The African Journal of Plant Science and Biotechnology ©2009 Global Science Books

Comparative Genotypic and Phenotypic Analysis of Tomato (*Lycopersicon esculentum*) Cultivars Grown under Two Different Seasons in Egypt

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ABSTRACT

Tomato (*Lycopersicon esculentum* Mill.) is considered as important and economic agricultural crop all over the world. For improving yield and yield components, varieties are often evaluated under different conditions. Morphological (growth and yield), biochemical (oil, moisture content, and radical scavenging activity) and molecular diversity (RAPD and ISSR) of 11 tomato (*L. esculentum*) cultivars ('Aledo VF', 'Carmeuco 201M', 'Castle-rock', 'Falkon', 'Money Maker', 'Peto 86', 'Red Star', 'Super Marmande', 'Super Queen', 'Super Strain -B' and 'UC97-3') were analyzed under heat stress in Egypt to assist breeders in selecting heat tolerant cultivars and nutritional quality. Cultivars 'Aledo VF', 'Peto 86' and 'Red Star' were found to have the most vigorous growth habit, while 'Super Queen' had the most significant average fruit weight, yield/plant and total yield/m^2 under heat stress. For nutritional quality, cultivars 'Super Marmande' and 'Aledo VF' showed the highest oil content, while 'Aledo VF' and 'Money Maker' showed the highest radical scavenging activity (RSA). Molecular diversity of cultivars was detected using two molecular markers systems of RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat) providing further facilities for molecular comparison.

Keywords: fresh-market tomato, molecular markers, molecular breeding

INTRODUCTION

Tomato (*Lycopersicon esculentum*, 2n = 2x = 24; 0.95×10^9 nt) is one of the most popular vegetable crops worldwide. Its origin and domestication started in the Andean region of South America and in Mexico from the wild ancestor of *L. e. cerasiforme*, (syn.: *Solanum lycopersicum cerasiforme*) (Bai and Lindhout 2007). Tomato entered Europe in the 16th century and spread first in the Mediterranean resulting in thousands of cultivars available today (Esquinas-Alcazar 1981; Pék and Helyes 2004). Breeding goals for tomato have gone through four phases starting with breeding for yield in the 1970s followed by breeding for resistance and long shelf-life in the 1980s, and for improved nutritional quality and taste from the 1990s. Breeding programs have also produced some unique varieties such as the dwarf 'Micro-Tom' variety (released in 1989) and the first transgenic tomato 'FlavrSavr' (1994-1997, Calgene Inc.) (Bai and Lindhout 2007). Nowadays, however, evaluating the chemical and nutritional quality of fresh-market tomatoes is an essential breeding goal for satisfying the market need. Because of the phenomena of global warming and rising temperature, developing crops that can tolerate high temperature and withstand climate changes is an international priority in the world. Unlike *L. chilense*, heat tolerance and adaptation of commercial tomatoes is limited. Heat stress is the rate limiting abiotic factor responsible for reducing tomato yield in Mediterranean and tropical countries. Tomato production under high temperature conditions, such as the summer in Egypt, sharply reduces quality and yield. For instance, low fruit setting, reduction in the flower fertilization rate, decrease in the lycopene content and high level of evaporation are all affiliated with high temperature stress (Al-Khatib and Paulsen 1999; Hall and Ziska 2000; Hall 2001). The structure of genetic variability among inbred tomato families at different generations of selfing depends on the way that genes act and varies according to the trait selected (Ismail 2003). Selection for heat tolerance under field conditions provides general data to identify potential tolerant germplasm (Blum 1988; Hall 2001).

Therefore, exploring the range of genetic diversity for heat tolerance in different fresh-market tomatoes is a very important strategy. The purpose of this study is to evaluate and rank 11 tomato cultivars for heat tolerance and nutritional quality by morphological, chemical and molecular markers characterization to be grown in tropical and subtropical region (i.e. Egypt).

MATERIALS AND METHODS

Plant material and cultivation

For evaluation trials, seeds of 11 fresh-market tomato cultivars, commercially provided by Korma seed Co., Cairo, Egypt, were sown on January 17th and March 29th (2007) in seeding trays under greenhouse conditions (**Table 1**). The growing medium consisted of peat moss (German beat) and vermiculite (1:1, v/v), Gaara seeds Co., Cairo, Egypt. The seedlings of each cultivar were grown and evaluated in the field under heat stress (**Table 2**) during two transplanting dates from March 1st (normal date) and May 5th (heat stress) date during the 2007 season. Plants were grown in clay soil conditions with a surface irrigation system. The 11 cultivars were arranged as a split plot in a randomized complete block design with three replicates. The field plots area was (22.5 m^2) in the Abu-Kabeer district, Sharkia, Egypt. Each plot consisted of 4 rows 6 m long and 0.9 m wide with plants transplanted 50 cm

Received: 25 November, 2008. Accepted: 26 March, 2009.

Original Research Paper

Table 1 Eleven fresh-market tomato (*L. esculentum*) cultivars studied for tolerance to heat stress.

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

Table 2 The mean and maximum temperature (T.) and relative humidity (RH) during tomato cultivation at ABU KABEER district from February to October.

RH. MIN	RH. MEAN	RH. MAX	T. MAX	T. MEAN	T. MIN		
42.36	62.20	82.04	21.62	16.61	11.60	Mean	February
6.13	5.04	6.27	3.42	2.26	1.88	STDEV	
35.61	59.41	83.21	25.52	19.40	13.27	Mean	March
5.79	3.65	3.99	2.43	1.46	1.37	STDEV	
31.20	57.26	83.32	32.65	21.96	15.26	Mean	April
5.28	2.54	1.79	2.89	2.16	2.25	STDEV	
24.61	54.35	84.10	34.20	25.68	19.15	Mean	May
4.50	2.43	1.19	3.05	2.45	2.47	STDEV	
35.33	59.81	84.28	36.89	29.23	23.58	Mean	June
5.09	2.64	1.32	1.68	1.26	1.40	STDEV	
46.48	65.63	84.77	36.71	30.18	25.65	Mean	July
4.03	1.99	0.60	1.11	0.55	0.52	STDEV	
48.98	66.98	84.98	37.73	30.18	25.63	Mean	August
2.40	1.26	0.64	0.73	0.51	0.58	STDEV	
40.88	62.66	84.45	35.34	29.03	23.72	Mean	September
3.62	2.00	1.08	1.16	0.84	1.16	STDEV	

*According to ABU KABEER climate station, Central Laboratory for Agricultural Climate, Agricultural Research Center, Ministry of Agriculture, Egypt.

apart within rows. Each plot contained almost 50 plants and the outer two rows in each plot were used for plant sampling for morphological studies. The inner two rows were used for yield estimations. The total amounts of mineral fertilizers were 240, 144 and 240 kg/ha of N, P and K, respectively. Agricultural sulphur at 240 kg/ha and one third of N, P and K fertilizers were added during soil preparation with Farm Yard Manure (FYM) (47.6 m^3/ha). The remaining amount of N, P and K fertilizers were divided into 4 equal portions and added at 15 days' intervals beginning 15 days after transplanting. The sources of N, P and K were ammonium sulphate (20.5% N) and ammonium nitrate (33.5%), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O), respectively. Other common agricultural practices for growing tomato plants under clay soil conditions in the district namely irrigation, micro-elements spraying, pest and weed control were carried out according to Egyptian Ministry of Agricultural Guidelines.

Field performance

Random samples of four plants were taken from each plot at the flowering stage (50 days from transplanting) to measure plant height (cm), number of branches and leaves per plant, and leaf area/leaf (cm^2) according to standards of the National Institute for Quality Control Egypt. At maturity, fruits from each plot were hand-harvested, and the total number of fruit, yield/plant and yield/m^2 was determined. The average fruit size of each cultivar was measured according to El-Mansi *et al*. (1986). Oil and moisture content were determined according to AOAC (1985).

Statistical analysis

All data obtained were subjected to analysis of variance according to Snedecor and Cochran (1980) and for comparison of cultivar the mean values were analyzed L.S.D. at the 0.05 level of probability, as mentioned by Cochran and Cox (1957).

DNA extraction

DNA samples were extracted from young, fresh leaves (0.1 g) (**Table 1**) 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 Gyulai *et al*. (2000). 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 30 ng/µl with ddH_2O and subjected to PCR amplification according to Gyulai *et al*. (2000).

PCR reactions

Amplification reactions were run in a volume of 25 µl with a Perkin Elemer 2400 thermocycler. The reaction mixture contained 0.4 µM of each of the four deoxynucleotides (dATP, dCTP, dGTP, dTTP); 2.0 mM $MgCl_2$; 0.5 U Taq-polymerase (Promega); 0.5-5.0 nM of primers (ISSR or RAPD/Operon Technologies) (**Table 3**);

Table 3 Sequence data of the ISSR primers applied

#	ISSR	Sequences (5′-3′)
1	814	$(CT)_8TG$ (#814)
2	844A	$(CT)_8AC$ (#844A)
3	844B	$(CT)_8G$ (#844B)
4	17898A	$(CA)_6AC$(#17898A)
5	17898B	$(CA)_6GT$ (#17898B)
6	17899A	$(CA)_6AG$ (#17899A)
7	17899B	$(CA)_6GG$ (#17899B)
8	HB8	$(GA)_6GG$ (#HB8)
9	HB9	$(GT)_6GG$ (#HB9)
10	HB10	$(GA)_6CC$ (#HB8)
11	HB11	$(GT)_6CC$ (#HB11)
12	HB12	$(CAC)_3GC$(#HB12)
13	HB13	$(GAG)_3GC$ (#HB13)
14	HB14	$(CTC)_3GC$ (#HB14)
15	HB15	$(GTG)_3GC$ (#HB14)

Table 3 (cont.) Sequence data of the RAPD primers applied

#	RAPD	Sequences (5′-3′)
1	P1	GTA GAC CCGG
2	P2	GGA CCC TTAC
3	P3	GTC GCC GTC A
4	P4	GGT CCC TGA C
5	P5	TGG ACC GGT G
6	P6	AGG GGT CTT G
7	P7	TTC CCC CGC T
8	P8	TTC CCC CCA G
9	P9	ACT TCG CCA C
10	P10	CAA TCG CCG T
11	P11	AGG GAA CGA G
12	P12	TGC GCC CTT C
13	P13	TTC GCA CGG G
14	P14	GTG AGG CGT C
15	P15	CAA ACG TCG G
16	P16	CTG CTG GGA C
17	P17	GTG ACG TAG G
18	P18	CCA CAG CAG T
19	P19	TGA GCG GAC A
20	P20	GTG AGG CGT C

2.5 µl of 10X thermophylic buffer (50 mM KCl, 10 mM TRIS-HCl, Promega); and 20 ng template DNA (Williams *et al*. 1990).

PCR amplification program

RAPD and ISSR amplification programs were determined for the thermal cycler at 94°C for 2 min; 35 cycles of: Denaturation at 94°C for 30 s, annealing for 45 s, extension at 72°C for 90 s; with a final extension at 72°C for 10 min; and 4°C soak. The annealing temperature varied according the melting temperature of each primer. The core program increased from 35 to 40 cycles, when amplification was weak, to increase the amount of PCR products.

Gel electrophoresis

Amplified fragments (10 µl) were separated by agarose (1.2%, SeaKem LE, FMC) gel electrophoresis, stained with ethidium bromide (0.5 ng/µl), run at 80 V in 1X TBE buffer and photographed on a UV transilluminator (Pharmacia) by a Canon S5 digital camera with a UV filter adaptor. A negative control which contained all the necessary PCR components except template DNA was included in the PCR runs.

Fragment analysis

Sharp PCR fragments were scored for the presence or absence (i.e. no "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.

Cluster analysis

Data of the similarity matrix were used for cluster analysis by using the unweighted pair-group method with arithmetic averages (UPGMA), (NTSYS-pc 2.02 software package; Numerical Taxonomy System, Exeter Software) (Rohlf 2000). Bootstrap analysis with 1000 replications was used (Winboot software) (Yap and Nelson 1996).

Radical scavenging activity (RSA) of tomato juice

The RSA of freshly prepared, blended and filtered by miracloth, tomato juice, was assayed with DPPH (2,2-diphenyl-1-picrylhydrazyl) (10^{-4} M) previously dissolved in methanol according to Ramadan *et al*. (2003) and Ramadan and Moersel (2007). DPPH, in the absence of antioxidant compounds, was stable for more than 2 h of a normal kinetic assay. For evaluation, 10 mg of juice was mixed with 390 µl methanolic DPPH radical and the mixture was vortexed for 20 s at ambient temperature (25°C). Against a blank of pure methanol without DPPH, the decrease in absorption at 515 nm was measured in 1-cm quartz cells after 30 and 60 min of mixing using a UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). The RSA of DPPH radicals were estimated from the differences in absorbance of methanolic DPPH solution with or without sample (control) and the inhibition percentage was calculated according to Ramadan *et al*. (2003). All measurements were performed in triplicate and mean values (± standard deviation) were calculated.

RESULTS AND DISCUSSION

Field performance under heat stress: effect of transplanting dates and genotypes

There was no significant effect of heat stress on morphological characters and yield components among the tomato cultivars, except for fruit size (**Fig. 1A, 1B**) and fruit number/plant (**Table 4**), which showed a linear correlation with transplantation time. Plants growing under heat stress (transplanted on May 5th) had reduced fruit production and

Table 4 Field performance data of fresh-market tomato cultivars (summer).

Characters / Cultivars	Morphological characters				Yield components			
	Plant height (cm)	Branch No./plant	Number of leaves /plant	Leaf area/leaf (cm^2)	Average fruit weight (g)	Fruit No./plant	Yield/plant (g)	Yield/m^2 (kg)
				Effect of transplanting dates				
*Normal date (control) (mean of all cultivars)	86.81	14.02	55.31	236.59	82.77	43.65	3461.54	7.691
**Heat stress date (mean of all cultivars)	82.30	13.88	58.42	239.85	80.30	39.81	3097.23	6.882
	NS	NS	NS	NS	NS	1.65	NS	NS
				Effect of cultivars				
Aledo V.F.	80.85	15.39	90.33	164.33	53.80	61.935	3348.84	7.441
Carmeuco 201-M	119.95	11.75	45.79	271.04	97.03	36.955	3590.57	7.978
Money Maker	94.08	12.75	49.31	226.89	53.00	59.585	3233.68	7.185
Super Marmande	84.685	18.41	60.68	267.97	100.64	34.615	3495.39	7.766
Castl rock	67.39	14.73	75.33	257.29	86.35	34.385	2973.68	6.607
Super Queen	86.23	12.24	42.35	238.93	99.23	42.055	4106.61	9.124
Red Star	81.83	17.85	84.98	263.20	81.22	34.355	2788.98	6.197
Peto 86	82.81	12.08	40.66	283.49	80.02	48.675	3877.31	8.615
UC 97/3	75.25	17.45	53.20	175.37	70.46	38.635	2720.23	6.044
Super Strain -B	67.75	11.02	29.37	221.90	85.46	31.66	2711.43	6.024
Falkon	89.24	9.74	53.68	264.08	89.66	36.035	3228.54	7.173
L.S.D. at 0.05 level	7.94	1.93	9.51	36.85	2.67	3.75	295.35	0.469

* Normal date (control) was transplanted on March 1st
** Heat stress date was transplanted in May 5th
NS: Not Significant

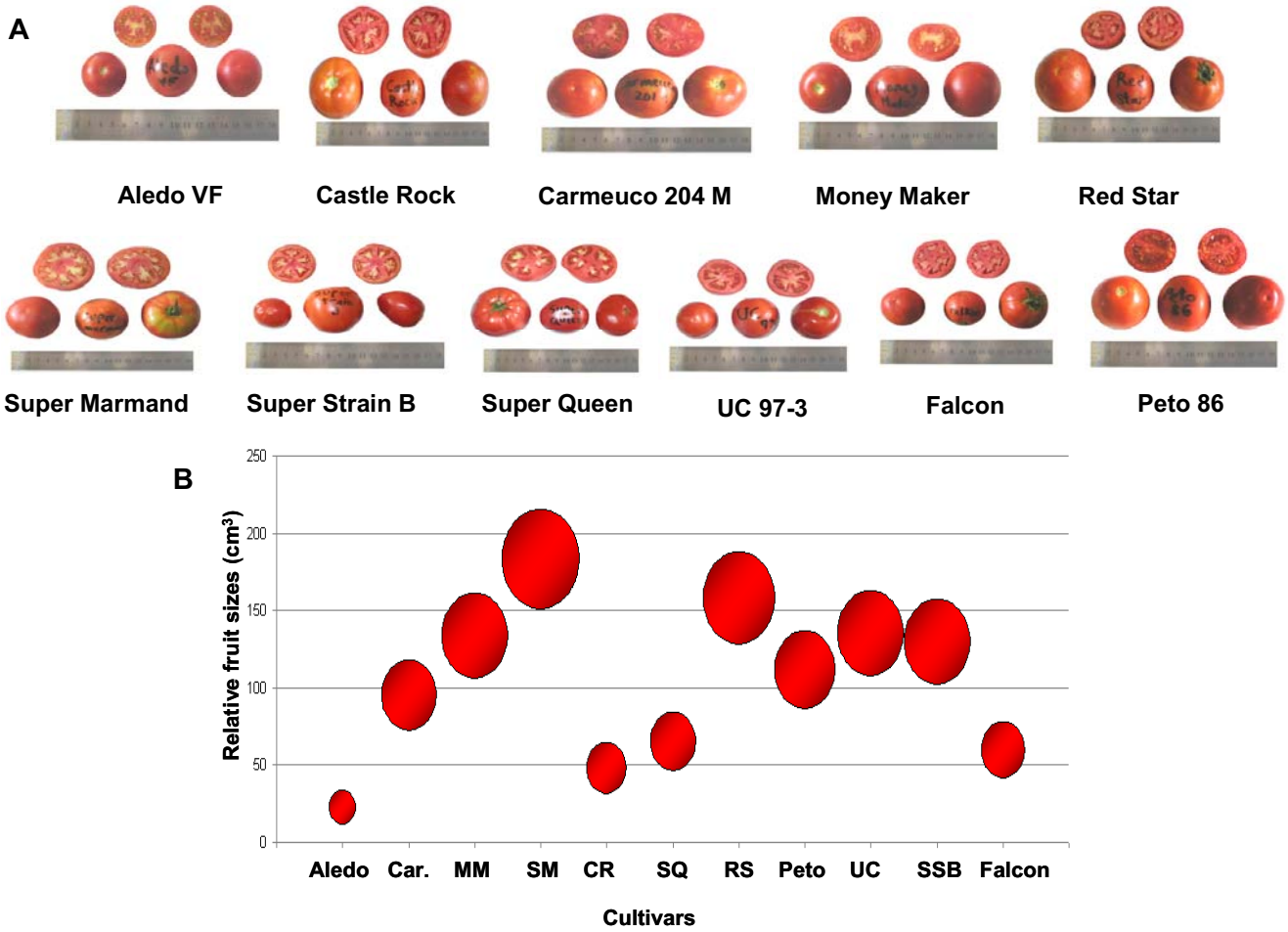

Fig. 1 Relative fruit sizes of tomato (*L. esculentum*) cultivars under heat stress. (**A**) Fruit characters (size, shape, color and flesh) of the tomato cultivars studied. (**B**) Comparative assessment of average fruit sizes (0-250 cm^3) of the cultivars (cultivar abbreviations are indicated in **Table 1**).

consequently total yield. This result is in agreement with other studies that confirmed that heat stress reduces yield (Al-Khatib and Paulsen 1999; Hall and Ziska 2000; Hall 2001). On the other hand, genotypic variation had a significant effect on all traits studied (**Table 4**). For instance, 'Super Queen' recorded the highest level of total yield/plant as well as yield/m^2. However, 'Red Star' recorded the lowest values, which allows these cultivars to be nominated for heat stress breeding programs.

Interaction between transplanting dates and genotypes

The interaction between transplanting dates had significant effects on morphological characters, (i.e., plant height, branch numbers, etc.) and yield components (i.e., average fruit weight, yield/plant, etc.) of the genotypes (**Table 4**). In this regard, 'Carmeuco 201-M' growing under heat stress was ranked first for plant height and average fruit weight (123.16 cm and 98.12 g, respectively). On the other hand, 'Castlerock' under non stressed date recorded the lowest values (62.21 cm). Meanwhile, 'Super Marmande' recorded the maximum value for branch number/plant (19). The minimum value of branch number/plant (9.33) was obtained in 'Falcon'. 'Aledo VF' and 'Red Star' growing under heat stress ranked first for the number of leaves/plant (89.66 and 84.50, respectively) without significant differences between them. Data also showed that leaf area/leaf of 'Peto 86', 'Carmeuco 201-M', 'Super Marmande', 'Falkon', 'Red Star', and 'Castlerock' increased significantly, while 'Aledo VF' and 'UC 97/3' gave the lowest value under heat stress conditions.

'Super Queen' growing under heat stress had significant increment in average fruit weight, yield/plant and total yield/m^2 (99.75 g, 4166.08 g/plant and 9.258 kg/m^2), followed by 'Super Marmande' (average fruit weight 100.64 g) and 'Carmeuco 201-M' (average fruit weight 98.12 g). In contrast, 'Super Strain–B', UC 97/3 and 'Red Star' recorded the lower values of yield/plant and total yield/m^2. On the other hand, 'Aledo VF' and 'Money Maker' recorded higher values of fruit numbers/plant under heat stress (66.66 and 64.81 respectively), while the lowest value was obtained by 'Super Strain–B' (29.02 fruit/ plant) under normal conditions.

Seed oil recovery and radical scavenging activity of tomato juice

Tomatoes are a dietary staple for humans in many parts of the world, ranking second only to potatoes. Tomato seeds were reported as an edible oil source. An earlier study (Al-Wandawi *et al*. 1985) showed that the extraction of tomato seed lipids by refluxing with hexane gave a total lipid concentration of 27.1%. Further extraction of the hexane-extracted flour with chloroform-methanol (2: l v/v) gave an additional lipid concentration of 3.5%. In our study, total lipids content extracted with hexane (Soxhlet extractor, for 6 h) was extremely low in comparison with results of Al-Wandawi *et al*. (1985). This variation might depend on the tomato cultivar and cultivation conditions. 'Super Marmand' and 'Aledo VF' had high oil content (**Fig. 2**).

There is convincing epidemiological evidence that the consumption of tomatoes is beneficial to health and contributes to the prevention of degenerative processes, particularly lowering incidence and mortality rate of cancer and cardio- and cerebrovascular diseases. The protection that fruits and vegetables provide against these diseases has been attributed to the various antioxidant phytonutrients contained in these foods (Ramadan and Moersel 2007). Tomatoes contain several micronutrients: besides minerals,

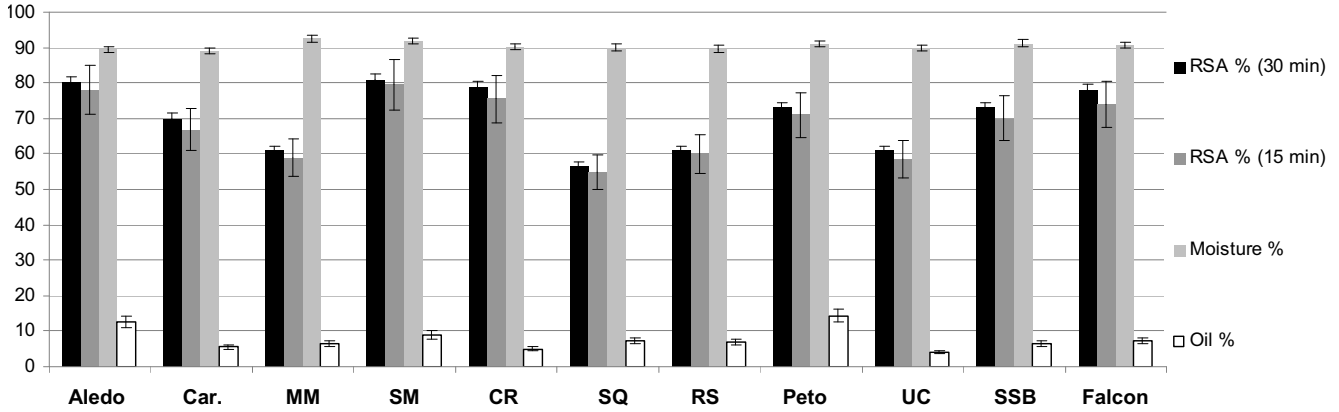

Fig. 2 Chemical composition (relative %) of fruits of tomato (*L. esculentum*) cultivars studied (cultivar abbreviations are indicated in Table 1) for heat tolerance including percentage of oil, moisture and radical scavenging activity (RSA).

flavonoids and vitamins E and C, the most pronounced are the carotenoids, particularly lycopene. The nutritional importance of lycopene has been neglected for many years because it has no pro-vitamin A activity. In the last 10 years, several studies have confirmed that lycopene has the highest antioxidant activity among tomato carotenoids, being the most efficient in quenching singlet oxygen (Graziani *et al*. 2003). We undertook this study to evaluate the antioxidant potential of tomato pulp and peel bioactive compounds. The improved understanding of these issues may favour marketing opportunities for the cultivars. All cultivars tested showed an evident antioxidant effect, wherein 'Aledo VF' and 'Money Maker' showed strong radical scavenging activity (**Fig. 2**).

Assessment of genetic diversity

The assessment of genetic diversity among tomato cultivars was evaluated by RAPD PCR-based markers (Williams *et al*. 1990) and ISSRs (Wang 2004; Zietkiewicz *et al*. 2004). Both methods provide quick, reliable and highly informative data for genotyping tomato cultivars (Levi and Rowland 1997; Nagaoka and Ogihara 1997; Bebeli and Mazzucato 2008). A set of 50 ISSR and 100 RAPD primers was used for initial screening. Only 15 ISSR primers and 20 RAPD primers detected intraspecific variations (**Table 5**). Genetic diversity parameters (average number of alleles per polymorphic locus, percent of polymorphism and marker index) were calculated for ISSR, RAPD and ISSR+RAPD approaches in all cultivars (**Table 6**). The results revealed high and clear reproducible fragment patterns for RAPD (256) and ISSR (185) in the range of 1500 bp to 100 kb. Genetic similarities were calculated from Nei's similarity indexes considering ISSR and RAPD approaches individually as well as together. Different dendrograms based on RAPD (**Fig. 3**), ISSR (**Fig. 4**) and a combined RAPD+ISSR (**Fig. 5**) revealed that molecular similarity and clustering is much dependant on the marker system used. This result might indicate that both markers systems target different sites in the genomes. However, the UPGMA dendrogram clustered tomato cultivars according to their geographical origins (**Figs. 3, 4** and **5**). The combined RAPD+ISSR dendrogram revealed that 'Castle Rock' (CR) and 'Super Queen' (SQ) have high similarity level with 'Super Strain B' (SSB), 'Aledo VF' and 'Red Star' in a further cluster. This data indicates close relationships between these cultivars in the pedigree. No further groups of cultivars were clustered (**Fig. 4**).

In conclusion, the study reflects the importance of combined studies of field performance, yield, origin and genotyping of tomato cultivars to assist growers in selecting the best heat-tolerant cultivars for market production.

Table 5 Sequence data of the RAPD and ISSR primers applied

#	ISSR	Sequences (5′-3′)
1	814	$(CT)_8TG$ (#814)
2	844A	$(CT)_8AC$ (#844A)
3	844B	$(CT)_8G$ (#844B)
4	17898A	$(CA)_6AC$(#17898A)
5	17898B	$(CA)_6GT$ (#17898B)
6	17899A	$(CA)_6AG$ (#17899A)
7	17899B	$(CA)_6GG$ (#17899B)
8	HB8	$(GA)_6GG$ (#HB8)
9	HB9	$(GT)_6GG$ (#HB9)
10	HB10	$(GA)_6CC$ (#HB8)
11	HB11	$(GT)_6CC$ (#HB11)
12	HB12	$(CAC)_3GC$(#HB12)
13	HB13	$(GAG)_3GC$ (#HB13)
14	HB14	$(CTC)_3GC$ (#HB14)
15	HB15	$(GTG)_3GC$ (#HB14)
#	**RAPD**	**Sequences (5′-3′)**
1	P1	GTA GAC CCGG
2	P2	GGA CCC TTAC
3	P3	GTC GCC GTC A
4	P4	GGT CCC TGA C
5	P5	TGG ACC GGT G
6	P6	AGG GGT CTT G
7	P7	TTC CCC CGC T
8	P8	TTC CCC CCA G
9	P9	ACT TCG CCA C
10	P10	CAA TCG CCG T
11	P11	AGG GAA CGA G
12	P12	TGC GCC CTT C
13	P13	TTC GCA CGG G
14	P14	GTG AGG CGT C
15	P15	CAA ACG TCG G
16	P16	CTG CTG GGA C
17	P17	GTG ACG TAG G
18	P18	CCA CAG CAG T
19	P19	TGA GCG GAC A
20	P20	GTG AGG CGT C

ACKNOWLEDGEMENTS

The project was granted by the by Bibliotheca Alexandrina (Egypt) - Center for Special Studies and Programs, Grant No. 050176. The authors would like to thank Prof. Dr. Luther Waters (Auburn University, Alabama, USA) for his comments on the manuscript and also Zoltán Tóth, Institute of Genetics and Biotechnology, St. Stephanus University, Gödöllő (Hungary) for his help in photo preparations.

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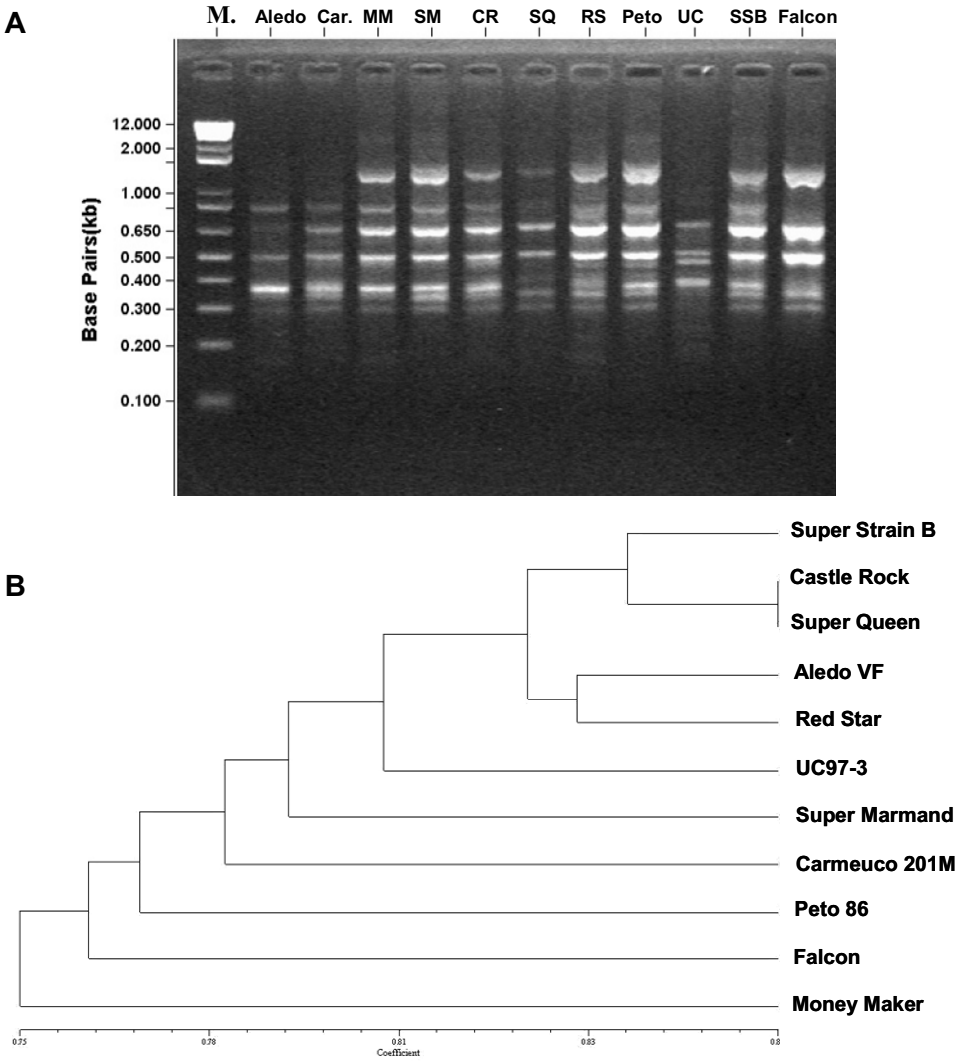

Fig. 3 Molecular polymorphism of different tomato cultivars revealed by RAPD. (**A**) PCR amplification with RAPD P15 primer. (**B**) Molecular dendrogram of tomato (*L. esculentum*) cultivars studied based on RAPD polymorphism.

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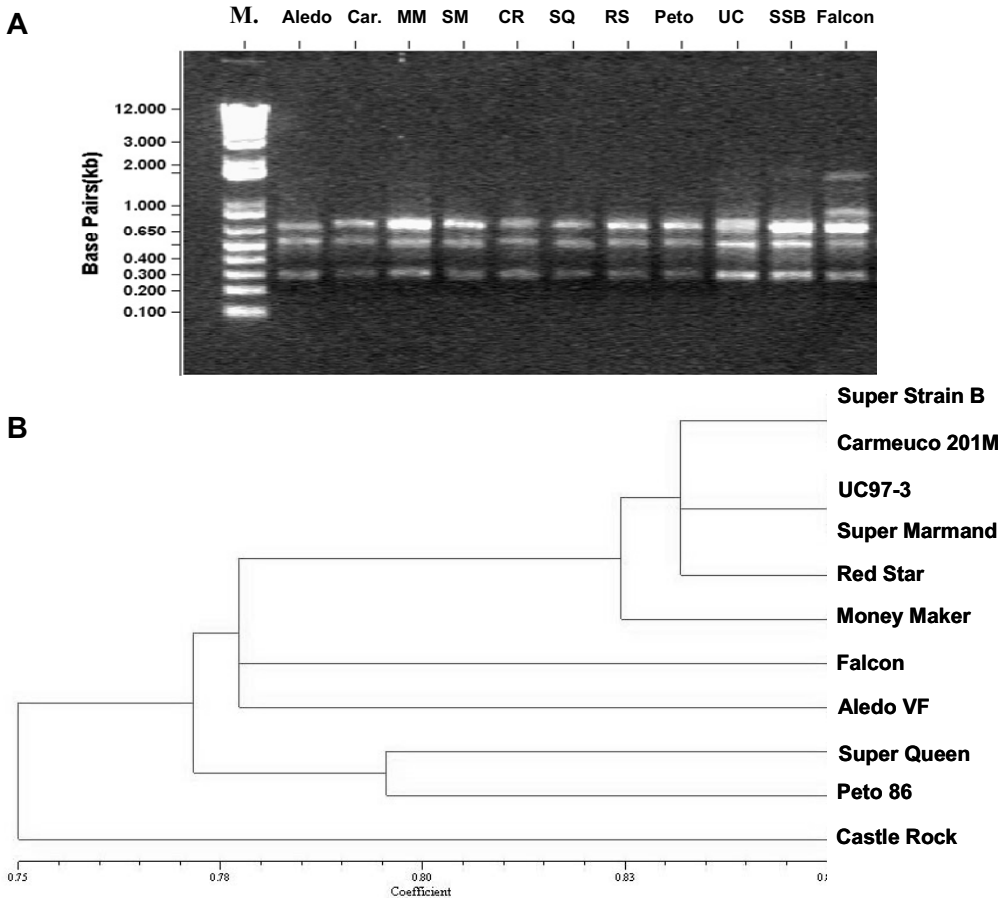

Fig. 4 Molecular polymorphism of different tomato cultivars revealed by ISSR. (**A**) PCR amplification with ISSR HP12 primer. (**B**) Molecular dendrogram of tomato (*L. esculentum*) cultivars studied based on ISSR polymorphism.

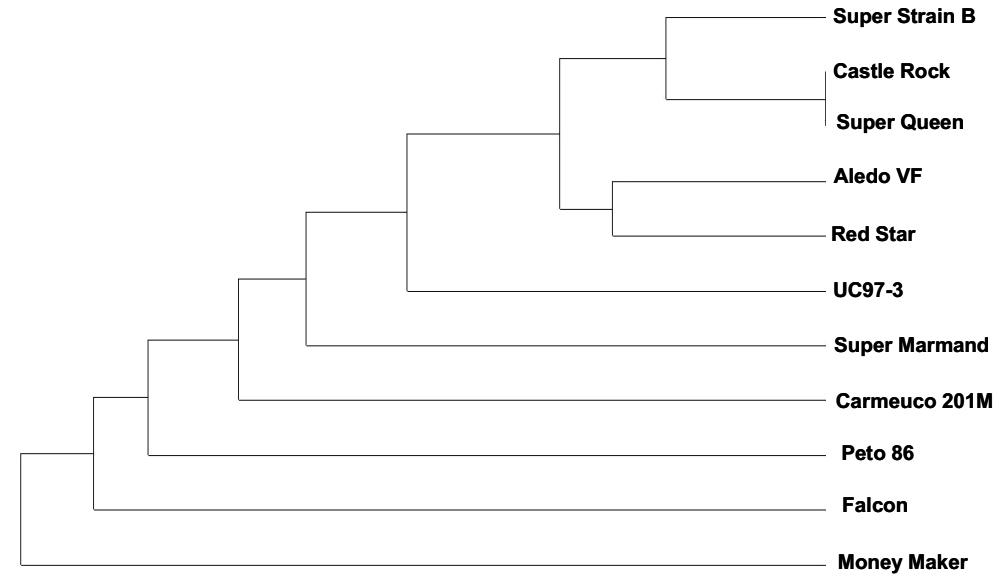

Fig. 5 Molecular polymorphism of different tomato (*L. esculentum*) cultivars studied based on combined results of ISSR+RAPD marker systems.

Table 6 Comparison of DNA marker systems in tomato (*L. esculentum*) cultivars.

Average № of bands/primer	Gel polymorphism			№ of primers	Marker system
	Polymorphic (with unique bands)	Unique bands	Polymorphic (without unique bands)		
25	25	18	7	20	RAPD
38	38	32	6	15	ISSR
31.5	31.5	25	6.5	35	RAPD+ISSR