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# Comparative Assessment of Genetic Diversity in Tomato Cultivars using IRAP, ISSR and RAPD Molecular Markers

**Ahmed Mansour[1*] • Jaime A. Teixeira da Silva[2] • Sherif Edris[3] • Rania A. A. Younis[3]**

[1] Genetics Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt
[2] Faculty of Agriculture and Graduate School of Agriculture, Kagawa University, Miki-cho, Ikenobe 2393, Kagawa-ken, 761-0795, Japan
[3] Genetics Department, Faculty of Agriculture, Ain Shams University, Cairo, Egypt

*Corresponding author*: * **alzohairy@yahoo.com**

## ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important agricultural and industrial crops. In this report, we investigated three different molecular marker systems, namely RAPD (randomly amplified polymorphic DNA), ISSR (inter-simple sequence repeat) and IRAP (inter-retrotransposon amplified polymorphism) to detect genomic variation within 10 tomato cultivars, which have different genotypic and phenotypic characters and are widely used in most breeding programs, namely 'Aledo VF', 'Carmeuco 201M', 'Castlerock', 'Money Maker', 'Peto 86', 'Red Star', 'Super Marmand', 'Super Queen', 'Super Strain B' and 'UC97-3'. Comparative assessments for using these three markers to study genetic diversity and analysis of molecular variance (AMOVA) among the 10 tomato cultivars are described. Different dendrograms constructed for the RAPD, ISSR and IRAP results individually and collectively reveal that similarity and clustering are highly dependant on the marker system used. This is the first ever such study in tomato to superimpose the results of three marker systems to verify their individual results.

**Keywords:** dendrogram, molecular breeding, molecular techniques, polymorphism

## INTRODUCTION

After the completion of the whole-genome sequencing project of the International *Solanaceae* Genome Project (SOL), it is becoming feasible to study the nature of variation at the nucleotide sequence level (Mueller *et al.* 2005). As a model plant for the *Solanaceae* family, which includes many vegetable, tomato crops, tomato is particularly interesting (see Passam 2008). Tomato is also a major vegetable crop for the world's Population (AVRDC - The World Vegetable Center 2009). Molecular breeding or marker-assisted selection (MAS) in tomato with quantitative trait loci (QTLs) provides plant breeders with the potential for increasing breeding efficiency; moreover, DNA markers which are genetically linked to a trait of interest can be used for gene cloning and trait introgression in tomato breeding programs (Saavedra and Spoor 2002; Labate *et al.* 2007; Passam *et al.* 2007; reviewed extensively in Bebeli and Mazzucato 2008).

In this regard, RAPD (randomly amplified polymorphic DNA) markers have been used in tomato species and cultivars to estimate genetic diversity and polymorphism (e.g. Klein-Lankhorst *et al.* 1991; Egashira *et al.* 2000) and to produce markers for important traits such as virus resistance (Dax *et al.* 1993; Śmiech *et al.* 2000), salt tolerance (Foolad and Chen 1998), drought tolerance (El-Sayed *et al.* 2002), callus induction (Mansour *et al.* 2005) and hybrid seed purity (Singh *et al.* 2007).

On the other hand, ISSR (inter-simple sequence repeat) markers have been used successfully and extensively in the genus *Lycopersicon* (Tikunov *et al.* 2003) to study genetic diversity among related tomato species, cultivars and landraces (Smolik *et al.* 2006; Terzopoulos and Bebeli 2008), assessment of genetic purity (Liu *et al.* 2007) and to evaluate the extent of genetic recombination (Toppino *et al.* 2008).

In contrast to other methods IRAP (inter-retrotransposons amplified polymorphism) is a marker system based on retrotransposable elements which detects their distribution in the genome as an important source of plant genomic diversity (Kalendar and Schulman 2006; Mansour 2008). Their distribution is influenced by element behavior and host-driven controls (Mansour 2007; Tam *et al.* 2007). Thus, IRAP is considered a valuable retrotransposon-based marker based on the fact that retrotransposons generally tend to cluster together in 'repeat seas' surrounding 'genome islands', and may even nest within each other (Kalendar *et al.* 1999; Bousios *et al.* 2010). IRAP detects retrotransposon insertional polymorphisms by amplifying the portion of DNA between two retroelements (Kalendar and Schulman 2006). IRAP was used to differentiate tomato from potato and *Datura*, closely-related species (Lightbourn *et al.* 2007).

The distribution of four *Copia*-type retrotransposons, Tnt1 (Grandbastien *et al.* 1989), ToRTL1 (Daraselia *et al.* 1996) and two other characterized retrotransposon sequences from tomato, namely T135 and T265, as described in Pearce *et al.* (1999) and Tam *et al.* (2005), were analyzed using IRAP (Kalendar *et al.* 1999) to generate and reveal their different distribution among the 10 studied tomato cultivars. Unlike RAPD and ISSR, which are commonly used in horticultural science, the use of IRAP remains fairly limited (Agarwal *et al.* 2008), and this constitutes the first study in which it has been applied in tomato.

In this investigation, we provided comparative assessments using ISSR, RAPD and IRAP for producing molecular markers to study the genetic diversity among 10 different commercially important tomato cultivars which have different genotypic and phenotypic characters and are widely used in most breeding programs. For this purpose, we analyzed each of those techniques individually and collectively with 10 different tomato cultivars from different genetic and geographic origins to compare their strength and reliability using analysis of molecular variance (AMOVA) as a comparative tool.

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**Table 1** Ten fresh-market tomato (*Lycopersicon esculentum*) cultivars uesd for assessting genetic diversity by three different molecular markers.

| Variety | Abbreviation | Origin | Growth habit |
|---|---|---|---|
| Aledo | AVF | Clause, France | Determinate |
| Carmeuco 201M | CAR | International Agricultural Research Center, Argentina | Indeterminate |
| Money Maker | MM | Yates Ltd., New Zealand | Indeterminate |
| Super Marmand | SM | Daehnfeldt, The Netherlands | Semi-determinate |
| Castle-Rock | CR | Castle Seeds, USA | Determinate |
| Super Queen | SQ | Sun Seed, USA | Determinate |
| Red Star | RS | Sun Seed, USA | Determinate |
| Peto 86 | Peto | Peto Seed, USA | Determinate |
| $UC_{97-3}$ | UC | Peto Seed, USA | Determinate |
| Super Strain B | SSB | Sun Seed, USA | Determinate |

## MATERIALS AND METHODS

### Plant material

Ten fresh-market tomato cultivars (**Table 1**) were sown in seeding trays. Seeds were supplied by a professional supplier, Gaara seeds Co., Cairo, Egypt. The growing medium consisted of peat moss (German beat Co., Germany) and vermiculite (1: 1, v/v) Gaara seeds Co. The seedlings of each cultivar were transplanted and evaluated in the field under normal conditions in clay soil with a surface irrigation system according to Egyptian Ministry of Agricultural Guidelines (EMAG).

### DNA extraction

DNA samples were extracted from young, fresh leaves (0.1 g) by the CTAB (cethyltrimethylammonium bromide) method followed by an RNase-A treatment (Sigma, St. Louis, MO; R-4875) for 30 min at 37°C in each case according to Mansour *et al.* (2005). The quality and quantity of extracted DNA was measured (2 µl) by a NanoDrop ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Delaware, USA). DNA samples were adjusted to a concentration of 50 ng/µl with $ddH_2O$ and subjected to PCR amplification.

### Inter-retrotransposons amplified polymorphism (IRAP)

The amplification reaction was performed according to the Kalendar *et al.* (1999) protocol. Four primers were designed from the long terminal repeats (LTR) of ToRTL1, Tnt1, T135 and T265P *copia*-like endogenous retrotransposons of tomato (**Table 2**).

IRAP amplifications were performed in a final volume of 20 µl containing 50 ng DNA, 1 × PCR buffer (20 mM Tris-HCl, pH 8.4, 50 mM KCl, 4 mM $MgCl_2$), 0.01% gelatin (w/v), 0.01% Triton X-100 (v/v), 300 nM dNTPs, 1U *Taq* DNA polymerase (Invitrogen), and 25 pmol of each LTR primer. Amplification was performed in a PTC-100™ thermocycler (MJ Research) in 0.2-ml microtubes. The amplification program consisted of initial denaturation at 94°C for 5 min, followed by 30 cycles composed of 94°C for 60 s, 50°C for 90 s, and 72°C for 120 s for denaturation, annealing, and extension, respectively. The annealing temperature varied according the melting temperature of each primer. After amplification, a final extension step was performed at 72°C for 10 min.

### Randomly amplified polymorphic DNA (RAPD)

Amplification reactions were performed according to (Williams *et al.* 1990) in volumes of 25 µl. Briefly, the reaction mixture containing 10 mM Tris-Cl, pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 0.001% gelatin, 50 µM of each dATP, dCTP, dGTP and TTP (Pharmacia), 25 pmol primer, 25 ng of genomic DNA, and 0.5 U of *Taq* DNA polymerase (Promega). The amplification was performed in a Perkin Elmer 2400 Thermal Cycler programmed for 5 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 34°C, 2 min at 72°C, using the fastest available transitions between each temperature (ramp time), followed by one cycle of 72°C for 20 min; and finally a 4°C soak indefinitely. The primer sequences are shown in **Table 3**. The core program increased from 40 to 45 cycles, if amplification was weak, to get a slight increase in the amount of PCR products.

**Table 2** Primers used for IRAP molecular markers analysis.

| IRAP primer | References |
|---|---|
| ToRL1 | Daraselia *et al*. 1996 |
| Tnt1 | Grandbastien *et al*. 1989 |
| T265P | Pearce *et al*. 1999 |
| T135 | Pearce *et al*. 1999 |

**Table 3** Sequence data of the RAPD and ISSR primers applied.

| Sequences (5′-3′) | ISSR |
|---|---|
| (CT)8TG (#814) | 814 |
| (CT)8AC (#844A) | 844A |
| (CT)8G (#844B) | 844B |
| (CA)6AC(#17898A) | 17898A |
| (CA)6GT (#17898B) | 17898B |
| (CA)6AG (#17899A) | 17899A |
| (CA)6GG (#17899B) | 17899B |
| (GA)6GG (#HB8) | HB8 |
| (GT)6GG (#HB9) | HB9 |
| (GA)6CC (#HB8) | HB10 |
| (GT)6CC (#HB11) | HB11 |
| (CAC)3GC(#HB12) | HB12 |
| (GAG)3GC (#HB13) | HB13 |
| (CTC)3GC (#HB14) | HB14 |
| (GTG)3GC (#HB15) | HB15 |
| **Sequences (5′-3′)** | **RAPD** |
| GTA GAC CCG | P1 |
| GGA CCC TTAC | P2 |
| GTC GCC GTC A | P3 |
| GGT CCC TGA C | P4 |
| TGG ACC GGT G | P5 |
| AGG GGT CTT G | P6 |
| TTC CCC CGC T | P7 |
| TTC CCC CCA G | P8 |
| ACT TCG CCA C | P9 |
| CAA TCG CCG T | P10 |
| AGG GAA CGA G | P11 |
| TGC GCC CTT C | P12 |
| TTC GCA CGG G | P13 |
| GTG AGG CGT C | P14 |
| CAA ACG TCG G | P15 |
| CTG CTG GGA C | P16 |
| GTG ACG TAG G | P17 |
| CCA CAG CAG T | P18 |
| TGA GCG GAC A | P19 |
| GTG AGG CGT C | P20 |

### Inter-simple sequence repeat (ISSR)

A set of 15 anchored microsatellite primers was procured from Metabion, Germany (**Table 3**). PCR amplification was performed according to Dangi *et al.* (2004). Briefly, 20 ng of DNA was added with 10 mM Tris-HCI pH 7.5, 50 mM KCI, 1.5 mM $MgCl_2$, 0.5 mM spermidine, 2% formamide, 0.1 mM dNTPs, 0.3 uM primer and 1 U of *Taq* DNA polymerase (Promega, USA) in a 25 µl reaction using a Perkin Elmer 2400 thermocycler. After initial dena-

turation at 94°C for 5 min, each cycle consisted of 30 sec denaturation at 94°C, 45 sec of annealing at 50°C, 2 min extension at 72°C along with 5 min extension at 72°C at the end of 40 cycles.

### Gel electrophoresis

Amplification products (15 µl) were separated by agarose (1.5%, SIGMA-Aldrich) gel electrophoresis stained with ethidium bromide (0.5 ng/µl) at 80 V in 1X TBE buffer and photographed on a UV transilluminator (Pharmacia) with a Canon S5 digital camera with a UV filter adaptor. A negative control which contained all the necessary PCR components except a template DNA was included in the PCR runs.

### Data analysis

Amplification products were scored independently as 1 and 0 for the presence or absence of bands, respectively, and the obtained binary data were used for the analyses. Only sharp PCR fragments were scored (not "ghost bands"). Fragments at low intensities were only scored as present when they were reproducible in repeated experiments using GelAnalyzer 3 (Egygene Co., Egypt) software. Unequivocally reproducible bands were scored and entered into a binary character matrix (1 for presence and 0 for absence). The genetic similarity among tomato cultivars was determined by Nei's genetic distance (Nei 1987) modified to accommodate dominant markers (Labate 2000) (e.g., RAPD, ISSR and IRAP). A dendrogram was constructed based on a distance matrix using Unweighted Pair Group Method with Arithmetic (UPGMA) averages. All calculations were performed with the NTSYS-pc version 2.02 software package (Numerical Taxonomy System, Exeter Software) (Rohlf 2000). In addition, correspondence of the RAPD, ISSR and IRAP similarity matrices was performed by means of MXCOMP procedure of NTSYS-pc with the null hypothesis that there is no association between these three similarity matrices. The statistical stability of the clusters was estimated by a bootstrap analysis with 1000 replications using Winboot software (Yap and Nelson 1996).

### AMOVA

An analysis of molecular variance (AMOVA) was performed using GENALEX 6 ('Genetic Analysis in Excel' (Peakall and Smouse 2006) in RAPD, ISSR and IRAP to partition the total molecular variance between and within populations (clusters).

## RESULTS AND DISCUSSION

### Diversity in tomato cultivar genomes revealed by IRAP

Polymorphisms in tomato cultivars were studied based on retrotransposons insertional diversity using four primers using tomato retrotransposable elements facing outwards from the LTRs. Namely, primers for ToRTL1, Tnt1, T135 and T265P retrotransposon were analyzed using IRAP to reveal their different distribution in the studied tomato cultivars (Tam *et al.* 2007). As different retrotransposons can produce variations in the quality of IRAP patterns (i.e. band intensity versus background intensity, scorability and abundance of bands; Leigh *et al.* 2003), we selected for retrotransposon primers that provided clear IRAP profiles in *Solanaceae* (Tam *et al.* 2009). Our results show that IRAP profiles obtained with the four different LTR primers yielded, on average, 36.3 bands per gel (**Fig. 1A**). There were 25 polymorphic (with unique bands), 17.3 polymorphic (without unique bands) and 17.3 unique bands among all the cultivars (**Table 4**). Shorter LTR fragments amplified from most or all cultivars, suggest conservation of the inter-

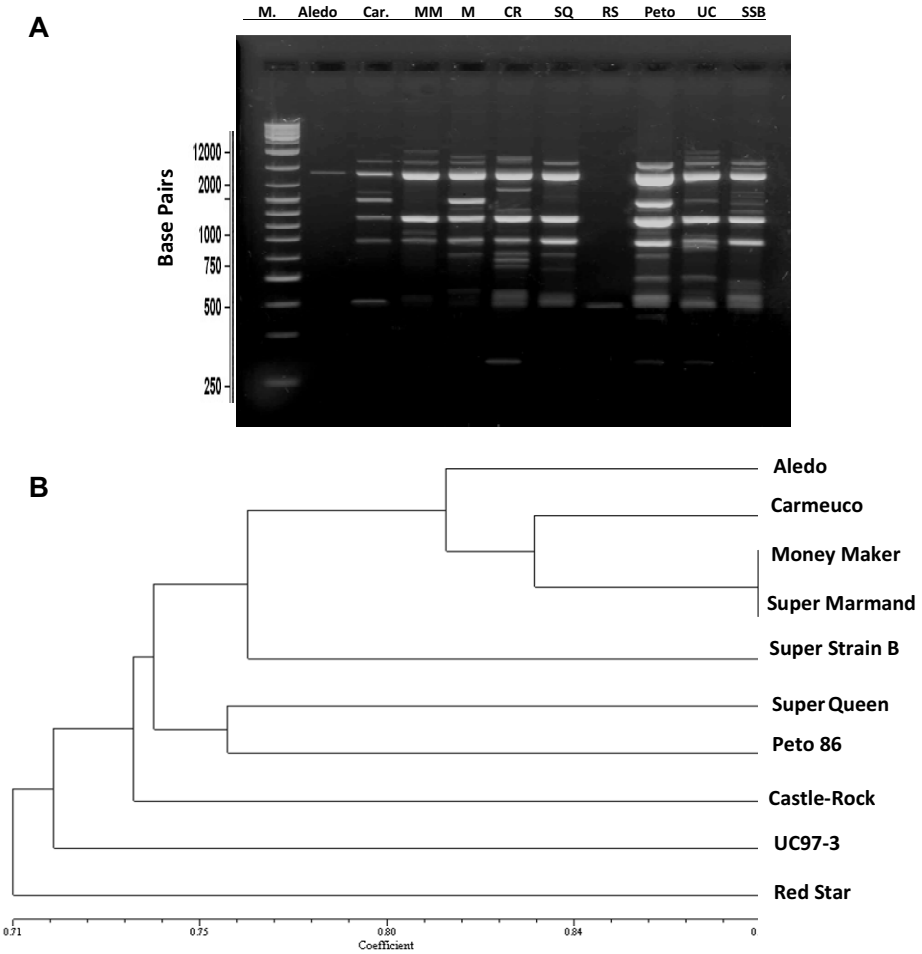


**Fig. 1 Example of IRAP gel profiles displaying genomic variations in the amplification pattern of specific retrotransposon in 10 tomato cultivars.** (**A**) IRAP gel profiles generated by using primer for Tnt1. (**B**) Dendrogram based on algorithm of unweighted pair group method with arithmetic averages using combined IRAP results.

**Table 4** Comparison of DNA marker systems in tomato (*Lycopersicon esculentum*) cultivars.

| Marker system | No. of primers | Gel polymorphism | | | Average No. of bands/primer |
|---|---|---|---|---|---|
| | | Polymorphic (without unique) | Unique bands | Polymorphic (with unique) | |
| RAPD | 20 | 8 ± 1 | 17 ± 1 | 23 ± 1 | 24 ± 1 |
| ISSR | 15 | 7 ± 1 | 30 ± 1 | 36 ± 1 | 37 ± 1 |
| IRAP | 4 | 17 ± 1 | 17 ± 1 | 25 ± 1 | 36 ± 1 |
| RAPD + ISSR + IRAP | 39 | 32.3 | 64.4 | 84 | 97.3 |

**Table 5** Analysis of molecular variance (AMOVA) of the three different techniques for producing molecular markers.

| PCR technique | Source | Df* | SS** | MS*** | Est. Var.**** | % |
|---|---|---|---|---|---|---|
| RAPD | Among samples | 9 | 2.362 | 0.262 | 0.001 | 1% |
| | Within samples | 2060 | 247.729 | 0.120 | 0.120 | 99% |
| | Total | 2069 | 250.091 | 0.383 | 0.121 | |
| ISSR | Among samples | 9 | 1.262 | 0.140 | 0.000 | 0% |
| | Within samples | 980 | 97.293 | 0.099 | 0.099 | 100% |
| | Total | 989 | 98.555 | 0.239 | 0.100 | |
| IRAP | Among samples | 9 | 3.425 | 0.381 | 0.002 | 2% |
| | Within samples | 990 | 147.350 | 0.149 | 0.149 | 98% |
| | Total | 999 | 150.775 | 0.529 | 0.151 | |

Df* Degrees of freedom
SS** Sum of squares
MS*** Mean square
Est. Var.**** Estimated variation

nal organization of some parts of retroelements. This also shows the transferable nature of the retrotransposon-based marker system (Bousios *et al*. 2010). Variation in retrotransposon insertions into the genome leads to a different number of amplified sites and fragments sizes of inter-retroelements that can be used as markers to detect genotype polymorphisms and to measure diversity or reconstruct phylogeny (Kumar and Hirochika 2001; Mansour 2008; Boronnikova and Kalendar 2010). Based on the combined IRAP gel results of each retrotransposon marker, a dendrogram was drawn using an algorithm of the unweighted pair group method with arithmetic averages in the 10 tomato cultivars (**Fig. 1B**). AMOVA analysis indicated that 2% of the genetic variation is attributable to differences among cultivars while 98% of the genetic variation is attributable to within-cultivar groups in IRAP data. The sum of squares was 3.425 and 147.350, respectively. Detailed results from AMOVA analyses are provided in **Table 5**.

Genetic analyses of IRAP polymorphisms separated 'Aledo' from 'Red Star' and provided a fairly good discrimination between other cultivars. The resulting dendrogram also shows that 'Money Maker' and 'Super Marmand' are clustered together, indicating that they are genetically closely related and may have a common ancestral origin. Moreover, both 'Aledo' and 'Carmeco' are close to the previous cluster. In addition, 'Super Queen' and 'Peto' clustered together.

## ISSR markers to assess diversity in tomato cultivars

ISSR uses microsatellites, usually 16–25 bp long, as primers in a single primer PCR reaction targeting multiple genomic loci to amplify mainly the ISSR sequences of different sizes (Ziêtkiewicz *et al.* 1994; Reddy *et al.* 2002). In this investigation, a set of 20 ISSR primers was used for initial screening of 10 tomato cultivars of which 25 could be amplified (**Fig. 2A**). However, only 15 ISSR primers detected intraspecific variation in these tomato cultivars generating clear and reproducible patterns and revealing on average 37 bands/gel/primer in the range of 300 bp to 2 kb (**Tables 3, 4**). Among these bands 7 were polymorphic and 30 were unique (**Table 4**). Based on ISSR gels patterns, similarity index values were used to construct a dendrogram using UPGMA (**Fig. 2B**). AMOVA of ISSR data indicated that 0% of the genetic variation was attributable to differences among accessions groups while 100% of the genetic variation was attributable to within-accessions groups. Sum of squares was 1.262 and 97.293, respectively. Detailed results from AMOVA are given in **Table 5**.

The resulting dendrogram shows three closely related clusters. The first cluster includes 'Super Strain B' and 'Carmeco'. The second cluster combines 'UC97-3' and 'Super Marmand'. Both the first and second cluster combined together in a distinctive cluster with 'Red Star' indicating similarity between 'Carmeco' and 'Super Marmand'. The third distinct cluster grouped 'Super Queen' and 'Peto'. The remaining cultivars were separated individually.

## Assessment of genetic diversity in tomato cultivars by RAPD

In RAPD analysis, 10-mer oligonucleotide primers of arbitrary sequence, but with a GC content of 50% or higher, were used to amplify segments of genomic DNA, as originally described by Williams *et al.* (1990). One year after RAPD assay was developed in 1990 it was used for producing molecular markers for tomato (Klein-Lankhorst *et al.* 1991). In this investigation, a set of 20 random primers were synthesized and used to amplify DNA from 10 different tomato cultivars (**Table 3**). Different primers generated different "fingerprints" of amplified DNA fragments (**Fig. 3A**) using amplification conditions as developed by Williams *et al.* (1990), and adjusting conditions as recommended by Klein-Lankhorst *et al.* (1991). A considerable amount of polymorphism was detected for all the primers used. The sizes of the amplified fragments ranged from 200 bp to 2 Kb. A total of 240 bands were scored, almost 24 bands/primer/gel, 8 polymorphic, 17 unique and 25 polymorphic bands (including unique). Some bands were monomorphic and were shared by all cultivars, and there were bands specific for each gel. Genetic similarity was calculated from Nei's similarity index value for all 10 tomato cultivars considering RAPD scoring results. Results from AMOVA analysis indicated that 1% of the genetic variation was attributable to differences among accessions while 99% of the genetic variation was attributable to within-accession groups in RAPD data. Sum of squares were 2.362 and 247.729, respectively. Detailed results from AMOVA are given in **Table 5**.

Based on RAPD marker results, the similarity index values were used to construct a dendrogram using UPGMA (**Fig. 3B**). The resulting dendrogram shows that 'Castle Rock' and 'Super Queen' were clustered together, while 'Aledo' and 'Red Star' were in another cluster, indicating their relatedness. The remaining 6 cultivars formed 6 individual clusters.

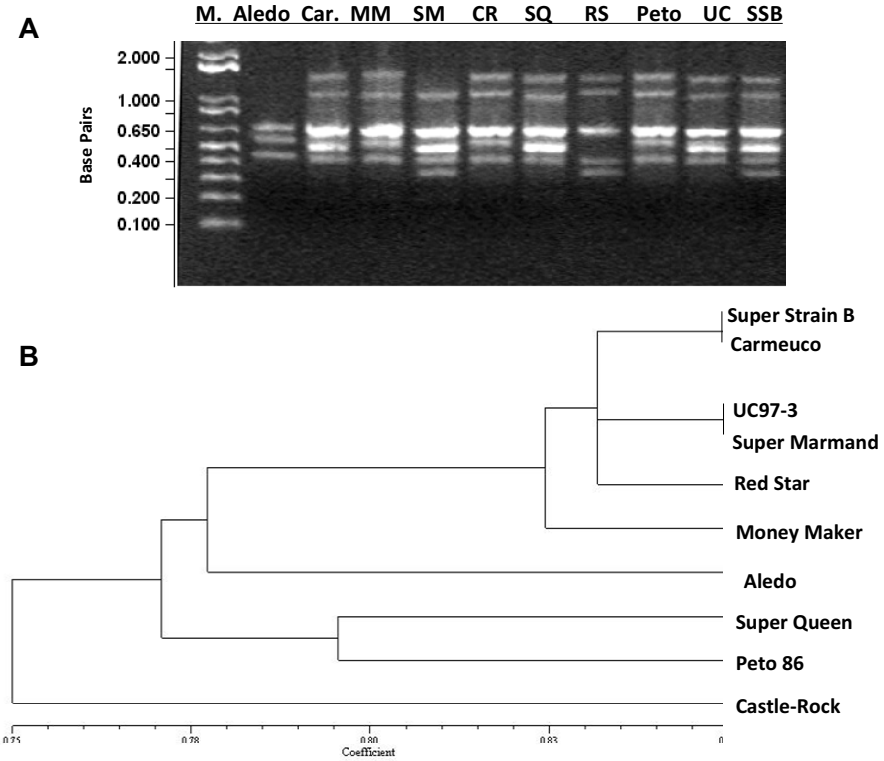


**Fig. 2 Example of ISSR markers detecting polymorphisms between 10 tomato cultivars.** (**A**) PCR amplification with 844A Primer. (**B**) Dendrogram based on algorithm of unweighted pair group method with arithmetic averages using ISSR results.

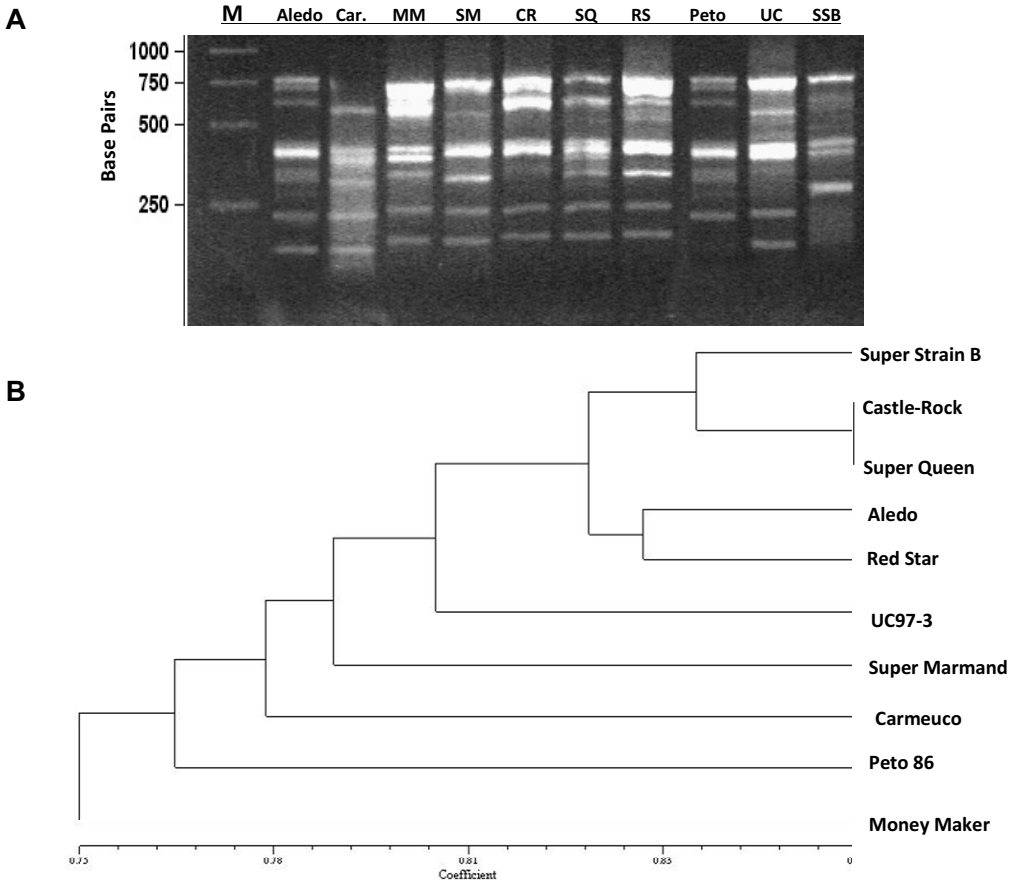


**Fig. 3 Example of 3 RAPD markers detecting polymorphisms between 10 tomato cultivars.** (**A**) PCR amplification with l0-mer oligonucleotide Primer P3. (**B**) Dendrogram based on algorithm of unweighted pair group method with arithmetic averages using RAPD results.

## Combined (IRAP, ISSR and RAPD) dendrogram analysis

Based on all the similarity indices produced by IRAP, ISSR and RAPD, genetic similarity was calculated from Nei's similarity index value considering three different marker systems individually as well as collectively. A dendrogram was constructed according to Jaccard's coefficient calculated by UPGMA based on the IRAP, ISSR and RAPD banding (**Fig. 4**). All calculations were performed by using the

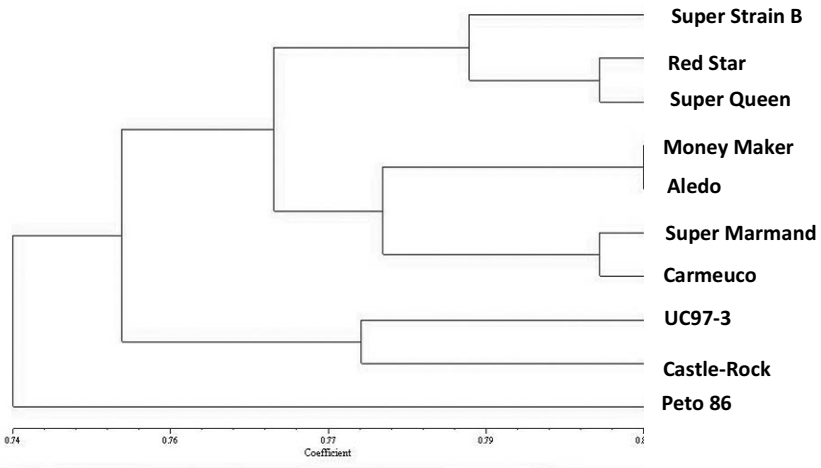


**Fig. 4 Dendrogram based on algorithm of unweighted pair group method with arithmetic averages using IRAP, ISSR and RAPD results between 10 tomato cultivars.**

NTSYS-pc 2.02 software package (Numerical Taxonomy System, Exeter Software) (Rohlf 2000). The resulting dendrogram clustered 'Money Maker' with 'Aledo' and 'Super Marmand' with 'Carmeco' in one cluster. Moreover, 'Red Star' with 'Super Queen' and 'UC97-3' with 'Carmeco' were each clustered in two distinct clusters (**Fig. 4**).

Amazingly, the collective dendrogram, even though apparently different, is in agreement with the other individual marker system dendrograms and confirms their individual results. For instance, it shows a close relation between 'Carmeco', 'Money Maker', and 'Super Marmand', as shown by IRAP (**Fig. 1B**) and ISSR (**Fig. 2B**). In addition, it shows a close relation between 'Super Strain B' and 'Super Queen', as shown by RAPD (**Fig. 3B**).

Findings from AMOVA supported these results by having estimated variances as 0.001 (with 9 df) and 0.120 (with 2060 df) for RAPD (**Table 5**), respectively. Results from AMOVA indicated that 0% of the genetic variation was attributable to differences among tomato cultivars in ISSR groups while it was 1 and 2% in RADP and IRAP, respectively which mean that the ISSR technique gives more resolution than RAPD and IRAP.

## CONCLUSION

Depending on the marker used, the genetic similarity analyses varied dramatically. ISSR and RAPD markers, in particular, have been used extensively as marker systems for evaluation of genetic variability in cashew (Archak *et al.* 2003), chickpea (Rao *et al.* 2006), strawberry (Kuras *et al.* 2004) and sorghum (Medraoui *et al.* 2007). However, each of them targets the genome differently and thus results in a different similarity matrix. For instance, RAPD primers target random homologous genomic regions (Williams *et al.* 1990) while ISSR primers amplify the highly repetitive inter-simple sequence repeats of the microsatellite region in the genome (Ziêtkiewicz *et al.* 1994). In contrast, IRAP primers amplify specific genomic retrotransposons inside the middle repetitive region (Kalendar *et al.* 1999). Each technique has been used individually to assess genetic variation in tomato cultivars (e.g. Klein-Lankhorst *et al.* 1991; Tikunov *et al.* 2003; Tam *et al.* 2005). However, each technique produces a different banding pattern and similarity dendrogram. In this regard, selecting only one technique is fairly inadequate for phylogenetic analysis. Assuming divergence from a common ancestor, it cannot correctly identify the relationships between individual genomes. The collective and close analysis of larger numbers of genome-specific regions may enable the relationships between individual genomes to be ascertained. This will help breeders to clearly identify and screen genotypes with better production value and manage the genotype resources of tomato, sometimes made difficult by synonymous names.

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