Genetic Evaluation and Molecular Markers for Heat Tolerance in Tomato (*Lycopersicon esculentum* Mill.)

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ABSTRACT

Genetic evaluation was performed on twenty three genotypes of tomato (*Lycopersicon esculentum* Mill.) under high temperature at summer season to determine the variation between them for heat tolerance. Heat tolerance related criteria, i.e., pollen viability, fruit setting, osmotic pressure and fruit yield per plant. LSSS1, Homestead 24, Black Russian Plum, Super Marmand and Money Maker possess more tolerance of heat. In contrast, Super Stain B, Castle Rock, Cherokee Purple, Moskvich and Nicholevna Pink were more susceptible of heat. The pollen grain viability and fruit setting criteria consider as suitable morphological markers for heat tolerance than other heat tolerant related criteria as osmotic pressure. Heritability was high and moderately whereas, the genetic improvement of new strains could be done. From previous evaluation, Lsss1 as tolerant line and Super Strain B as sensitive cultivar of heat tolerance was crossed for study of molecular markers related to heat tolerance by using bulk segregate analysis (BSA). Crossing was carried out between these two genotypes to obtain the F_1seeds which were left for selfing to obtain the F_2 seeds. The two selected genotypes, their F_1 and F_2 plants were evaluated for their response to heat stress by recording some heat stress related traits. Bulk of the two extremely F_2 plants (most tolerant and most sensitive F_2 groups), the two contrasting parents and their F_1, were used to develop some molecular genetic markers associated with heat tolerance in tomato by using ten RAPD and six ISSR primers. two RAPD markers (with molecular sizes of 100 bp for primers A16 and 500 bp for primer Z13) and one ISSR marker (with molecular size of 650 bp) were considered as reliable markers for heat tolerance as well as susceptible genotypes possessed eight RAPD markers (with

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molecular sizes 500 and 1500 bp for primer C02, 1750 and750 bp for primer C03, 2400 bp for primer C05, 550 bp for primer C08, 400 bp for primer C14 and 650 bp for primer C15). [Journal of American Science 2010; 6(12):364-374]. (ISSN: 1545-1003).

Keywords: Tomato, Heat stress, Heat related traits, Molecular markers, RAPD-PCR, ISSR-PCR. Bulked segregant analysis (BSA), Marker assisted selection (MAS).

Introduction:

Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperatures. However, while some researchers believe that night temperatures are major limiting factors others have argued that day and night temperatures do not affect the plant independently and that the diurnal means temperature is a bitter predictor of plant response to high temperature with day temperature having a secondary role [Peet and Willits, 1998].

Heat stress due to high ambient temperatures to a serious threat to crop production worldwide [Hall, 2001]. Gaseous amissions due to human activities are substantially adding to the existing concentrations of greenhouse gases, particularly CO_2, methane, chlorofluorocarbons and nitrous oxides.

Different global circulation models predict that greenhouse gases will gradually increase world´s average ambient temperature. According to a report of the Intergovernmental panel on Climatic Change (IPCC), global mean temperature will rise 0.3°C per 1 and 3°C above the present value by years 2025 and 2100, respectively, and leading to global warming. Rising temperatures may lead to altered geographical distribution and growing season of agricultural crops by allowing the threshold temperature for the start of the season and crop maturity to reach earlier [Porter,2005].

Tomato (Lycopersicon esculentum Mill.) belongs to the family Solanaceae and is an important vegetable crop all over the world. Tomatoes, although originating from elevated regions of the Andes, can be adapted to various conditions. In the last years, interest through the cultivation of this produce has steadily increased in tropical and subtropical zones. Therefore, tomatoes from these regions should be resistant or tolerant to heat, while the most common problem is the abortion of flowers due to high temperatures.

A threshold temperature refers to a value of daily mean temperature at which a detectable reduction in growth begins upper unde lower developmental threshold temperature have been determined for many plant species through controlled laboratory and field experiments. In tomato, for example, when the ambient temperature exceeds 35 C, its seed germination, seedling and vegetative growth, flowering, fruit set and fruit ripening are adversely affected [Miller *et al*., 2001]. The criteria, which more affecting than the other for heat stress are, pollen grain viability, osmotic pressure, fruit setting and fruit yield [Saeed *et al*. 2007, Abdul-Baki 1991, Peter *et al*. 2002, Adul-Baki and John 1995, Firon *et al*., 2006].

Traditional breeding methods provide little information on the chromosomal regions controlling these complex traits, the simultaneous effects of each chromosomal region on other traits (epistasis, pleiotropy, or linkage), or the genetic basis of such yield related traits (dominance or over-dominance) [Semel *et al*. 2006]. If based only on phenotype analyses, selection by traditional breeding methods is difficult under conditions of large genotype– environment interactions. There is no reliable field screening technique that can be used year after year or generation after generation.

Molecular markers can be used not only for estimating the genetic diversity of germplasm collections but also for distinguishing genotypes within population. [Kantety *et al*., 1995] showed that ISSR technology was able to detect differences between the closely related inbred lines of corn. Thin ISSR should be very useful for studying tomato genotypes.

One approach to facilitate the selection and breeding of polygenic traits is to identify genetic markers linked to the traits of interest. DNA markers have facilitated quantitative trait locus (QTL) mapping studies in segregated populations, and showed that certain genomic regions derived from wild germplasm have the potential to improve fruit-related traits [Gur and Zamir 2004]. The application of molecular markers in plant breeding programs facilitates the improvement of many crop species [Williams *et al*., 1990]. The detection of RAPD markers on the genomic map of different field crop is beneficial to improve breeding programs of these crops. It offers the simplest and fastest method for detecting a great number of genomic markers in less period of time [Edwards *et al*., 1992; Michelmore *et al*., 1991] developed the bulked segregant analysis of F_2 plants as a simpler alternative technique to isogenic line analysis where the highest and

lowest extremes of the F_2 population are bulked for the development of RAPD and SSR molecular markers needed for QTL-assisted selection. ISSR markers have recently found to be highly variable, require less investment in time, money and labor than other methods, and have the ability to be inherited [Wolfe and Liston, 1998].
Therefore the present study aimed to genetic evaluation of twenty three introduced local lines and cultivars for heat tolerance, as well as trial to discovery of some molecular genetic markers associated with heat stress (RAPD and ISSR markers) using bulk segregant analysis (BSA) to be used in marker assisted selection (MAS) program and to develop a database which will enable the utilization of genetic markers as selection tools to improve crop characterization.

Materials and Methods:

1- Evaluation of genotypes for heat tolerance:

1.1. Materials

Twenty cultivars and three lines of tomato were used for this study Table (1). These genotypes were evaluated for heat tolerance during late summer season 2007 at experimental farm of the Faculty of Agriculture, Zagazig University. The seeds of twenty cultivars were kindly obtained from Horticulture Department, and three lines were selected previous study at Genetic Department, Faculty of agriculture, Zagazig University, Egypt.

1.2. Methods:

Seeds of genotypes were sown on may 15^{th}, 2007 in nursery in multi-pot transplant trays field with a mixture of peat-moss and vermiculite (1:1, v/v) medium. After one month from sowing, transplants were transferred to the filed. The mean monthly air temperature in the cultivated area during the growing season is indicated in Table (2).

1.3.Measured characters osmotic pressure:

The osmotic pressure was estimated from transforming the total soluble solids (TSS) to osmotic pressure as air pressure (bar) and multiplied by the factor (1.013) to represent osmotic pressure (bar). The total soluble solids was determined as refraction index using zeiss refractometer after 90 days from transplanting at room temperature [Morgan, 1977].

Table 1: Name , source and characterization of tomato genotypes.

NAME	source	Characterization
MANITOBA	Roguelands seeds, UK	Determinate, red fruit color, fruit weight 90 g
MARION RED	Roguelands seeds, UK	Determinate, red fruit color, fruit weight 85 g
MOSKVICH	Roguelands seeds, UK	Determinate, red fruit color, fruit weight 110 g
BLACK RUSSIAN PLUM	Roguelands seeds, UK	Indeterminate, orange fruit color, fruit weight 25 g
CHEROKEE PURPLE	Roguelands seeds, UK	Semi-determinate, red fruit color, fruit weight 185 g
HOMESTEAD 24	Roguelands seeds, UK	Determinate, red fruit color, fruit weight 195 g
KAZAHK SCHALAVIJE	Roguelands seeds, UK	Semi-determinate, red fruit color, fruit weight 225 g
PLUM LEMON	Roguelands seeds, UK	Indeterminate, yellow fruit color, fruit weight 50 g
NICHOLEVNA PINK	Roguelands seeds, UK	Determinate, red fruit color, fruit weight 150 g
WALTER RED	Roguelands seeds, UK	Determinate, red fruit color, fruit weight 125 g
SUPER STRAIN B	Sun seed, USA	Determinate, red fruit color, fruit weight 140 g
CASTLE ROCK	Castle s, USA	Determinate, red fruit color, fruit weight 125 g
SUBER MARMAND	Daehnfeldt, Holland	Semi-determinate, red fruit color, fruit weight 110 g
MONEY MAKER	Yates, New Zealand Ltd	Indeterminate, red fruit color, fruit weight 40 g
FALCON	Antakya seed, Turkey	Determinate, red fruit color, fruit weight 65 g
ALEDO	Clause, France	Determinate, red fruit color, fruit weight 55 g
RED STAR	Sun seed, USA	Determinate, red fruit color, fruit weight 150 g
PETO 86	Peto Seed, USA	Determinate, red fruit color, fruit weight 85 g
SUPER QUEEN	Sun seed, USA	Determinate, red fruit color, fruit weight 125 g
VF145-B52	Commercial Egypt	Determinate, red fruit color, fruit weight 125 g
LSSS1	New developed strain*	Determinate, red fruit color, fruit weight 85 g
LSSS2	New developed strain*	Determinate, red fruit color, fruit weight 110 g
LSSS3	New developed strain*	Determinate, red fruit color, fruit weight 165 g

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Percentage of fruit set per plant was determined as the total number of fruit divided by the total flower number on clusters 2-6 of plant.

Pollen viability by staining of pollen grains with acetocarmine dye and estimation of pollen viability according to the method followed by [Moreira and Gurgel 1941].

Total yield (kg/plant) was estimated from total weight of harvested fruits from each plant.

Table 2: The mean monthly air temperatures in the cultivated area during the growing season 2007 and 2009.

Month	Temperature (C^{o})			
	2007		2009.	
	Max.	Min.	Max.	Min.
June	48	20	40	20
July	45	17	40	19
August	45	19	39	19
September	40	16	35	16

1.4. Statistical analysis

Collected data were analyzed using the statistical software SPSS version 9.0. One-way analysis of variance (ANOVA) was used to determine differences among genotypes. Relationships between variable characters were estimated as correlation coefficient. Heritability in broad sense was done as follows:

$$h^2 \text{ (in broad sense)} = \frac{\text{genotypic variance}}{\text{genotypic variance + environmental variance}} \times 100$$

2. Bulk Segregant analysis (BSA):

2.1. Materials

Two genotypes of tomato namely; Lsss1 line (heat tolerant) and Super Strain B (heat sensitive) were chosen after evaluation for heat tolerance of twenty three genotypes in above experiment. The seeds of these genotypes were kindly obtained from Horticulture Department, Faculty of Agriculture, Zagazig Universtiy, Egypt.

2.2 Methods:

2.2.1. Sand culture experiment

The two selected genotypes (Lsss1 and Super Strain B) were grown in field and crossed to obtain the F_1 seeds. Some of the F_1 seeds were transplanting in the field and selfed to obtain the F_2 seeds.

Seeds of genotypes, i.e., two parents, F_1'S and F_2 generations were sown on may 15th, 2009 in nursery in multi-pot transplant trays field with a mixture of peat-moss and vermiculite (1:1, v/v) medium. After one month from sowing, transplants were transferred to the filed. The mean monthly air temperatures in the cultivated area during the growing season are indicated in Table (2).

Data were recorded for ten plants for each genotype of the following

related traits; Percentage of fruit set, Pollen viability and total yield per plant. The F_2 plants represented by 200 plants were classified into groups according to their behavior under heat stress.

Samples of the two parents, their F_1 and the two extreme groups of F_2 individuals (the most ten plants heat tolerant and the most ten plants heat sensitive) were taken for further molecular analysis.

2.3 Molecular genetic studies:

2.3.1 Genomic DNA extraction

DNeasy™ Plant Mini Kit (Qiagen Inc., Cat. No. 69104) was used for DNA isolation as described in the manufacturer manual from plant samples (the two parents; F_1 and the two extreme groups of F_2 plants) using bulked segregant analysis (BSA) technique.

2.3.2. RAPD-PCR analysis

PCR reactions were performed according to [Williams *et al.,* 1990] using ten 10-mer primers (Operon Technology, USA) table (3).

Amplification reactions were performed in a volume of 25 µl containing 1X Reaction buffer, 2 mM MgCl2, 0.2mM dNTPS, 0.2 mM primers, 25 ng of genomic DNA, and 1 units of Taq DNA polymerase.

Amplification was performed on a top quality thermal cycler programmed for 45 cycles of 1 minute at 94°, 1 minute at 36°, and 2 minutes at 72°. Amplification products were analyzed by electrophoresis in 1.4% agarose gels and detected by staining with ethidium bromide.

2.3.3. ISSR-PCR analysis

ISSR-PCR reactions were conducted according to [Sharma et al., 1995] using six primers Table (3). Amplification reactions were performed in a volume of 25 µl containing 1X Reaction buffer, 1.5 mM MgCl2, 0.2mM dNTPS, 50 pmol primers, 25 ng of genomic DNA, and 1 units of Taq DNA polymerase.

PCR amplification was performed in a hybrid Cycler programmed to fulfill 35 cycles after an initial denaturation cycle for 4 min at 94ºC. Each cycle consisted of denaturation step at 94ºC for 45 sec, an annealing step at 50ºC for 30 sec. and an elongation step at 72ºC for 2 min and 30 sec. The primer extension segment was extended to 5 min at 72ºC in the final cycle. Agarose gel (1.4%) electrophoresis was used for separating the PCR products.

2.3.4. Desitometric scanning:

All bands resulting from RAPD and ISSR gels were detected on an UV-trans illuminator filter. All gels were photographed under UV light with Polaroid film 667 and scanned with Bio-Rad video densitometer Model 620 at wavelength of 577. Software data analysis for Bio-Rad Model 620 USA densitometer and computer were used.

No.	Primer	Sequence (5' to 3')
		RAPD
1	A16	5'-AGCCAGCGAA-3'
2	B01	5'-GTTTCGCTCC-3'
3	C02	5'-GTGAGGCGTC-3'
4	C03	5'-GGGGGTCTTT-3'
5	C05	5'-GATGACCGCC-3'
6	C08	5'-TGGACCGGTG-3'
7	C14	5'-TGCGTGCTTG-3'
8	C15	5'-GACGGATCAG-3'
9	C19	5'-GTTGCCAGCC-3'
10	Z13	5'-GACTAAGCCC-3'
		ISSR
1	844A	5' (CT)8AC 3'
2	844B	5'(CT)8GC 3'
3	17899B	5'(CA)6AC 3'
4	HB08	5'(GA)6GG 3'
5	HB10	5'(GA)6CC 3'
6	HB13	5'(GAG)3GC 3'

Percentage of fruit set per plant was determined as the total number of fruit divided by the total flower number on clusters 2-6 of plant.

Pollen viability by staining of pollen grains with acetocarmine dye and estimation of pollen viability according to the method followed by [Moreira and Gurgel 1941].

Total yield (kg/plant.) was estimated from total weight of harvested fruits from each plant.

3. Results and Discussion:

1. Genetic evaluation of heat tolerance related traits:

1.1 Genetic variation and heritability of tomato heat tolerance:

Highly significant differences between studied genotypes were recorded for osmotic pressure, pollen viability, fruit setting and total yield per plant, as well as high heritability in broad-sense (h^2) $_{bs}$) for these traits (Table 4).These results confirmed that genetic improvements were done of heat tolerance through related characters of it. These results are similar to those obtained by [Grilli *et al*.,2003], where the fruit set of various tomato genotypes at high temperatures had about 89.5 % broad sense heritability, thus suggesting that the selection of individuals based on characteristic evaluated can be efficient.

Table 4: The mean squares of four studied characters of twenty three genotypes in tomato.

S.V.	d.f.	Osmotic pressure	Pollen viability	Fruit setting	Total yield/plant
Replicate	2	0.547101	0.461449	0.58971	0.009275
Genotypes	2	6.868248**	1095.465**	1139.682**	4.171357**
Error	4	0.399374	4.603874	3.581528	0.025033
h^2		0.8438	0.9875	0.9910	0.9787
C.V %		8.50	4.20	4.00	8.80

h^2_{bs} = Broad sense heritability cv= Coefficient of variability

1.2 Mean performance for studied traits of twenty three genotypes in tomato:

The mean performance for four studied characters of twenty three genotypes (Table 5) confirmed the wide difference between genotypes under study for heat tolerance related characters. Higher values of four traits were recorded for Black Russian Plum, Homstead 24, Super Marmand, Mony Maker, LSSS1 and LSSS3 than the other genotypes suggested as a donor for heat tolerance by using of them in hybridization, with remark to some more tolerant cultivars possess small fruit size as Black Russian Plum and Mony Maker. In contrast several heat susceptible genotypes were recorded, i.e., Moskvich, Nicholevna Pink, Super Strain B, Castle Rock. In addition, the most of studied genotypes consider as intermediate heat tolerance as Manitoba, Marion Red, Khazahk Schalavije, Plum Lemon, Walter Red, Falcon, Aledo, Red Star, Peto 86, Super Queen, VF145-B52 and LSSS2.

From these results may be concluded that the high tolerant genotypes possess high values for four studied traits, and vise versa, susceptible heat genotypes possess low values for four studied traits. No obvious trend were recorded in intermediate heat tolerant genotypes for four studied traits. Comparable study was done by [Saeed et al.,2007] who suggested that genotype, which will produce better yield under high temperature conditions, would be heat tolerant. The value of high broad sense heritability (0.9715) that showed that about 90% of the variation observed was genetically determined. [Abdul-Baki, 1991] who assessed fruit yield of tolerant and sensitive tomato lines and cultivars in field under high temperature condition. The heat tolerant lines produced higher fruit yield than heat sensitive cultivars.
[Peter et al., 2002] who reported that high temperatures fruit set (heat tolerance) was a critical trait of tomato. In the same trend for pollen grain viability, [Adul-Baki and John, 1995] demonstrated that using pollen viability as a selection criterion for high temperature tolerance was genotype effect as well as [Firon et al., 2006] reported that heat stress caused a reduction in number of pollen grains in heat – sensitive cultivars, caused reduced fruit set. In heat tolerant cultivars, however, number and quality of pollen grains, number of fruits were less affected by high temperatures.

1.3 Relationship between four studied criteria related to heat tolerance in tomato:

Correlation coefficient (r) for four characters in tomato is shown in (Table 6). Osmotic pressure was positively and significantly correlated to pollen viability (r = 0.46), fruit set (r = 0.48) and yield (r = 0.54). positive correlation was also observed between pollen viability and both of fruit set and yield (r = 0.95 and 0.77 respectively). As well as correlation between fruit set and yield were positive and highly significant (r = 0.74). These results confirmed that, increased yield under heat stress might be obtained by breeding genotypes that were high in osmotic pressure, pollen viability or fruit set under heat stress. These results agreed with [Weaver and Timm, 1989] whose reported a positive correlation between heat tolerance and pollen viability maintenance after briefly exposing flowering tomato plants in the greenhouse to high temperatures.

2. Bulk Segregant Analysis (BSA)

2.1 Responses of the F_2 plants:

F_2 plants presented by 200 individuals were classified into groups according to their behavior under high temperature stress. The first group refers to the best growing F_2 plants and the last group refers to the worst ones under high temperature stress. The F_2 plants were arranged in descending order according to their frequency, so plants with high frequency in group one were chosen as the most tolerant F_2 plants. While the plants in the last group were taken to represent the most sensitive F_2 plants.

According to these classifications, ten F_2 plants were taken to represent the most tolerant and the most sensitive ones to high temperature stress for each trait as shown in Table (7).

These twenty plants were used for bulked segregant analysis to obtain molecular (RAPDS and ISSRS) markers linked with high temperature stress.

2.2 Molecular genetic markers for heat tolerance:

2. 2.1 RAPD molecular markers

DNA isolated from the two contrasting parents, LSSS1 as a heat tolerant parent and Super Strain B as a heat sensitive parent, their subsequent F_1 and DNA bulks of the tolerant and sensitive groups of F_2 segregating population were tested against ten preselected primers. All primers gave polymorphisms with the studied genotypes, while 8 primers developed molecular markers for heat and sensitive tolerance as shown in table (8).

Primers A16 and Z13 exhibited 2 positive molecular markers which were found only in the tolerant parent (LSSS1), F_1 and the tolerant F_2 bulk with molecular sizes of 100 bp for primers A16 and 500 bp for primer Z13, while there were absent in the sensitive parent (Super S train B) and the sensitive F_2 bulk Fig (1). On the other hand, primers C02, C03, C05, C08, C14 and C15 exhibited eight molecular markers which were found only in the sensitive F_2 bulk with molecular sizes 500 and 1500 bp for primer C02, 1750 and 750 bp for primer C03, 2400 bp for primer C05, 550 bp for primer C08, 400 bp for primer C14 and 650 bp for primer C15 Fig (2).

Table 5: Mean performance and least significant difference (LSD) of four studied characters of twenty three genotypes in tomato under heat stress at summer season, 2007.

Name	Osmotic pressure	Pollen viability %	Fruit setting %	Total yield/plant (Kg)
MANITOBA	6.78	53.7	40.5	1.3
MARION RED	7.345	41.8	35.2	1.1
MOSKVICH	7.91	32.4	29.3	0.6
BLACK RUSSIAN PLUM	11.3	75.7	68.1	3.5
CHEROKEE PURPLE	7.345	22.0	23.7	1.0
HOMESTEAD 24	9.605	76.8	88.5	3.2
KAZAHK SCHALAVIJE	6.78	44.4	33.7	1.5
PLUM LEMON	6.215	62.7	61.2	2.1
NICHOLEVNA PINK	6.215	17.3	14.8	0.7
WALTER RED	6.78	42.6	48.2	1.4
SUPER STRAIN B	6.215	20.7	19.3	0.5
CASTLE ROCK	9.605	24.2	16.3	0.6
SUBER MARMAND	9.04	77.6	63.1	4.1
MONEY MAKER	10.17	77.5	74.7	3.8
FALCON	10.735	68.6	66.3	1.8
ALEDO	6.78	57.1	48.2	1.1
RED STAR	10.735	42.3	40.4	1.2
PETO 86	6.78	51.0	43.1	0.9
SUPER QUEEN	8.0	43.0	47.7	1.2
VF145-B52	7.571	71.2	67.2	1.1
LSSS1	10.735	61.6	58.1	3.3
LSSS2	10.17	49.2	46.0	1.4
LSSS3	9.04	59.0	53.1	3.8
L.S.D 5%	1.07	3.63	3.20	0.27
L.S.D 1%	1.45	4.94	4.36	0.36

Table 6: Correlation coefficient (r) among different related characters to heat tolerance in tomato.

Trait	Osmotic	Pollen viability	Fruit set	Yield/plant
Osmotic pressure	1			
Pollen viability	0.46^{*}	1		
Fruit set	0.48^{*}	0.95^{**}	1	
Yield/plant	0.54^{**}	0.77^{**}	0.74^{**}	1

Table 7: The most tolerant and the most sensitive F_2 plants according to some heat tolerance related traits.

	Plant no.	Fruit setting (%)	Pollen viability (%)	Yield/plant (Kg)
Most tolerant	156	68.5	70.4	4.1
	189	67.9	70.1	3.9
	45	66.7	69.3	3.8
	78	66.6	69.6	3.5
	7	66.4	69.5	3.7
	16	65.8	68.4	3.5
	122	65.8	68.2	3.3
	82	64.3	66.9	3.1
	95	64.2	66.7	3.0
	110	64.1	66.4	3.0
Most sensitive	33	18.5	20.4	0.4
	64	18.4	20.6	0.4
	151	18.4	20.3	0.4
	154	18.1	19.9	0.4
	12	17.9	18.7	0.3
	69	17.8	18.2	0.3
	130	17.6	18.5	0.3
	173	17.2	18.0	0.3
	54	17.1	18.1	0.3
	28	16.8	17.8	0.2

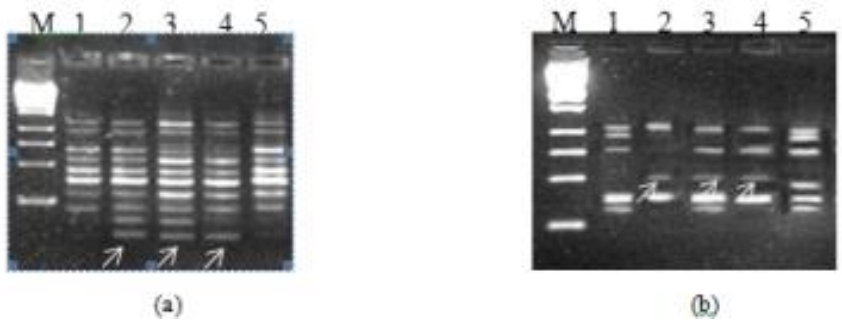

Fig. 1: Amplification patterns using RAPD primers to identify the a) A16and b) Z13 markers linked to resistance alleles. Lane M 1-kb molecular- weight ladder,1) susceptible parent Super Strain B, 2) tolerant parent LSSS1, 3) F_1, 4) tolerant F_2 bulk, 5) susceptible F_2 bulk.

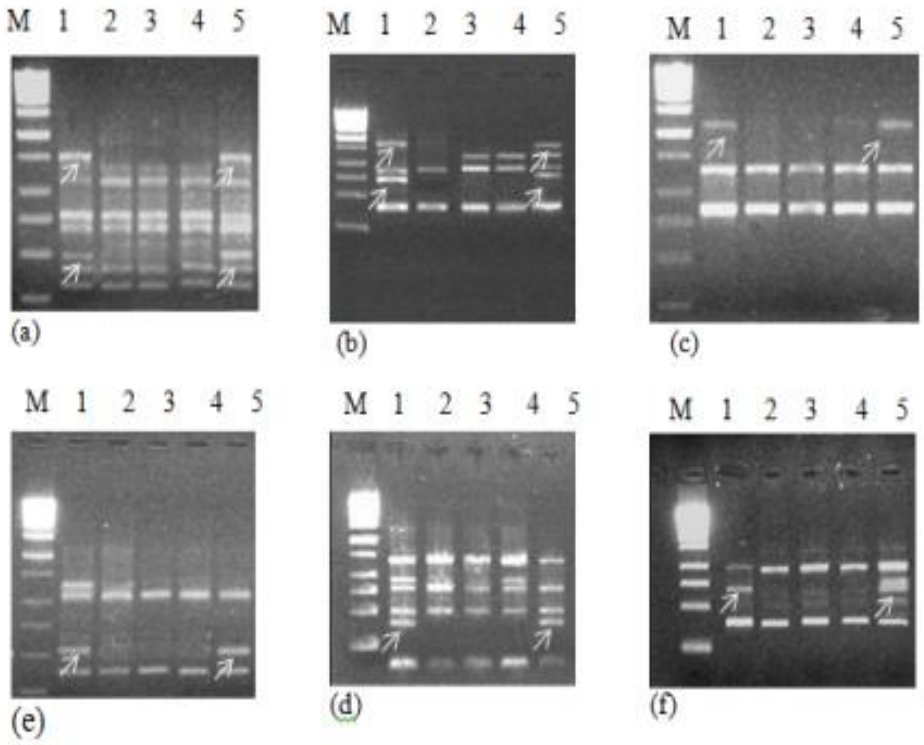

Fig. 2: Amplification patterns using RAPD primers to identify the a) C02, b) C03, c) C05, d) C08, e) C14 and f) C15 markers linked to resistance alleles. Lane M 1-kb molecular- weight ladder, 1) susceptible parent Super Strain B, 2) tolerant parent LSSS1, 3) F_1, 4) tolerant F_2 bulk, 5) susceptible F_2 bulk.

These two positive and eight negative RAPD markers could be considered as reliable markers for heat tolerance in tomato. These results agreed with [Zhang *et al.* 1994 and Mackay and Caligari 2000] whose reported that RAPD analysis combined with BSA has been used to screen for markers linked to genes of interest. Moreover [Lin *et al.* 2006] identified random amplified polymorphic DNA (RAPD) markers linked to heat tolerance traits in tomatoes under heat stress by using the bulked segregant analysis. In addition, bulked segregant analysis was used to identify RAPD markers linked to the Sw-5 gene for resistance to tomato spotted wilt virus (TSWV) in tomato [Chague *et al.* 1997].

Table 8: RAPD-PCR polymorphic bands of eight primers linked to heat and sensitive tolerance with the two parents, their subsequent F_1 and two bulks of F_2.

Primer name	PBN	M.W (bp)	SP	TP	F_1	Tb	Sb	MT
A16	1	2000	0	0	1	0	1	-
	4	750	0	0	1	0	0	-
	11	150	0	1	1	0	0	-
	12	100	0	1	1	1	0	P
Z13	1	1200	1	1	0	0	0	-
	2	950	1	0	1	1	1	-
	3	850	0	0	0	0	1	-
	4	775	1	0	1	1	1	-
	5	500	0	1	1	1	1	P
	6	450	0	0	0	0	1	-
	8	350	1	0	1	0	1	-
C02	1	1500	1	0	0	0	1	N
	5	500	1	0	0	0	1	N
C03	1	1750	1	0	0	0	1	N
	2	1250	0	0	1	1	1	-
	4	750	1	0	0	0	1	N
C05	1	2400	1	0	0	0	1	N
C08	1	1300	1	0	0	0	0	-
	3	550	1	0	0	0	1	N
C14	2	900	1	0	0	1	0	-
	5	400	1	0	0	0	1	N
C15	2	750	0	0	0	0	1	-
	3	650	1	0	0	0	1	N
	4	550	0	0	0	0	1	-

PBN: polymorphic band number Tb: tolerant bulk P: positive

SP: sensitive parent Sb: sensitive bulk N:

TP: tolerant parent MT: marker type

2.2.2 ISSR molecular markers

DNA isolated from the two contrasting parents, LSSS1 as a heat tolerant parent and Super Strain B as a heat sensitive parent, their subsequent F_1 and DNA bulks of the tolerant and sensitive groups of F_2 segregating population were tested against six preselected primers.

All primers gave polymorphisms among the studied genotypes, while only one primer developed molecular markers for heat tolerance Table(9). Primer 844A showed one positive molecular marker which were found only in the tolerant parent (LSSS1),F_1

and the tolerant F_2 bulk with molecular size of 650 bp. Fig. (3). gene that determined the ratio of fructose to glucose in mature tomato fruits [Levin *et al.*, 2000].

Our results were in harmony with those of [Lin *et al.*, 2010] who used the 160 F_2 tomato plants segregating population to identification of ISSR markers linked to fruit related traits in the tomato subjected to high temperatures. ISSR were useful for finding markers associated with major and minor genes controlling agronomical important traits in wheat [Ammiraju *et al.*, 2001]. Also several ISSR markers had been found to be tightly linked to the

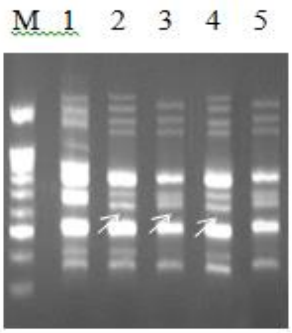

Fig. 3: Amplification patterns using ISSR primers to identify the 844A markers linked to resistance alleles. Lane M 100 bp molecular-weight ladder, 1 susceptible parent Super Strain B, 2 tolerant parent LSSS1, 3 F_1, 4 tolerant F_2 bulk, 5 susceptible F_2 bulk.

These results confirmed that the possibility for breeding of new Egyptian lines and hybrids possess high tolerance of heat and simultaneously high fruit yield for cultivation of summer season, which the temperature up to 40 °C in about two months July and August per year.

The present discovered molecular markers for heat tolerance and sensitively in Egyptian cultivars will be acceleration of breeding program for development of new lines and subsequently new hybrids having more tolerance to heat and high fruit yield at the summer season.

Table 9: ISSR-PCR polymorphic bands of one primers linked to heat tolerance with the two parents, their subsequent F_1 and two bulks of F_2.

Primer name	PBN	M.W (bp)	SP	TP	F_1	Tb	Sb	MT
	1	1250	1	1	0	1	0	-
	4	9	0	1	1	1	1	-
844A	5	9	1	0	0	0	0	-
	8	6	0	1	1	1	0	P
	1	4	1	1	0	1	0	-

PBN: polymorphic band number Tb: tolerant bulk P:

SP: sensitive parent Sb: sensitive bulk

TP: tolerant parent MT: marker type

The highly significant correlation between four heat tolerance related characters under study by using discovered molecular markers for pollen grain viability, osmotic pressure, fruit set and fruit yield per plant and recent study trial to determine the relationships between new molecular markers and quantitative trait loci (QTL) from database and subsequently, helpful to determine the controlling genes for heat tolerance, as well as the study by [Lin *et al.*, 2010], which to determine the quantitative trait loci influencing fruit- related characteristics of tomato grown in high temperature.

In conclusion, our goal was to find RAPD and ISSR markers linked to heat tolerance genes in order to use them in marker-assisted breeding programs. BSA allowed us to rapidly find marker linked to heat tolerance. The results showed that only two RAPD and one ISSR markers were linked to heat tolerance. Thus, BSA allowed us to directly target the gene, as demonstrated by [Michelmore et al., 1991]. The level of polymorphism detected in molecular marker followed by using marker-assisted selection (MAS) has been proven to be good alternative method of the agronomic selection, where it provides plant breeders with environmental- independent genetic markers for certain economic traits.

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