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Full Length Research Paper

Genetic diversity and DNA fingerprint study in tomato (*Solanum lycopersicum* L) cultivars grown in Egypt using simple sequence repeats (SSR) markers

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A collection of ten cultivars of tomato grown in Egypt were screened with 20 simple sequence repeat (SSR) primers in order to determine genetic identities, genetic diversity and genetic relationships among these cultivars. On an average, 38 alleles were amplified using SSR primers with scorable fragment sizes ranging from approximately 75 to 275 bp. 23 alleles were polymorphic thus revealing 60.5% of polymorphism. The genetic similarity estimated according to SSR data was scaled between 17.6 and 93.2%, suggesting the potential of SSR markers in discriminating among plants of close or distant genetic backgrounds. Unweighted pair group method with arithmetic mean (UPGMA) clustering grouped the cultivars into two groups where the two Egyptian cultivars Edkawy and Giza 80 were clustered in different group. In addition, clustering was found consistent with the known information regarding growth habit. The genetic distance information obtained in this study might be useful to breeder for planning crosses among these cultivars.

Key words: Tomato cultivars, diversity, Simple sequence repeats (SSR), Egypt.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.*,* formerly *Lycopersicon esculentum* L.) is one of the most economically important and widely grown plants in *Solanaceae* family. In addition to its worldwide agricultural and economic importance as a crop, tomato is a preeminent model system for genetic studies in plants. Tomato is one of the most important vegetable in Egypt where it is grown all year round.

Molecular genetic diversity estimates are extremely useful for intellectual property protection, particularly in the determination of essential derivation. Measurements of genetic diversity can be used in breeding programs to increase the genetic variation in base populations by crossing cultivars with a high level of genetic distance as well as for the introgression of exotic germplasm. The genetic diversity estimates based on molecular marker data may be compared to a minimum genetic distance which indicates that two cultivars are not essentially derived (Lefebvre et al., 2001).

During recent decades, Simple sequence repeats (SSR) also known as microsatellites have become the most popular source of genetic markers owing to their high reproducibility, multi-allelic nature, co-dominant inheritance, abundance, and wide genome coverage. SSR markers have been successfully adopted to analyze genetic diversity in a variety of different plant species (McCouch et al., 1997; He et al., 2003; Frary et al., 2005; Sarıkamış et al., 2006, 2009, 2010). It was long assumed that SSRs were primarily associated with non-coding DNA, but it is now clear that they are also abundant in the single- and low- copy fraction of the genome commonly

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Table 1. Names of tomato cultivars that were used in this investigation with their source, growth habit, seed type and fruit size and color.

Cultivar	Source	Growing habit[a]	Seed type	Fruits size and color
Edkawy	Egypt	Indeterminate	Open-pollinated (not hybrid)	Medium, red
Giza-80	Egypt	Determinate	Open-pollinated (not hybrid)	Medium, red
Manapal	Gene bank of Mexico	Indeterminate	Open-pollinated (not hybrid)	Medium, red
Jefferson	Gene bank of Mexico	Indeterminate	Open-pollinated (not hybrid)	Medium, pink
Meral	USA	Determinate	Open-pollinated (not hybrid)	Unknown
Roma-VF	USA	Determinate	Open-pollinated (not hybrid)	Medium, red
Pakmor	Asgrow USA	Determinate	Open-pollinated (not hybrid)	Medium, red
Peto-86	Peto-seed (USA)	Determinate	Open-pollinated (not hybrid)	Medium, red
Flora-Dade	Peto-seed (USA)	Determinate	Open-pollinated (not hybrid)	Large, red
VG-151	Heirloom-seed project (Germany)	Indeterminate	Open-pollinated (not hybrid)	Small, red (Cherry)

[a]Indeterminate: climbing, fruit ripens over an extended period of time and determinate: bush type, fruit ripens in a concentrated time period.

referred to as genic SSRs or EST-SSRs.

In tomato, only limited genetic variation is found between varieties (Tanksley et al., 1992). Cultivated tomato (*S. lycopersicum* L.) is known to be highly monomorphic at the molecular level although it is phenotypically very diverse (Labate and Roberts, 2002). Crosses between tomato cultivars and wild species were used for identification of agriculturally important traits as well as for gene mapping (Majid, 2007). A number of SSR markers have been identified in *Solanaceae* including tomato (Broun and Tanksley, 1996; Areshchenkova and Ganal, 1999; Yi et al., 2006; Bindler et al., 2007). Some conserved regions were found between different *Solanaceae* species, such as tomato, potato and tobacco, allowing the application of the same SSRs between these species (Areshchenkova and Ganal, 1999). Analysis of genetic diversity in tomato will be useful in the selection of parental genotypes for mapping populations and breeding programmers attempting to broaden the genetic base of future tomato cultivars. In particular, this opens up significant opportunities for the development of intraspecific mapping populations that will be highly relevant to modern tomato breeding programmes.

The aim of this research was to characterize different selected origin tomato genotypes grown in Egypt and to assess the genetic diversity within this germplasm using SSR markers. Assessment of genetic diversity is important for breeding purposes, and the utilization of molecular markers helps accelerate the evaluation process.

MATERIALS AND METHODS

Plant materials

The plant material for the study consisted of ten various cultivars of tomato (*S. lycopersicon*) that have different origin and grown under Egyptian environment (Table 1). The seeds were kindly provided by Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.

SSR primers

20 microsatellite primer pairs were used for the genotyping assays (Sasaki et al., 2002). Primer names, sequences and corresponding annealing temperatures and the amplified fragments are listed in Table 2.

Genomic DNA extraction

Seeds were grown in a growth chamber at 25°C with a 12/12 h day/night photoperiod. Genomic DNA was extracted in bulk from young fresh leaves (10 plants for each cultivar) using the plant isolation kit (Jena Bioscience, Germany) and extraction procedure was based on the manufacturer's protocol.

Microsatellite genotyping

Genomic DNA samples were diluted in 0.1 mM TE buffer (10 mM Tris pH 8.0 and 0.1 mM EDTA) to 25 ng/µl before amplification by polymerase chain reaction (PCR). Amplification was carried out in 25 µl of reaction mixture, containing 7 µl distilled water, 12.5 µl of 10x assay buffer with 15 mM $MgCl_2$, 2 µl of 50 ng template DNA, 1.5 µl of each forward and reverse primer (1 µm), 1 µl (200 Mm of each dNTP) and 0.25 µl (1 U) Go Taq DNA Polymerase (Promega). Reactions were conducted in Eppendorf thermal cycler system (Germany). The PCR profile starts with initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 1 min., annealing as per the primers for 1 min., extension at 72°C for 2 min. followed by a final extension at 72°C for 5 min. The products were size-separated on 8% polyacriamid gels and stained with ethidium bromide (1 $\mu g.ml^{-1}$) and visualized on gel documentation system (Gel Doc 2000 Bio-Rad).

Band scoring and cluster analysis

The SSR gel images were scanned using the Gel Doc 2000 Bio-Rad system and all the genotypes were scored for the presence and absence of the SSR band using Quantity One Software v. 4.0.1 (Bio-Rad Laboratories, Hercules, CA USA). The bands were sized

Table 2. List of SSRs primers with their sequences, the annealing temperature used in the PCR reaction, the total (T) amplified fragments and the polymorphic (P) amplified fragments.

S/N	Primer	Sequence	Repeat	Annealing temperature (°C)	Fragment size	Fragment (T)	Fragment (P)
1	Tom 39A-40A	5'-TAACACATTCATCAAAGTACC -3' 5'-TTGCGTGATAATCCAGTAAT- 3'	AATT4	45	160-220	2	-
2	Tom 8-9	5'GCATTGATTGAACTTCATTCTCGTCC- 3' 5'-ATTTTTGTCCACCAACTAACCG -3'	ATT7	48	175-246	4	4
3	Tom 11-28	5'-ATTGTA ATGGTGATGCTCTTCC -3' 5'-CAG TTA CTACCAAAAATAGTCAAACAC -3'	CTT5/CT5	48	207-254	2	-
4	Tom 41-42	5'-GAAATCTGTTGAAGCCCTCTC -3' 5'-GAC TGT GAT AGT AAG AAT GAG -3'	TCC6	48	164-196	2	1
5	Tom 31A-32A	5'-AATGTC CTTCGTATCCTTTCGT -3' 5'-CTC GGTTTTAAT TTTTGTGTCT -3'	TA11	45	182-210	2	2
6	Tom 43-44	5'-GCAGGAGATAATAACAGAATAAT -3' 5'-GGTAGAAGCCCGAATATCATT -3'	TCC6	40	205-232	2	-
7	Tom 47-48	5'-CAAGTTGATTGCATTACCTATTG -3' 5'-TACAACAACATTTCTTCTTCCTT -3'	AT10	48	75-95	2	-
8	Tom 49-50	5'-AAGAAACTTTTTGAATGTTGC -3' 5'-ATTACAATT TAGAGGTCAAGG -3'	AT10	48	232-285	2	-
9	Tom 55-56	5'-ATTTCTGTAACTCCT TGT TTC -3' 5'-TGACTTCAACCCGACCCCTCT T -3'	ATTT5	48	160-200	2	1
10	Tom 57-58	5'-TCTAAGTGGATGACCATTAT -3' 5'-GCAGTGATAGCAAATGAAAA -3'	CT8	49	216-246	2	2
11	Tom 59-60	5'-TAACACATGAACATTAGTTTG A -3' 5'-CAC GTA AAA TAAAGA AGG AAT- 3'	C16	48	196-253	2	2
12	Tom 61-62	5'-GGCAAAGAAGGACCC AGAGC- 3' 5'-GGT GCC TAAAAAAGT TAAAT- 3'	CAC6	48	187-215	2	2

Table 2. Continued.

13	Tom 63-64	5'-TCGTAATTGTTTGTCATGTTGC- 3' 5'-TCATTGTAGTGAGGTGCTAGTG -3'	AATT2T/AATT2	48	173-224	2	2
14	Tom 65-66	5'-AGATAAAGAAACTCTTGGTTGTC -3' 5'-GATGGGATATGGAACAATTC -3'	TAA7	48	164-208	2	1
15	Tom 67-68	5'-TCCAACACCCCCTACACCAT -3' 5'-TAACACGTCCACACAAGGAC -3'	CAC6	48	125-156	2	1
16	Tom 144-145	5'-CTGTTTACTTCAAGAAGGCTG -3' 5'-ACTTTAACTTTATTATTGCGACG -3'	TAT15/TGT4	48	164-222	2	2
17	Tom 160-161	5'-TGCTGAAGAATACAATGTTACC -3' 5'-ATTGTTGGATGCTCAGTTTG -3'	AT8	48	194-229	2	2
18	Tom 146-147	5'-TTATCAATTCATCATTGTGGC- 3' 5'-ATTGTTGGATGCTCAGTTTG- 3'	CTT6	45	195-237	2	1
19	Tom 152-153	5'-ATTCAAGGAACTTTTAGCTCC -3' 5'-TGCATTAAGGTTCATAAATGA -3'	TA9	46	-	-	-
20	Tom 95-96-	5'-GTGGATGGATATGTGTGA -3' 5'-GCACGGTAGGTCGCAGGCA -3'	GT	46	-	-	-
Total						38	23

and were entered into a binary matrix as discrete variables; 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis.

The software NTSYSpc version 2.1 (Rohlf, 2000) was used to calculate the pair wise differences matrix and plot The dendrogram among tomato cultivars (Yang and Quiros, 1993). Cluster analysis was based on similarity matrix obtained with the unweighted pair-group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

RESULTS

Characteristics of SSR markers

20 microsatellite markers were used to test the genetic diversity of ten tomato cultivars. Two primers (10%) failed to amplify the expected PCR fragments and five markers (25%) amplified monomorphic banding patterns. The remaining 13 markers (65%) were generated polymorphic banding patterns (Table 2). A total of 38 alleles were detected by the SSR markers and 23 alleles were polymorphic thus revealing 60.5% of polymorphism. The majority of polymorphic SSR loci (13) generated two alleles; only one loci (Tom 8-9) generated four alleles with an average of 2.1 alleles per locus and the size of the amplified fragments ranged from 75 to 285 bp.

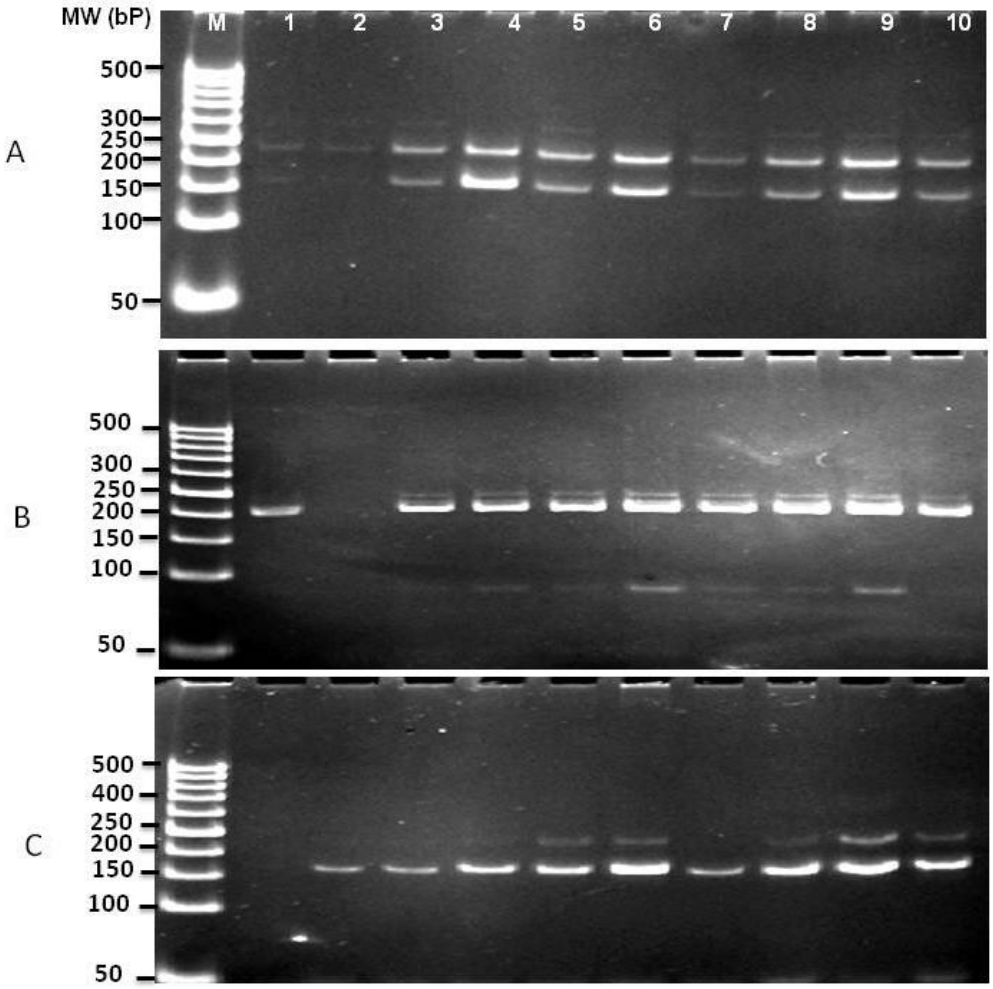

Figure 1. Profiles of the ten tomato cultivars as revealed by SSRs. A, B and C show the allelic segregation of the SSR markers Tom 39A-40A, Tom 57-58 and Tom 144-145, respectively, in the analyzed tomato cultivars. Lanes 1 to 10 represent Edkawy, Manapal, Jefferson, Meral, Giza-80, Roma-VF, Pakmor, Peto-86, Flora-dad and VG -151, respectively; M, DNA size marker (50-bp)

Some minor bands were produced by some SSR markers (Figure 1B). The existence of these minor bands may have been affected, but were not considered during, the allele scoring process.

Moreover, the SSR markers Tom 57-58 and Tom 144-145 (Figure 1B and C) were among the seven SSR markers that showed null alleles (no amplification product) for at least one of the ten fingerprinted tomato accessions. The other five SSR markers were Tom 31A-32A, Tom 49- 50, Tom 63-64, Tom 65-66 and Tom 160-161 (data not shown).

Genetic diversity and relationships of tomato cultivars

To examine the genetic relationships among the ten tomato cultivars under study based on the SSR results, the data scored from the 16 primers were compiled and analyzed using the Dice similarity coefficient. The genetic dis-similarity (GD) matrices based on the Dice coefficients are shown in Table 3. Similarities among the ten tomato cultivars ranged from 17.6 to 93.2%. The highest value of 93.2% was observed between Flora-dad and both of Roma-VF and Peto-86, while the lowest value of 17.6% was observed between Manapal with both of Pakmor and VG-151 in addition to Edkawy with Roma-VF and Peto-86. The relationships between the Egyptian tomato cultivars Giza-80 and Edkawy with other cultivars of different origins cultivated in Egypt was estimated based on the data in Table 3. The local cultivar Edkawy is relatively closely related to those of Flora-dad (78.9%) and Jefferson (75.3%), but distantly related to those of Roma-VF and Peto-86 (17.6%). Giza-80 showed highest

Table 3. Genetic dissimilarity (GS) matrices computed according to Dice coefficient from the 16 SSR markers of ten tomato cultivars.

	Edkawy	Manapal	Jefferson	Meral	Giza-80	Roma-VF	Pakmor	Peto-86	Flora-dad	VG -151
Edkawy	0.0000									
Manapal	0.3241	0.0000								
Jefferson	0.2471	0.5068	0.0000							
Meral	0.6181	0.7489	0.2471	0.0000						
Giza-80	0.5068	0.6181	0.2471	0.1773	0.0000					
Roma-VF	0.8240	0.3658	0.3658	0.1447	0.1447	0.0000				
Pakmor	0.5604	0.8240	0.3658	0.2114	0.2846	0.2471	0.0000			
Peto-86	0.8240	0.3844	0.3658	0.1447	0.1447	0.1134	0.1134	0.0000		
Flora-dad	0.2114	0.3844	0.5068	0.2471	0.2471	0.0834	0.2114	0.0834	0.0000	
VG -151	0.6807	0.8240	0.2846	0.2114	0.1447	0.1134	0.3241	0.1447	0.1447	0.0000

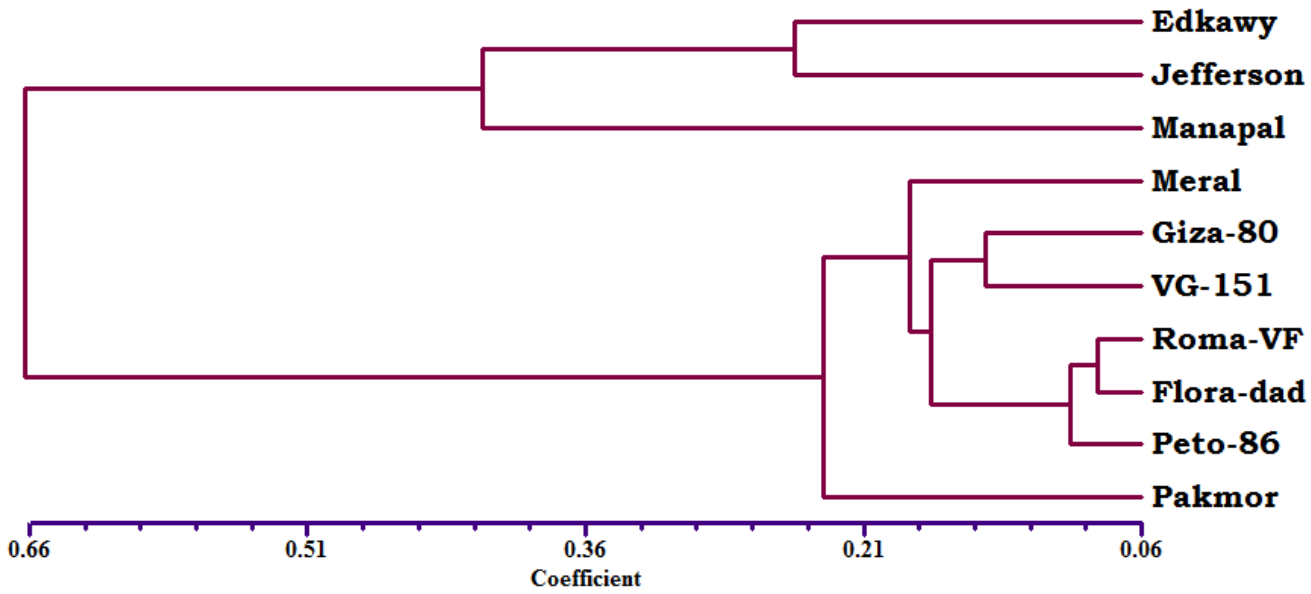

Figure 2. Dendrogram for the ten tomato cultivars constructed from SSRs data analysis using unweighed pair group with arithmetic average similarity matrices computed according to Dice coefficients.

similarity with those of VG-151, Roma-VF, Peto-86 (85.5%), followed by Flora-dad (75.3), but distantly related to those of Manapal (38.2%) and Edkawy (49.4%).

The distance matrix based on SSR data sets was used to construct a dendrogram, which is shown in Figure 2. The dendrogram is divided into two main clusters; the first main cluster is divided into two sub-clusters, one contains the American cultivar, Manapal. The second sub-cluster contains the two cultivars Edkawy and Jefferson, each in one branch. The second main cluster was divided into two sub-clusters; the first contained only Pakmor. The second subcluster was further divided into two sub-clusters. One of these sub-clusters contained only the cultivar Meral. While, the second was divided into several branches, the first contained Giza-80 and VG-151 and the second contained Roma-VF, Flora-dad and Peto-86. Noticeably, the indeterminate cultivars Edkawy, Manapal and Jefferson were clustered in the same main cluster, with the exception of the Cherry cultivar, VG-151 that branched in the other main cluster with the determinate cultivars.

Genotype identification by SSR markers as unique markers

Unique markers are defined as bands that specifically identify varieties from the others by their presence or absence. The bands that are present in one variety but

Table 4. Tomato cultivars characterized by unique positive (PUM) and/or negative SSR markers (NUM), marker size and total number of markers identifying each cultivar.

Cultivar	UNM		UPM		Total
	Primer	Size of the marker band (bp)	Primer	Size of the marker band (bp)	
Manapal	Tom 8-9; Tom 57-58	194 and 217; 216 and 246	Tom 8-9	175 and 246	6
Roma-VF	Tom 61-61.	187	-		1

not found in the others are termed positive unique markers (PUM), opposite to the negative unique markers (NUM). Unique DNA markers were obtained by SSR and were used to characterize the ten tomato cultivars of different origins. In the present study, three primers out of the 16 revealed seven unique SSR alleles (2 positive and 5 negative) as recorded in Table 4. Manapal was characterized with the highest number of unique markers (6), two positive with primer Tom 8-9 and four negative as 2 with Tom 8-9 and 2 with primer Tom 57-58. While the cultivar Roma-VF was characterized by one negative unique marker with primer Tom 61-62.

DISCUSSION

The present study revealed the genetic diversity within a group of tomato genotypes of different origins and cultivated in Egypt using molecular (SSR) approaches (Figures 1 and 2). The possibility and application of the SSR technique in varietal identification of tomato have been well explored (Hokanson et al., 1998; Smulders et al., 1997; He et al., 2003; Rajput et al., 2006; Pritesh et al., 2010)

90% of the selected primers produced amplicons, even when using modified amplification conditions. Four markers out of the 18 SSR markers that produced scorable and reproducible were monomorphic, and the majority of polymorphic SSR loci (13) generated only two alleles. It was reported that in solanaceous plants, a low frequency of polymorphism among cultivars and intraspecific (Smulders et al., 1997; Nunome et al., 2003; Stàgel et al., 2008), is probably due to its autogamous nature. In addition, cultivated tomato is known to be highly monomorphic at the molecular level although it is phenotypically very diverse (Labate and Roberts, 2002).

The relatively high polymorphism that was recorded in this study (60.5%) was due to the occurrence of the null allele's segregation (no amplification product) in seven markers for at least one of the ten fingerprinted tomato accessions (Figure 1B and C). Mutations within the SSR primer region may yield null alleles, whereas a mutation between the primer regions may result in new alleles. The natural mutation rate for genomic non-repetitive DNA is estimated to range from 10^{-8} to 10^{-6} per locus and generation (Drake et al., 1998). However, SSRs showed higher mutation rates than non-SSR regions ranging from approximately 10^{-6} per locus and generation for *Saccharomyces cerevisiae* (Sia et al., 2000) up to 10^{-3} in the pipefish *Syngnathus typhle* (Jones et al., 1999). In addition, Schloetterer (2000) reported that the mutation rate of SSRs was found to be dependent on the repeat type, the repeat number, and the sequence of the repeat motif or the flanking sequence. Mutations within SSR markers were mostly insertions and deletions of mainly complete repeats (Twerdi et al., 1999). Unequal crossover in SSR regions is another genetic reason for the unexpected variation in GD as reported in wheat (Plaschke et al., 1995). The genetic similarity estimated according to SSR data was scaled between 17.6 and 93.2%, suggesting the potential of SSR markers in discriminating among plants of close or distant genetic backgrounds.

The detection of minor and nonspecific products that could be shadow, heteroduplex or faint bands (Figure 1B) may affect the allele scoring process and increases the difficulty of legitimate allele identification. We did not considered these minor bands during allele scoring however, Wang et al. (2003) and Rodriguez et al. (2001) reported that the minor bands can be useful during gel scoring for genotype verification, because they are generally consistent.

In the present study, SSR gave definite identification of two cultivars of tomato, that is, seven unique SSR bands that characterize the two cultivars Manapal (four negative and two positive) and Roma –VF (one negative) (Table 4). These unique bands could have a number of potential applications including the determination of cultivar purity, efficient use and management of genetic resources collection and the establishment of property rights.

The obtained data confirmed the efficacy of the SSR markers as a highly variable markers that detect the codominant single locus and suitable to distinguish between the genetically related genotypes.

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