J. Verbr. Lebensm. 4 (2009): 118 – 127
1661-5751/09/020118-10
DOI 10.1007/s00003-009-0474-5
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Variations in tomato (*Lycopersicon esculentum*) cultivars grown under heat stress

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Received: January 27, 2009; accepted: February 12, 2009
Online First 7 April 2009

Key words: Fresh-market tomato, heat stress, Egypt, comparative analysis, molecular markers.

Abstract: Tomato (*Lycopersicon esculentum* Mill) is considered as important and economic agricultural crop all over the world. For improving the yield and yield attributes, varieties are often produced and evaluated under different growth condition. In the study presented morphological (growth and yield parameters), biochemical (oil, moisture content and radical scavenging activity) and molecular diversity (RAPD and ISSR) of eleven fresh-market 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, Peto86 and Red Star were found to have the most vigorous growth habit, while cv. Super Queen has the most significant average fruit weight, yield/plant and total yield/m^2 under heat stress. For nutritional quality cv. Super marmande and cv. Aledo showed the highest oil content while cv. Aledo and cv. Money Maker showed the highest radical scavenging activities (RSA). Molecular polymorphism among cultivars was detected using two molecular markers systems, RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat), providing further facilities for molecular comparison.

1. Introduction

Tomato (*Lycopersicon esculentum*, $2n = 2x = 24$; 0.95 x 109 nt) is one of the most popular vegetable crops worldwide. Its origin and domestication started in 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 1970 s, followed by resistance breeding and long shelf-life in the 1980 s, then for nutritional quality and taste from the 1990 s until now. Currently breeding programs have produced unique varieties such as the dwarf 'Micro-Tom' variety (released in 1989) and the first transgenic tomato 'FlavrSavr' (Bai and Lindhout, 2007). Nowadays, however, evaluating the chemical and nutritional quality of fresh-market tomatoes is an essential breeding goal for satisfying market need. Because of the phenomena of global warming and temperature rising, 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 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, reduces the product quality and yield. For instance, low fruit setting, reduction in the flower fertilization rate, decrease in the lycopine content and high evaporation are all related to high temperature stress (Al-Khatib and Paulsen, 1999; Hall and Ziska, 2000; Hall, 2001). The structure of genetic variability among inbred families at different generations of selfing depends on the way genes act and varies according to the trait selected (Ismail, 2003). Selection for heat tolerance under field conditions provides breeders with general data to identify potentially tolerant germplasm (Blum, 1988; Hall, 2001). Therefore exploring the range of genetic diversity for heat tolerance in different fresh-market tomato is very important strategy. The purpose of this study is to evaluate and rank eleven tomato cultivars for heat tolerance

Tab. 1 List and data of the eleven fresh-market tomato (*L. sesculentum*) cultivars studied for tolerance to heat stress.

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

and nutritional quality by morphological, chemical and molecular marker characterization to be grown in tropical and subtropical region (i.e. Egypt).

2. Materials and Methods

2.1 Plant material and cultivation

For the evaluation trial, seeds of eleven fresh-market tomato cultivars (Tab. 1) were sown on January 17th and March 29th in speeding trays under greenhouse conditions. The growing medium was consisted of peat mass and vermiculite (1:1, v/v). The seedlings of each cultivar were grown and evaluated in the field under heat stress, (Tab. 2) during two transplanting dates at March 1st (normal date) and May 5th (heat stress) date during 2007 season. The plants were grown in clay soil conditions with surface irrigation system. These eleven cultivars were arranged as split plot in randomized complete block design with three replicates. The field plots area were (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 apart within rows. Each plot contained 50 plants and the outer two rows in each plot were used for left to samples determination. The other rows were used for yield determination. The total amounts of mineral fertilizers were 100, 60 and 100 kg/fed of nitrogen (N), phosphorus (P) and potassium (K), respectively. Agricultural Sulphur at 100 kg/fed and one third of N, P and K fertilizers were added during soil preparation with farmyard manure FYM (20 m^3/fed). The rest amounts of N, P and K fertilizers were divided into 4 equal portions and added 15 days intervals beginning after 15 days of 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 of growing tomato plants under clay soil conditions in the district, i.e. irrigation, micro-elements spraying, pests control and weed control were carried out.

2.2 Field performance

A random sample 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 total number of fruit, yield/plant and yield/m^2 were determined. Oil and moisture content were determined according to AOAC (1985).

2.3 Statistical analysis

All obtained data in the experiment was subjected to proper statistical analysis of variance according to Snedecor and Cochran (1980) and the comparison of cultivar means was done using LSD test at the 0.05 level of probability as mentioned by Cochran and Cox (1957).

2.4 DNA extraction

DNA samples were extracted from young, fresh leaves (0.1 g) (Tab. 1) by the CTAB 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).

2.5 PCR reactions

Amplification reactions were run at a volume of 25 µ by Perkin Elmer 2400 thermocycler. The reaction mixture contained 0.4 µ 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) (Tab. 3), 2.5 µl of 10 x thermophylic buffer (50 mM KCl, 10 mM TRIS-HCl, Promega), and 20 ng template DNA (Williams et al., 1990).

2.6 PCR amplification program

RAPD and ISSR amplification programs were settled on for the thermal cycler as 94 °C for 2 min., 35 cycles [of 94 °C for 30 sec., annealing for 45 sec., 72 °C for 90 sec.], 72 °C for 20 min., and

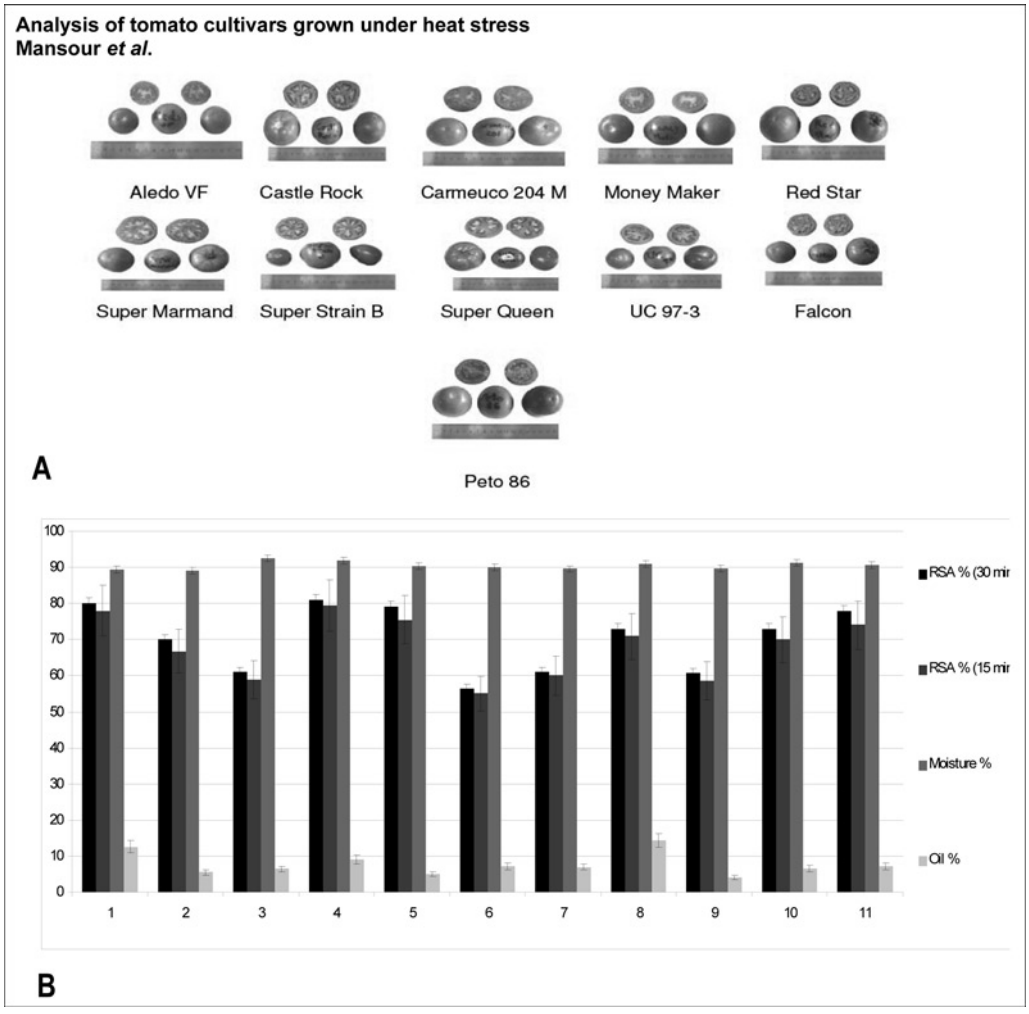

Fig. 1 (A) Fuit characters (size, shape, color and flesh) and **(B)** chemical composition (percentage of oil, moisture) and radical scavenging activity-RSA of the eleven tomato cultivars grown under heat stress (RAS = radical scavening activity).

hold at 4 °C. The annealing temperature varied according to the melting temperature of each primer. The core program was increased from 35 to 40 cycles, when amplification was weak, to get increase in the amount of PCR products.

2.7 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) at 80 V in 1 X TBE buffer and photographed on a UV transilluminator (Pharmacia) by Canon S5 digital camera with UV filter adaptor. A negative control which contained all the necessary PCR components except template DNA was included in the PCR runs.

2.8 Fragment analysis

Sharp PCR fragments were scored for the presence *versus* absence (not "ghost"). Fragments at low intensities were only scored as present when they were reproducible in repeated experiments using (GelAnalyzer 3, Egygene) software.

2.9 Cluster analysis

Genetic similarity was estimated on the basis of Jaccard coefficient (Jaccard, 1908). Data from the similarity matrix were used for cluster analysis by the unweighted pair-group method with arithmetic averages (UPGMA). All the calculations were performed by using the NTSYS-pc version 2.02 software package (Numerical Taxonomy System, Exeter Software) (Rohlf, 1997).

2.10 Radical scavenging activity (RSA) of tomato juice

The RSA of freshly prepared 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 normal kinetic assay. For evaluation, 10 mg of juice was mixed with 390 µl methanolic DPPH radical and the mixture was vortexed for 20 sec 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 UV-260 visible recording spectrophotometer (Shimadzu, Kyoto, Japan). RSA of DPPH radicals was estimated from the differences in absorbance of methanolic DPPH solution with or without sample (control) and the inhibition percent was calculated according to Ramadan et al. (2003). All experimental procedures were performed in triplicate and mean values (± standard deviation) were calculated.

Tab. 2 Data of the mean and maximum temperature (T) and relative humidity (RH) of growing period of tomato cultivation at ABU KABEER district from February to October 2007.

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

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

Tab. 3 Field performance data of eleven fresh-market tomato cultivars (summer season 2007).

Cultivars	Characters							
	Morphological characters				Yield and its components			
	Plant height (cm)	Branch No. / plant	Number of leaves /plant	Leaf area /leaf (cm^2)	Average fruit weight (gm)	Fruit No. /plant	Yield /plant (gm)	Yield / m^2 (kg)
	Effect of transplanting dates							
*Normal date (control)	86.81	14.02	55.31	236.59	82.77	43.65	3461.54	7.691
**Heat stress date	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 in March 1st, ** Heat stress date was transplanted in May 5th.

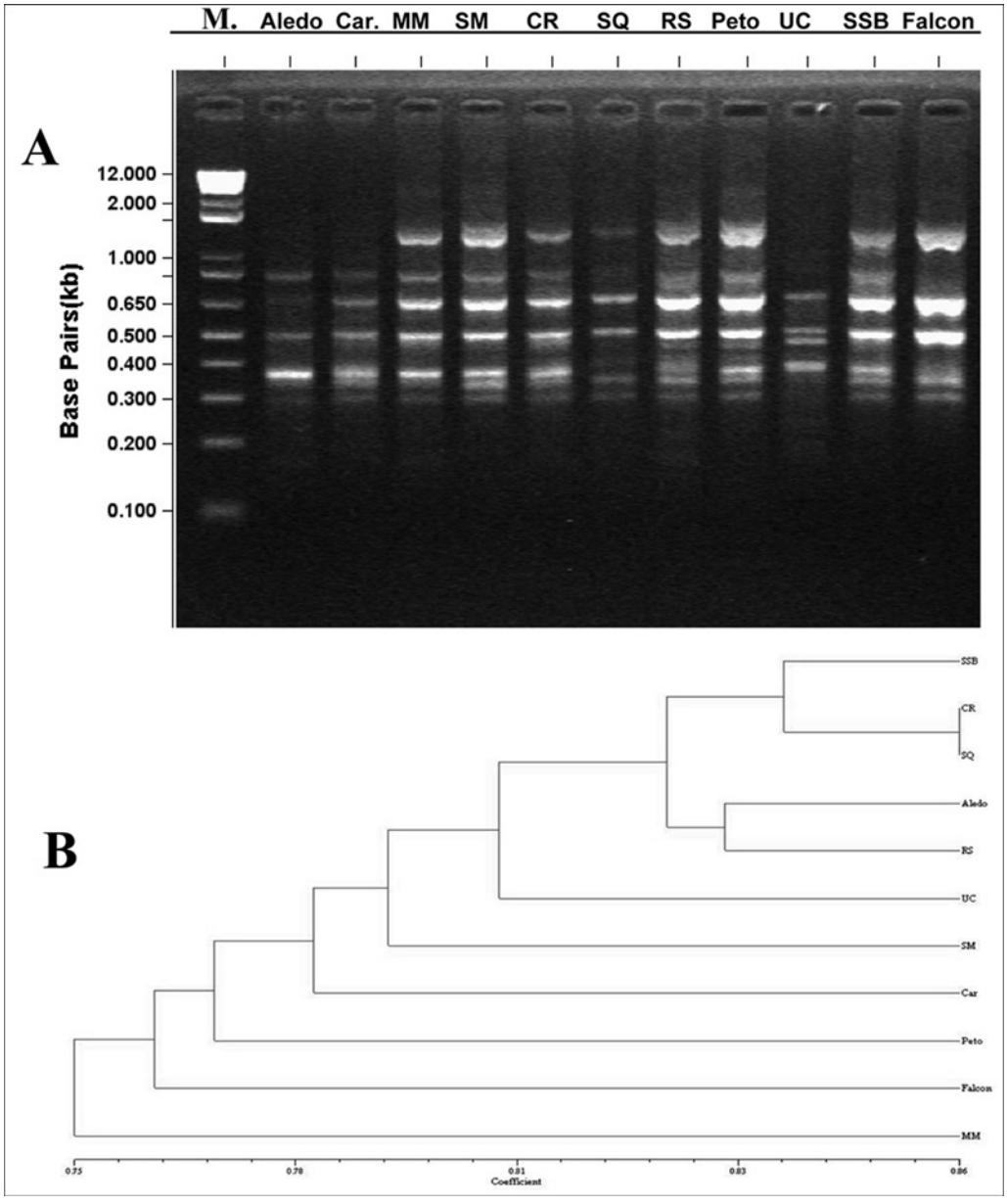

Fig. 2 Samples of RAPD polymorphism (primer RAPD P-15) of eleven fresh-market tomato cultivars (A). Molecular dendrogram of tomato (*L. esculentum*) cultivars based on total RAPD polymorphism (B)

3. Results and Discussion

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

The results show no significant differences in morphological characters and yield components, except fruit number/plant (Tab. 3). It is also shown that the transplanting date has significant effect on fruit numbers. This indicates that plants growing under heat stress had reduced number of produced fruits and thus reduced total yield. This result is in agreement with other investigations on the effect of heat stress (Al-Khatib and Paulsen, 1999; Hall and Ziska, 2000; Hall, 2001). On the other side, Tab. 4 shows that tomato genotypic variation has significant effect on all studied traits. For instance, Super Queen recorded the highest total yield/plant as well as yield/m^2. However, Red Star recorded the lowest value. This significant variation between different genotypes nominates these cultivars for breeding programs of heat stress. Thus, for production purposes, cultivation of these cultivars is advisable for tomato breeders in such hot climate areas.

3.2 Interaction between transplanting dates and genotypes

The results show that the interaction between transplanting dates and genotypes had significant effects on morphological characters, (i.e. plant height and branch numbers) and yield components (i.e. average fruit weight and yield/plant) (Tab. 4). In this regard, cv. Carmeuco 201-M growing under heat stress recorded the first rank for plant height and average frit weight (123.16 cm and 98.12 g, respectively). On the other hand cv. Castlerock under normal planting conditions shows the lowest

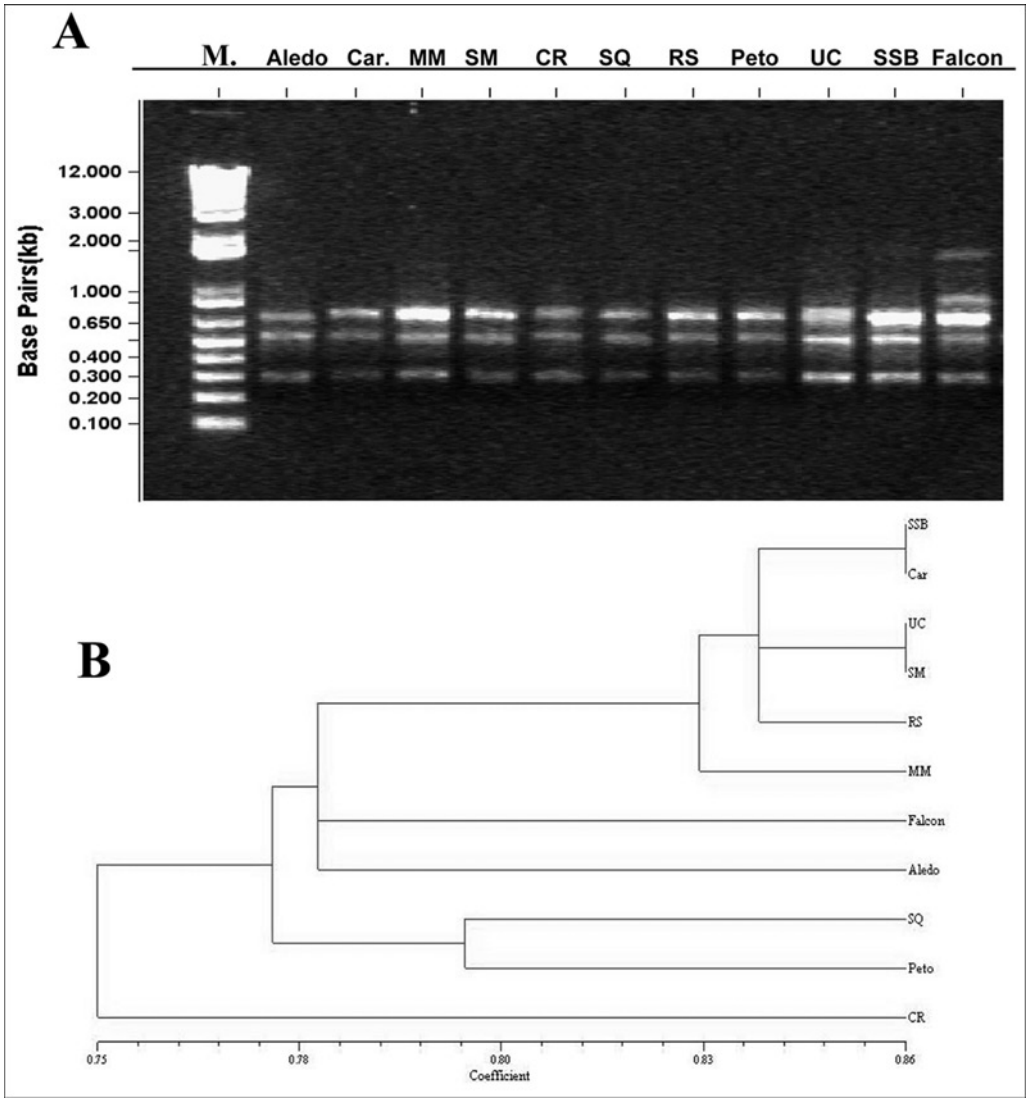

Fig. 3 Samples of ISSR polymorphism (primer ISSR HP12) of eleven fresh-market tomato cultivars (A) PCR amplification with ISSR HP12 Primer, (B) Molecular dendrogram of tomato (*L. esculentum*) cultivars based on total ISSR polymorphism.

values (62.21 cm). Meanwhile cv. Super Marmande shows the maximum value for branch number/plant (19 branch), while the minimum value for branch number/plant (9.33 branch) was obtained by cv. Falcon. Cv. Aledo V.F. and cv. Red Star growing under heat stress came in the first rank as for number of leaves/plant (89.66 and 84.50 leaf/plant) without significant differences between them. Data also show that cv. Peto 86, Carmeuco 201-M, Super Marmande, Falkon, Red Star, and Castlerock significantly increased leaf area/leaf without significant differences between them, while cv. Aledo V.F. and UC 97–3 have lower values under heat stress conditions.

The cv. Super Queen growing under heat stress had significantly increased average fruit weight, yield/plant and total yield/m^2 (99.75 gm, 4166.08 gm/plant and 9.258 kg/m^2), and without significant differences between cv. Super Marmande (100.64gm) and cv. Carmeuco 201-M (98.12 gm) for average fruit weight (Figure 1A). In contrast cv. Super Strain–B, UC 97–3 and Red Star show the lowest values yield/plant and total yield/m^2. On the other hand cv. Aledo V. F. and Money Maker show the highest value of fruit numbers/plant under heat stress (66.66 and 64.81 fruits), while the lowest value was obtained by cv. Super Strain-B (29.02 fruit/ plant) under normal condition.

3.3 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 total lipid concentration of 27.1%. Further extraction of the hexane-extracted flour with chloroform-methanol (2:1, v/v) gave an additional lipid concentration of 3.5%. In our study, total lipids content extracted with hexane (Soxhlet extractor, 6 h) was extremely low in comparison with Al-Wandawi et al. (1985) results. This variation might depend on the tomato cultivar and cultivation conditions. Figure 1B showed that cultivars Super Marmand and Aledo had the highest oil recovery.

There is convincing epidemiological evidence that the consumption of fruits and vegetables is beneficial to health

Tab. 4 Field performance data of eleven fresh-market tomato cultivars (summer season 2007).

Cultivars	Characters															
	Morphological characters								Yield and ist components							
	Plant height (cm)		Branch No./ plant		Number of leaves /plant		Leaf area /leaf (cm2)		Average fruit weight (gm)		Fruit No./plant		Yield / plant (gm)		Yield / m2 (kg)	
	Normal date (control)	Heat stress date	Normal date (control)	Heat stress date	Normal date (control)	Heat stress date	Normal date (control)	Heat stress date	Normal date (control)	Heat stress date	Normal date (control)	Heat stress date	Normal date (control)	Heat stress date	Normal date (control)	Heat stress date
Aledo V.F.	80.37	81.33	15.87	14.91	91.00	89.66	174.04	154.62	52.03	55.58	57.21	66.66	2969.71	3727.97	6.598	8.283
Carmeuco 201-M	116.75	123.16	11.75	11.75	46.00	45.58	265.83	276.25	95.94	98.12	34.33	39.58	3295.71	3885.44	7.323	8.633
Money Maker	93.00	95.16	10.50	15.00	51.71	46.91	221.70	232.08	54.86	51.14	54.36	64.81	2979.47	3487.90	6.620	7.750
Super Marmande	84.96	84.41	17.83	19.00	62.79	58.58	272.62	263.33	99.50	101.78	33.79	35.44	3368.25	3622.53	7.484	8.049
Castl rock	62.21	72.58	14.96	14.50	83.33	67.33	257.29	241..58	80.31	92.40	34.33	34.44	2760.60	3186.77	6.134	7.081
Super Queen	87.71	84.75	12.58	11.91	42.71	42.00	246.20	231.66	98.71	99.75	41.00	43.11	4046.42	4166.80	8.991	9.258
Red Star	77.08	86.58	17.54	18.16	85.46	84.50	250..75	263.20	81.85	80.60	34.41	34.30	2813.83	2764.13	6.252	6.141
Peto 86	76.62	89.00	12.91	11.25	42.75	38.58	284.70	282.29	81.88	78.17	46.37	50.98	3783.58	3971.04	8.407	8.823
UC 97/3	72.25	78.25	17.25	17.66	53.08	53.33	172.00	178.75	69.22	71.70	37.50	39.77	2591.98	2848.48	5.759	6.329
Super Strain -B	65.00	70.50	11.29	10.75	30.50	28.25	229.22	214.58	85.03	85.89	29.02	34.30	2474.72	2948.14	5.498	6.550
Falkon	89.33	89.16	10.16	9.33	53.29	54.08	264.00	264.16	83.96	95.37	35.59	36.48	2985.32	3471.76	6.633	7.714
L.S.D. at 0.05 level	11.24		2.74		13.45		52.13		3.77		5.31		417.70		0.991	

Tab. 5 Sequence data of the ISSR and RAPID primers rapplied.

Sequences (5' – 3')	ISSR	#	Sequences (5' – 3')	RAPD	#
$(CT)_8$TG (#814)	814	1	GTA GAC CCG	P1	1
$(CT)_8$AC (#844A)	844A	2	GGA CCC TTAC	P2	2
$(CT)_8$G (#844B)	844B	3	GTC GCC GTC A	P3	3
$(CA)_6$AC(#17898A)	17898A	4	GGT CCC TGA C	P4	4
$(CA)_6$GT (#17898B)	17898B	5	TGG ACC GGT G	P5	5
$(CA)_6$AG (#17899A)	17899A	6	AGG GGT CTT G	P6	6
$(CA)_6$GG (#17899B)	17899B	7	TTC CCC CGC T	P7	7
$(GA)_6$GG (#HB8)	HB8	8	TTC CCC CCA G	P8	8
$(GT)_6$GG (#HB9)	HB9	9	ACT TCG CCA C	P9	9
$(GT)_6$GG (#HB10)	HB10	10	CAA TCG CCG T	P10	10
$(GT)_6$GG (#HB11)	HB11	11	AGG GAA CGA G	P11	11
$(GT)_3$GG (#HB12)	HB12	12	TGC GCC CTT C	P12	12
$(GT)_3$GG (#HB13)	HB13	13	TTC GCA CGG G	P13	13
$(GT)_3$GG (#HB14)	HB14	14	GTG AGG CGT C	P14	14
$(GT)_3$GG (#HB15)	HB15	15	CAA ACG TCG G	P15	15
			CTG CTG GGA C	P16	16
			GTG ACG TAG G	P17	17
			CCA CAG CAG T	P18	18
			TGA GCG GAC A	P19	19
			GTG AGG CGT C	P20	20

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

Marker system	No. of Primers	Gel Polymorphism			Average No. of bands/ Primer
		Polymorphic (without Unique)	Unique bands	Polymorphic (with Unique)	
RAPD	20	7	18	25	25
ISSR	15	6	32	38	38
RAPD+ISSR	35	6.5	25	31.5	31.5

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, 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 have undertaken 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 in our study show an evident antioxidant effect, wherein cv. Aledo and Money Maker show the strongest antiradical action (Fig. 1).

3.4 Assessment of genetic diversity

The genetic diversity between different fresh-market tomato cultivars were investigated by using RAPD PCR-based markers (Williams et al., 1990) and ISSR (Zietkiewicz et al., 2004; Wang, 2004). Both methods provide quick, reliable and informative data for genotyping tomato cultivars (Nagaoka and Ogihara, 1997; Levi and Rowland, 1997). A set of 50 ISSR and 100 RAPD primers were used for initial screening between eleven different fresh-market tomato cultivars. However, only 15 ISSR and 20 RAPD primers detected intraspecific variations (Table 5). Genetic diversity parameters (average number of alleles per polymorphic locus, percent polymorphism and marker index) were calculated for ISSR, RAPD and the combined ISSR+RAPD experiments in all the cultivars (Tab. 6). Dendrograms were constructed using Unweighted Pair Group Method with Arithmetic averages (UPGMA) algorithm based on the similarity index values for RAPD results (Fig. 2), ISSR results (Fig. 3) and combined RAPD+ISSR analysis (Fig. 4). The UPGMA analysis show that different fresh-market tomato cultivars from different geographical regions are distributed in different groups (Fig. 2, 3 and 4). The results revealed high and clear reproducible fragment patterns for RAPD (256) and ISSR (185) in the range of 1500 bp to 100 kb. Different dendrograms con-

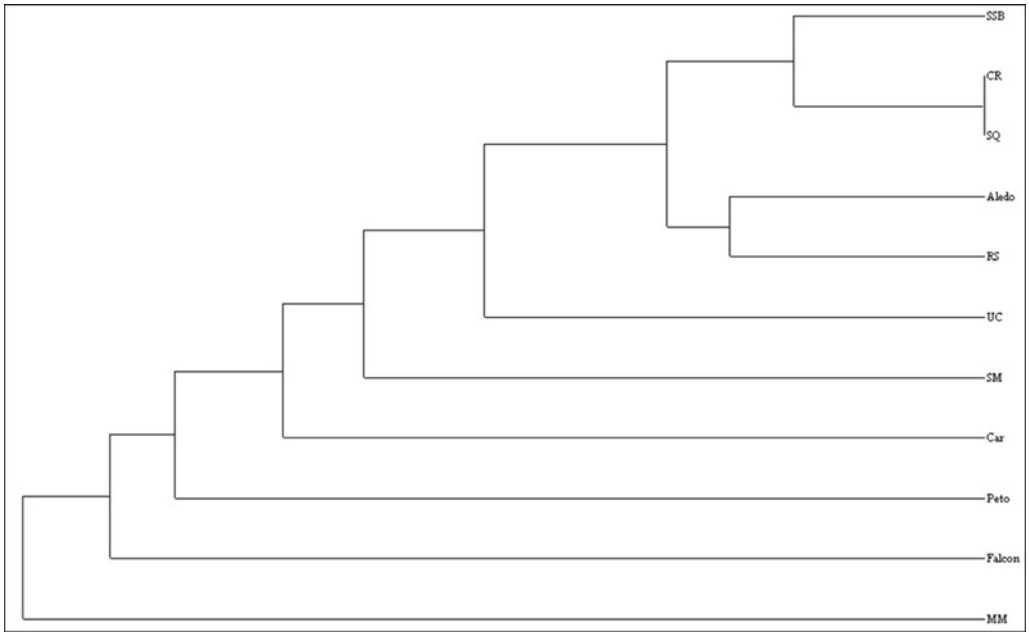

Fig. 4 Molecular dendrogram of eleven fresh-market tomato (*L. esculentum*) cultivars studied based on combined pattern of ISSR and RAPD polymorphism.

structed for RAPD markers (Fig. 2), ISSR markers (Fig. 3) and (RAPD+ISSR) analysis (Fig. 4) reveal that similarity and clustering is much dependent on the marker system used, because both markers systems target the genome differently. The dendrogram, based on combined analysis of both marker systems, shows that cultivars Castle Rock (CR) and Super Queen (SQ) have the highest similarity value. Moreover, they group together with cultivars Super Strain B (SSB), Aledo VF and Red Star in one cluster. These data indicate close relationships between these cultivars in the pedigree. No further groups of cultivars were clustered (Fig. 3). Genetic similarity was calculated from the Nei's similarity index value considering ISSR and RAPD approaches individually as well as together. The genetic similarity matrices generated by ISSR and RAPD markers are highly correlated indicating congruence between these two systems. In the UPGMA analysis, no significant correlation is observed between geographic distances and genetic diversity.

4. Acknowledgment

The project was granted by the by Bibliotheca Alexandrina (Egypt), Center for Special Studies and Programs, Grant No. (050176). Authors thank Prof. Luther Waters (Auburn University, Alabama, USA) for his comments on the manuscript.

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