0	1	0	0	0
	748.621	0	0	0	0	0	0	0	0	0	0	1
	624.300	0	0	0	0	0	0	0	0	0	0	1
	494.691	0	0	0	0	0	0	0	0	0	0	1
	437.892	0	0	0	0	0	0	0	0	0	0	1
ISSR-2	1190.000	0	0	0	0	0	0	0	0	0	0	1
	949.226	0	0	0	0	0	0	0	1	0	0	0
	881.060	0	0	0	0	0	0	0	0	0	0	1
	826.480	0	0	0	0	0	0	0	0	0	0	1
	677.984	0	0	0	0	0	0	0	0	0	0	1
	637.544	0	0	0	0	0	0	0	0	0	0	1
	528.214	0	0	0	0	0	0	0	0	0	0	1
	489.093	0	0	0	0	0	0	0	0	0	0	1
	442.640	0	0	0	0	0	0	0	0	0	0	1
890	889.967	0	0	0	0	0	0	0	0	0	0	1
	825.861	0	0	0	0	0	0	0	0	0	0	1
	557.333	0	0	0	0	0	1	0	0	0	0	0
	522.538	0	0	0	0	0	0	0	0	0	0	1
	246.696	0	0	0	0	0	0	0	0	0	0	1
891	900.000	0	0	0	0	0	0	0	0	0	0	1
	673.929	0	0	1	0	0	0	0	0	0	0	0
	326.266	0	0	0	0	0	0	0	0	0	0	1
	221.487	0	0	0	0	0	0	0	0	0	0	1
807	662.013	0	0	0	0	0	0	0	0	0	0	1
	338.309	0	0	0	0	0	0	0	0	0	0	1
	261.695	0	0	0	0	0	0	0	0	0	0	1
	219.594	0	0	0	0	0	0	0	0	0	0	1
811	630.036	0	0	0	0	0	0	0	1	0	0	0
	420.909	0	0	0	0	0	0	0	0	0	0	1
AW-3	929.429	0	0	0	0	0	0	0	0	0	0	1
	565.039	0	0	0	0	0	0	0	0	0	0	1
	419.584	0	0	0	0	0	0	0	0	0	0	1
3	1300.000	0	0	0	0	0	0	0	0	0	0	1
	1183.333	0	0	0	0	0	0	0	0	0	0	1
	1116.667	0	0	0	0	0	0	0	0	0	0	1
	758.181	1	0	0	0	0	0	0	0	0	0	0
	711.103	0	0	0	0	0	0	0	0	0	0	1
	230.687	0	0	0	0	0	0	0	0	0	0	1
	138.412	0	0	0	0	0	0	0	0	0	1	0

Table (6): Cont'

16	1058.300	0	0	0	0	0	1	0	0	0	0	0
	733.905	0	0	0	0	0	1	0	0	0	0	1
	676.748	0	0	0	0	0	0	0	0	0	0	1
	496.103	0	0	0	1	0	0	0	0	0	0	0
	459.572	0	0	0	0	0	0	0	0	0	0	1
	389.313	0	0	0	1	0	0	0	0	0	0	1
	259.680	0	0	0	0	0	1	0	0	0	0	0
17	383.022	0	0	0	0	1	0	0	0	0	0	1
	48.889	0	0	0	0	0	0	0	0	0	0	1
DAT	353.869	0	0	0	0	0	0	0	1	0	0	0
ISSR-34	427.393	0	0	0	0	0	0	0	0	0	0	1
ISSR-35	647.821	0	0	0	0	0	0	0	0	0	0	1
	514.017	0	0	0	0	1	0	0	0	0	0	0
	248.491	0	0	0	0	0	0	0	0	0	0	1
834	1550.000	0	0	0	0	0	0	0	0	1	0	0
	1458.000	0	0	0	0	0	0	0	0	1	0	0
	667.114	0	0	0	0	0	0	0	0	0	0	1
	532.476	0	0	0	0	0	0	0	0	0	0	1
	433.230	0	0	0	0	0	0	0	0	0	0	1
	320.370	0	0	0	0	0	0	0	0	0	0	1
841	862.300	0	0	0	0	0	0	0	0	0	0	1
	655.200	0	0	0	0	0	0	0	1	0	0	0
	443.681	0	0	0	0	0	0	0	0	0	0	1
	252.200	1	0	0	0	0	0	0	0	0	0	0
842	1230.000	0	0	0	0	0	0	0	0	1	0	0
	1200.000	0	0	0	0	0	0	0	0	0	1	0
	951.774	0	0	0	0	1	0	0	0	0	0	1
	881.974	0	0	0	0	0	0	0	0	1	0	0
	589.047	0	0	0	0	0	0	0	0	0	0	1
	385.120	0	1	0	0	0	0	0	0	0	0	0
	374.939	0	0	0	0	0	0	0	0	0	0	1
	239.141	0	0	0	0	0	1	0	0	0	0	0
	220.554	0	0	0	0	0	0	0	0	0	0	1
	208.670	0	0	0	0	0	0	0	0	1	0	0

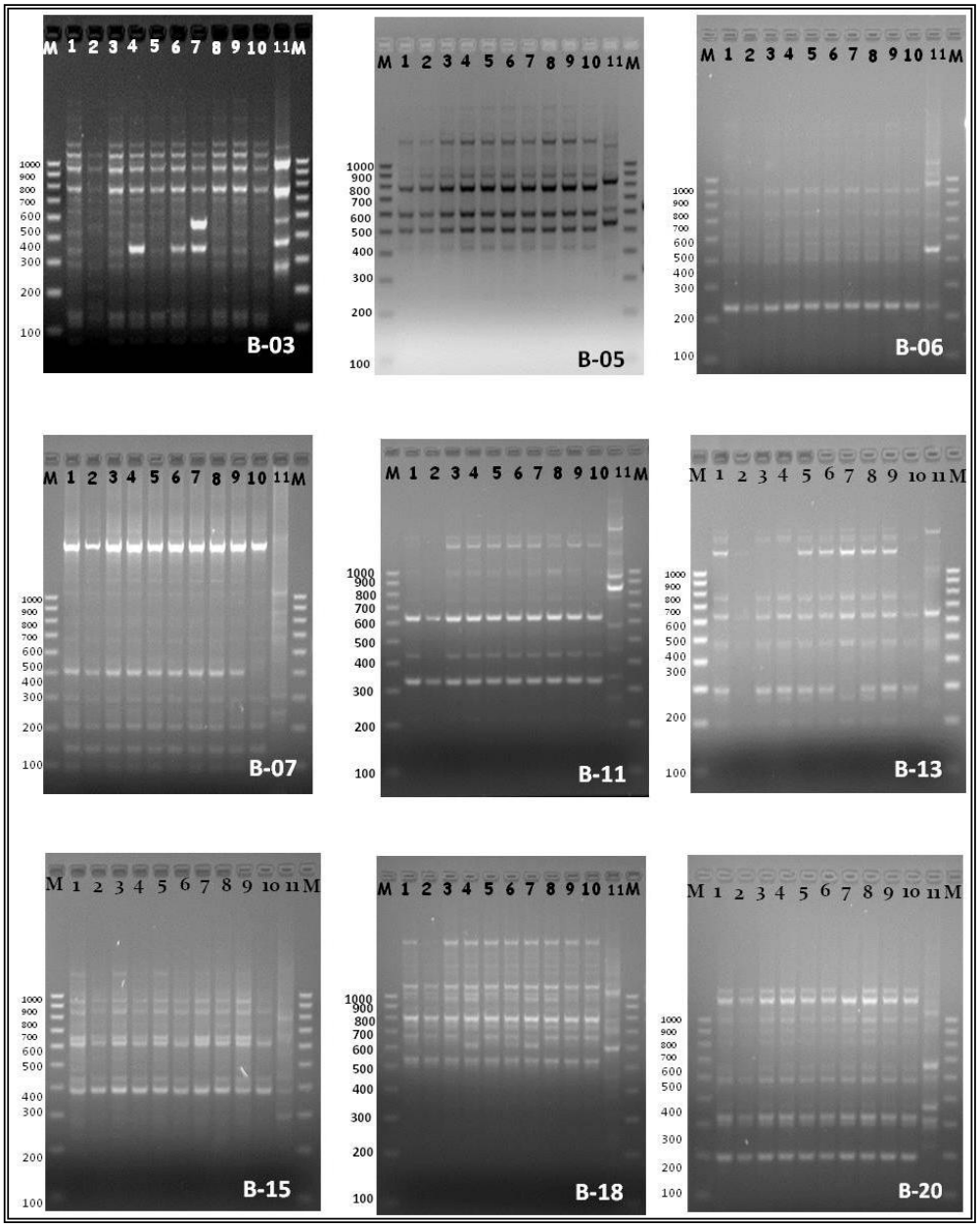


Fig. (1): RAPD profiles of the eleven tomato varieties as detected by different RAPD primers (1) Super marmand, (2) Floradade, (3) Juhilee, (4) Peto86, (5) Edkawy, (6) Strain B, (7) Pakmore, (8) Castle Rock, (9) VFNT, (10) Cherry tomato and (11) Tomatillo as detected by different RAPD primers, M = 100 bp marker.

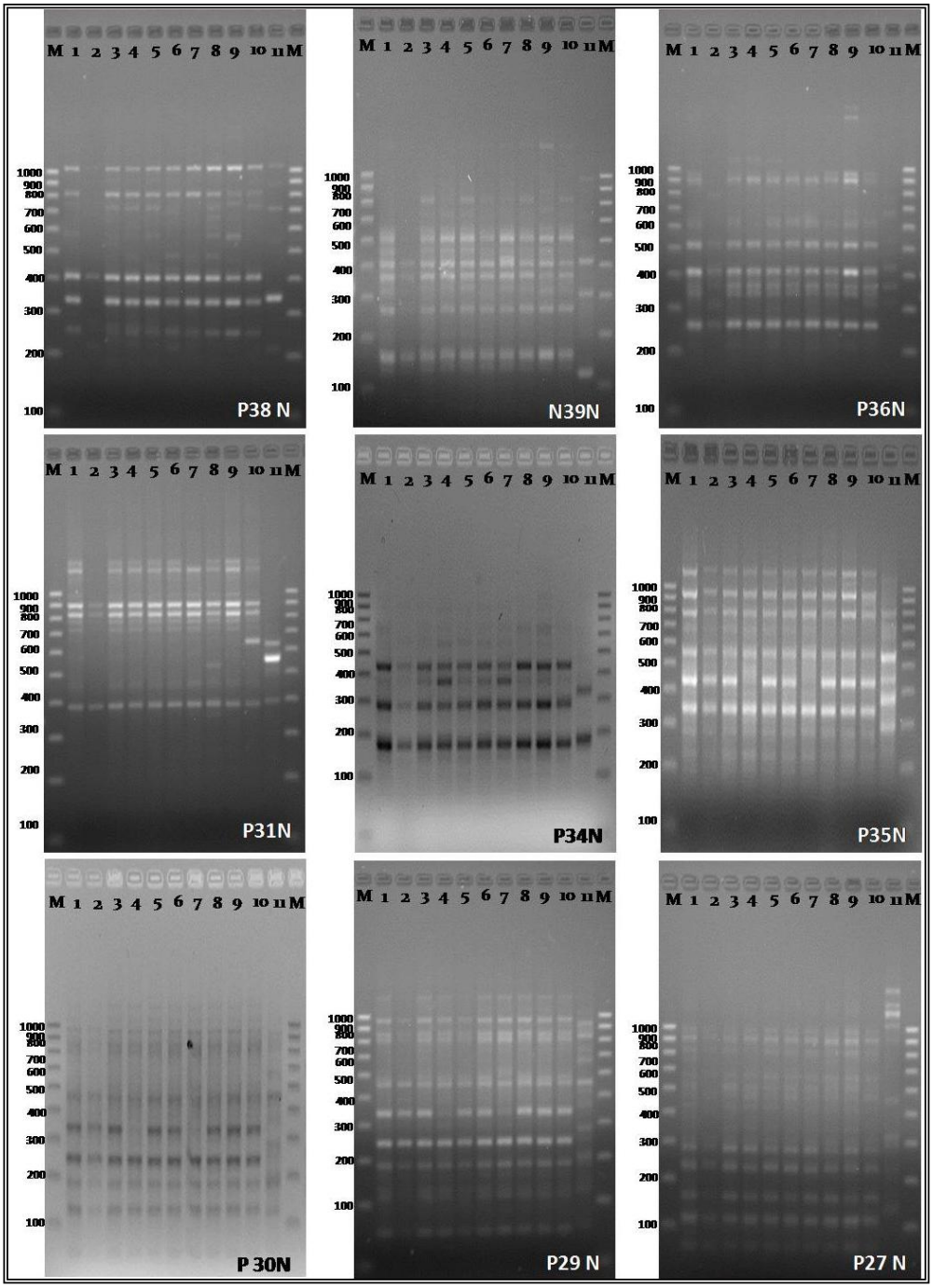
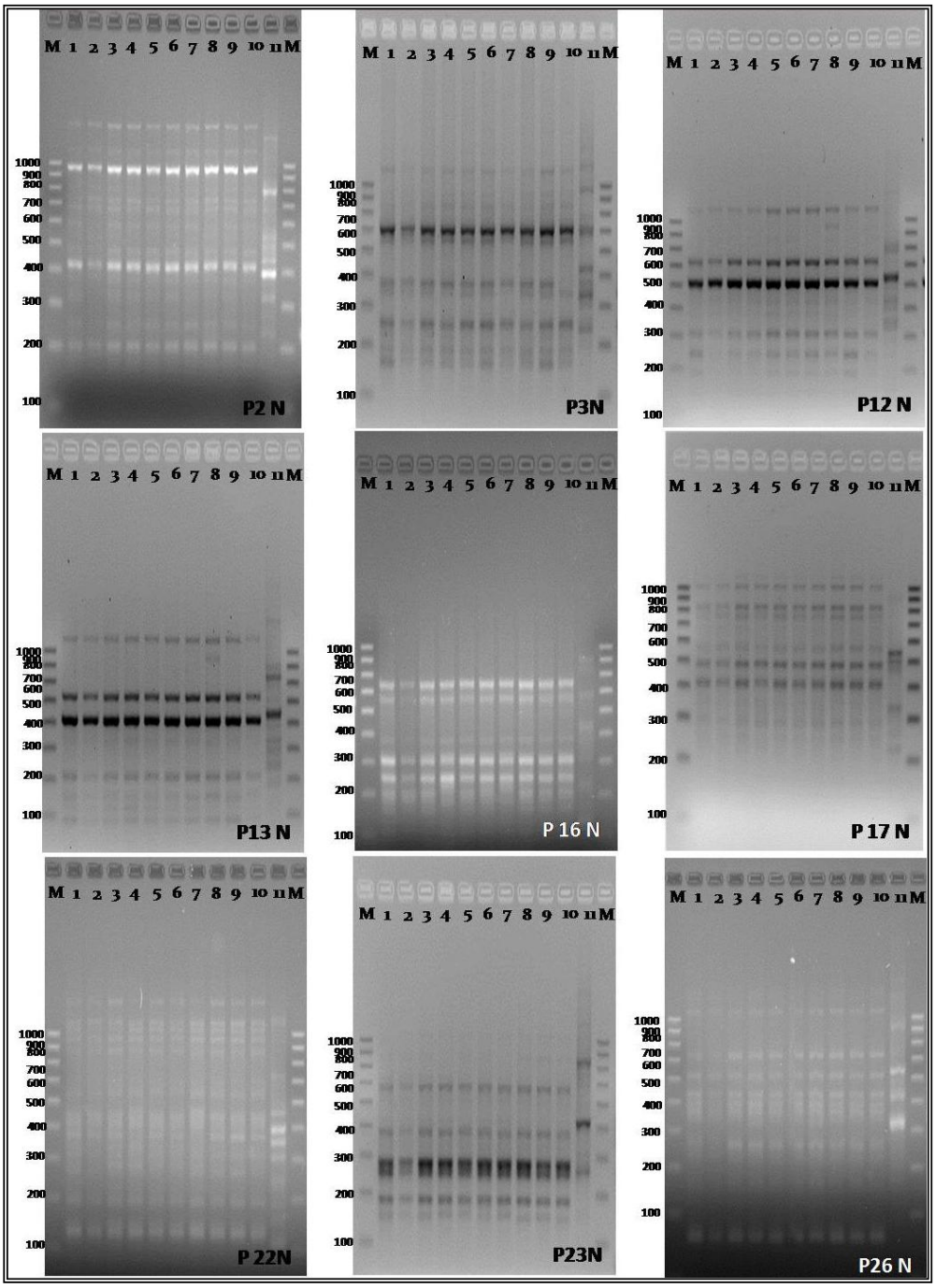


Fig. (2): ISSR profiles of the eleven tomato varieties as detected by different ISSR primers (1) Super marmand, (2) Floradade, (3) Juhilee, (4) Peto86, (5) Edkawy, (6) Strain B, (7) Pakmore, (8) Castle Rock, (9) VFNT, (10) Cherry tomato and (11) Tomatillo as detected by different ISSR primers, M = 100 bp marker.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1000
900
800
700
600
500
400
300
200
100
P2 N
P3N
P12 N
P13 N
P 16 N
P 17 N
P 22N
P23N
P26 N

Fig. (2): Cont'

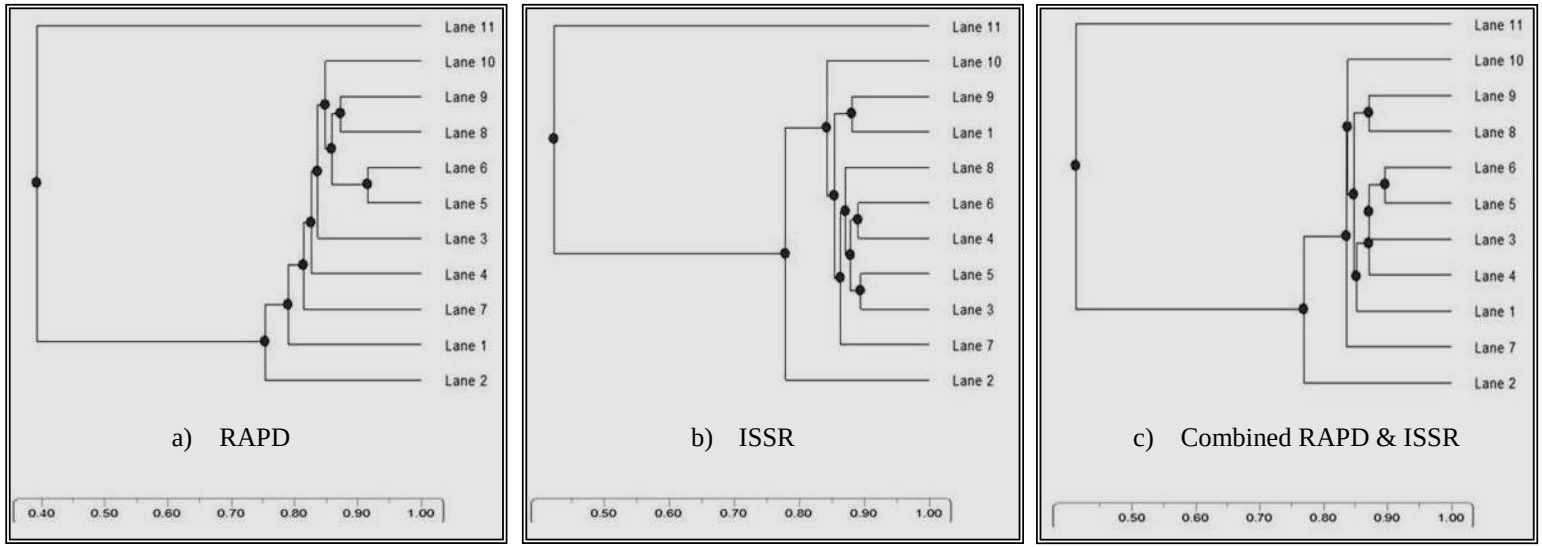

Fig. (3): Dendrogram showing the genetic relatedness of eleven tomato varieties based on RAPD (a), ISSR (b) and combined RAPD and ISSR data (c). (1) Super marmand, (2) Floradade, (3) Juhilee, (4) Peto86, (5) Edkawy, (6) Strain B, (7) Pakmore, (8) Castle Rock, (9) VFNT, (10) Cherry tomato and (11) Tomatillo.