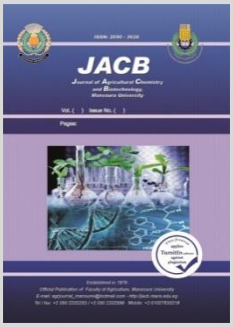
Journal of Agricultural Chemistry and Biotechnology

Journal homepage: www.jacb.mans.edu.eg
Available online at: www.jacb.journals.ekb.eg

Enhancement of Chilling Tolerance and Efficiency Using Start Codon Targeted (SCoT) Technique for Molecular Assessment in some Tomato Genotypes.

Rehab M. Habiba[1*]; A. Y. A. M. Elsayed[2] and Hasnaa A. Saleh[2]

[1]Department of Genetics, Faculty of Agriculture, Mansoura University.
[2]Horticulture Research Institute, Agriculture Research Center, Giza, Egypt.



ABSTRACT

Significant reduction in tomato yield and its fruit quality occurs annually in Egypt during winter and early summer seasons as a result of chilling stress. In this study, a set of tomato lines and cultivars were estimated against their response to treatment by ATP and glycine betaine, in order to select the most promising genotypes for further breeding programs. The Field experiment was carried out at Genetics Department farm, Faculty of Agriculture, Mansoura University during the winter season of 2017/2018. A Randomized Complete Blocks Design in three replicates was adopted in current investigation. The Data were recorded on different vegetative, fruit quality and yield component traits. The results showed that the Edkawy cultivar had the most useful yield components parameters affected by the exogenous treatment followed by S. Marmande and the line KL-45. This cultivar was characterized by a large number of fruits per inflorescence and average fruit weight around 90 grams. The genetic diversity using SCoT marker technique between 11 tomato genotypes was conducted for selecting the promising genotypes for further breeding programs. The SCoT primers produced a total of 94 bands with an average 11.75 bands per primer ranged from seven (SCoT-6 and SCoT-9) to 22 (SCoT-1) per primer. Among them 84 bands were polymorphic. The highest molecular distance was among KL-41 and KL-03 (0.482). While the lowest MD was between Floradade and Edkawy (0.215). The 11 genotypes showed 14 marker loci, these markers were spread over these genotypes variously differentiating each genotype from the other.

***Keywords*:** *Lycopersicon esculentum*, chilling, tolerance, ATP, Genetic diversity, SCoT primers

INTRODUCTION

Tomato (*Solanum lycopersicun* L.) belongs to the *Solanaceae* family is one of the most important vegetable crops grown in Egypt for fresh as well as processed food industries uses. Tomatoes also produce large amounts of important primary and secondary metabolites which can serve as intermediates or substrates for producing valuable new compounds. As a model crop, tomato already has a broad range of tools and resources available for biotechnological applications (Li *et al.*, 2018).

Temperature is the main environmental factor affecting plant growth and development as well as physiological and biochemical characters *(*Nahar and Ullah, 2012; Bita and Gerats, 2013). The temperature which is considered optimal for the production of tomato ranging from 21 to 28 °C during the day and from 15 to 20°C during the night. The tomato grown under temperatures below 13 °C may inhibit fruit-set (Atherton and Rudich 1986), while extended exposure below 6 °C could destroy the whole plant. Thus, significant reduction in fruit yield and quality happens every year in Egypt during winter and early summer seasons where level of night temperature drops several times below 10 °C. Both genetic improvement and cultural practices such as planting time, plant density, and soil and irrigation managements are among the popular approaches that should be employed simultaneously to reduce the negative impacts of abiotic stresses. The genetic management approach as development of chilling tolerant genotypes for once would be a cheap input technology that would play a vital role in lessening the harmful impacts of climate change on agricultural production of low income and small land holding farmers (Tester and Langridge, 2010).

Assessment of the genetic diversity within crop germplasm is essential for breeding and conservation of genetic resources, and is mostly useful as a general guide in the select of parents for breeding hybrids (Talebi *et al.*, 2008). Molecular genetic markers have been widely used in the last years for both assessment of original material and search for valuable plant phenotypes. Genetic diversity in tomato has been explored using a range of molecular markers such as SSR, RAPD and AFLP. SCoT marker polymorphism was described by Collard and Mackill (2009), based on the short conserved regions of plant genes that are surrounded by the ATG translation start codon. The principle of SCoT marker is the single primer amplified region since it uses a single primer as a forward and reverse primer, like the RAPD or ISSR technique.

This investigation aimed to study the effect of exogenous components i.e., ATP and glycine betaine on stimulation of chilling tolerance stress in tomato and assay the genetic diversity using SCoT marker technique between five varieties and six advanced inbred lines of tomato (*Lycopersicon esculentum*) for selecting the promising genotypes for further breeding programs.

* **Corresponding author.**
E-mail address: rehab74@mans.edu.eg
DOI: 10.21608/jacb.2020.123557

MATERIALS AND METHODS

The experiment was carried out at Genetics Department farm, Faculty of Agriculture, Mansoura University, Egypt during winter season of 2017/2018**.**

Genetic materials

The plant materials used in this study included five varieties and six advanced inbred lines of tomato (*Lycopersicun esculentum*). They named Edkawy, Floradade, Castle Rock, Super marmande, NC 1 CELBR, KL-45, KL-41, KL- 46, KL- 47, KL-03, and KL-52. These genotypes were kindly provided from Vegetable Breeding Department, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt. Seeds were sown in 209-cell trays filled with a commercial substrate. After 35 days, seedlings were transplanted to field at 35 cm apart on one side of ridge 4.5m long and 1m. A Randomized Complete Blocks Design (RCBD) in three replicates was adopted in current investigation. During this experiment, plants were grown in low temperature condition where level of night temperature drops several times below 10 C° during a season. The agents ATP and Glycine betaine were used in concentrations of 100 ppm and 50 ppm, respectively as foliar spray. Plants were sprayed four times with assigned treatment, the first one was at 20 days after transplanting and repeated each 15 days. Untreated plants for each variety and advanced line of tomato were used for comparison. All agricultural practices were applied according to pamphlet guidance of Ministry of Agriculture.

Data were recorded on the following traits; plant height in centimeter (P.H), number of branches, fruit quality traits (total soluble solids (TSS), fruit firmness, lycopene content and ascorbic acid) and yield components (fruit set %, number of clusters / plant, average fruit weight in grams and total yield/plant (kg / plant)).

Statistical analysis:

In this study, different forms of analysis of variance were employed in order to test the significance of the difference between the genotypes in each treatment In addition, a combined analysis of variance for genotypes over the treatments was made for the studied traits according to Steel and Torrie (1960). Then, it could be calculated the heritability in broad sense from the combined data according to the following formula:

$$H_b\% = (\sigma^2g/\sigma^2ph) \times 100$$

Where, σ^2ph **is phenotypic variance and calculated from the following formula:**

$$\sigma^2ph = \sigma^2g + \sigma^2gt/t + \sigma^2e/r$$

Where:

t, r, g are number of treatment, replicates and genotypes, respectively.

σ^2e, σ^2gt, σ^2g **are error, genotypes by treatments and genotypic variances, respectively.**

Based on the phenotypic data of 11 genotypes of tomato (untreated plants), Euclidian distances ED were calculated and clustering dendrogram was drawn using the statistical approach based on the Euclidian distance algorithm using XLSTAT.7 software.

Molecular diversity assessment:

Eight Start Codon Targeted Polymorphism (SCoT) primers were used to study the genetic diversity among 11 genotypes of tomato. Genomic DNA was isolated from fresh leaves by DNeasy plant mini kit (QIAGEN). DNA quality was checked using spectrophotometer and agarose gel electrophoresis. Genomic DNA was used as a template for Polymerase chain reaction (PCR) amplification using eight SCoT primers in molecular assessment for the 11 genotypes of tomato. Amplification reactions for SCoT techniques were carried out in Techni TC-512 Thermal Cycler accotding to Abd El-Aziz *et al*. (2019). Amplified products were separated on a 1.5% agarose gel with ethidum bromide and 100 bp to 3000 bp ladder markers. The run was carried out for about 30 min at 80 V in mini submarine gel BioRad. DNA banding pattern photos were photographed using Bio-1D Gel Documentation system and were analyzed by GelAnalyzer 3 software. Clear amplicons were scored as present (1) or absent (0) for each primer and entered in the form of a binary data matrix.

From this matrix, DNA profiles were performed according to Adhikari *et al*. (2015). Also, from binary data, the capability of each primer to differentiate among studied genotypes was evaluated according to Resolving power (Rp) value calculated as described by Prevost and Wilkinson (1999). According to binary data matrix, Molecular distances MD (Dissimilarity) were calculated by Dice coefficient according to Nei and Li (1979) and cluster analysis was performed agglomerative hierarchical clustering (AHC) analysis derived from Unweighted pair-group average UPGMA method using XLSTAT.7 software.

RESULTES AND DISCUSSION

Analysis of variance

The analysis of variance and the mean squares of all genotypes over treated and untreated plants for vegetative and fruit quality traits are presented in Table 1.

According to the analysis of variance, highly differences were observed among genotypes for all studied traits. The mean square of treatment was highly significant for all studied traits except of TSS. On the other hand, the magnitudes of the mean squares for genotypes by treatments interaction were non-significant for all studied traits except of lycopene and ascorbic acid.

Table 1. Analysis of variance and the mean squares of some vegetative and fruit quality traits for tomato genotypes.

S. O. V.	d.f	P.H.	N. of branches	TSS.	Firmness (inch/cm^2)	Lycopene	Ascorbic acid
Rep. /Treat.	4	26.92	1.94*	0.264*	0.470*	0.25	1.03*
Treat. (T)	1	3505.5**	16.50**	0.073	2.584**	3.71**	16.28**
Genotypes (G)	10	1489.5**	6.01**	2.754**	15.414**	45.29**	2.25**
G X T	10	61.14	1.07	0.069	0.365	0.69**	1.11*
Error	40	87.82	0.64	0.10	0.18	0.21	0.40

***,** significant at 0.05 and 0.01 levels of probability, respectively.**

The analysis of variance and mean squares for yield components traits are presented in Table 2. The results revealed that the mean square of treatments were highly significant for all traits except for no. of clusters/ plant. The mean square of genotypes has highly significant differences for all traits. While, the mean square of treatments x genotypes interactions were significant differences for all traits expect for no cluster / plant. The presence of significant differences would indicate that the comparison among the means of these genotypes would be a valid test. These results agree with Kuar *et al.* (2018) who reported that yield components showed highly significant differences among the genotypes.

Table 2. Analysis of variance and the mean squares for yield components traits of tomato genotypes.

S. O. V.	d.f	Fruit set %	No. of clusters/Plant	Average fruit weight	Total yield/ plant
Rep. / Treat.	4	22.15	2.08	10.46	0.09
Treat. (T)	1	967.92 **	8.02	1600.37**	9.45*
Genotypes (G)	10	433.89**	136.13**	1833.36**	2.83**
G X T	10	41.05*	1.58	135.45**	0.31**
Error	40	16.78	2.06	42.45	0.063

***,** significant at 0.05 and 0.01 levels of probability, respectively**

Mean performance of crosses for some vegetative and fruit quality traits at untreated and treated plants with anti-oxidant components are presented in Table 3. The results of means showed that no specific variety was superior or inferior for all studied traits. However, KL-41 was the lowest line at untreated and treated plants for plant height (77.67 and 86.67, respectively) and fruit lycopene content (1.31 and 1.24 mg/100 gm, respectively). The line KL-45 was the best for plant height at untreated and treated plants. For TSS, no significant differences were observed among the genotypes in untreated and treated genotypes.

Whereas, the genotype KL-45 was the best one for TSS and fruit firmness at all cases. The Castle Rock had the greatest value for fruit lycopene content at untreated and treated with means 8.71 and 10.64 mg/100 gm, respectively. In addition, the KL-03 had the greatest mean for treatment at ascorbic acid at treatment with mean 8.98.

Table 3. Mean performance of tomato genotypes for some vegetative and fruit quality traits

Genotypes		Plant height (cm)		N. of baranches		TSS. (%)		Firmness (inch/cm^2)		Lycopene (mg/100gm)		Ascorbic acid	
		Untreated.	Treat.	Untreated.	Treat.	Untreated.	Treat.	Untreated.	Treat.	Untreated.	Treat.	Untreated.	Treat.
Edkawy		82.00	103.7	5.33	7.67	5.01	5.07	3.13	2.93	2.61	2.65	7.84	8.77
Floradade		88.33	105.0	7.33	8.67	5.69	5.33	3.53	3.10	3.36	3.47	7.02	7.32
Castle Rock		108.7	129.3	5.67	8.00	4.33	4.40	3.97	3.50	8.71	10.64	5.85	7.15
S. marmande		105.3	121.3	6.67	7.00	4.35	4.07	5.43	4.47	7.18	7.78	6.30	8.85
NC1 CELBR		89.00	101.7	6.67	6.67	4.49	4.37	3.53	2.93	5.34	5.10	6.87	7.00
KL-45		126.7	137.7	5.67	6.00	6.52	6.33	7.50	7.67	5.39	5.55	7.55	7.99
KL-41		77.67	86.67	5.67	7.00	5.52	5.70	3.60	2.83	1.31	1.24	5.99	6.88
KL- 46		121.0	135.0	8.33	10.00	5.18	4.80	6.59	6.80	3.19	3.40	5.90	7.63
KL- 47		104.3	108.3	7.00	7.00	4.33	4.30	5.11	4.07	5.65	6.97	7.13	6.91
KL-03		92.67	101.7	6.33	6.67	4.61	4.83	6.03	6.47	7.28	8.31	6.98	8.98
KL-52		102.3	128.0	8.00	8.67	5.12	5.23	6.03	5.33	1.35	1.48	6.84	6.81
LSD	0.05	12.42	18.85	1.37	1.36	0.49	0.61	0.81	0.63	0.84	0.73	1.01	1.15
	0.01	16.93	25.71	1.86	1.85	0.66	0.83	1.09	0.85	1.14	0.99	1.38	1.56

In general, Figure 1 illustrated the relative increase over untreated plants for some vegetative and fruit quality traits. For plant height from Edkawy, KL-52 and Castle Rock were 26.46%, 25.12% and 18.95%, respectively. While the lines KL-47 and KL-45 recorded the least relative increase over their relative untreated plants (3.84, 6.68%). A similar trend was observed by Hoda *et al.* (2016) on tomato which had taller plant estimated by 28.53% over untreated plants. Whereas, the relative increase ratio over untreated plants ranged from 0.00 to 43.90 % for number of branches per plant. In this respect, Edkawy followed by Castle Rock recorded the highest increase comparing to corresponding untreated plants (43.90 and 41.09%, respectively). On the other hand, no differences were found in number of branches among KL-47 and NC 1 CELBR. It could observed that the treatment by ATP and glycine betaine decreased the general mean performances of the treated plants comparing with untreated for both TSS and fruit firmness. Significant and positive relative increases over untreated plants were observed for lycopene content and ranged from 3.27% to 23.36%. Since the line KL-47 and Castle Rock have the highest response comparing to their relative untreated plants (23.36, 22.16%). For ascorbic acid, a great response was observed among the treated and untreated plants ranged from -3.09 to 40.48% over untreated plants. The cultivar S. Marmande gave the highest relative increase with percentage of 40.48%.

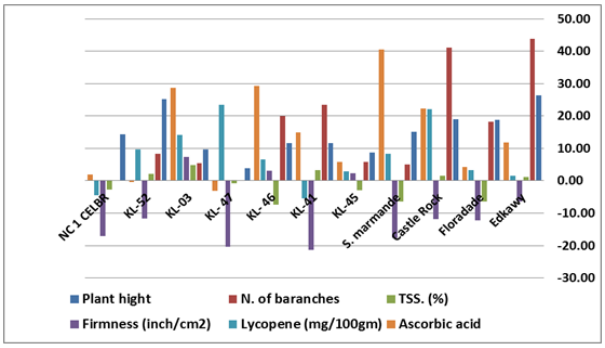


Fig .1. Relative increase over untreated plants for some vegetative and fruit quality traits

The mean performances for yield and its components are presented in Table 4. The results showed that the genotype Kl-03 was the best genotypes for fruit set (86.23) under the effect of antioxidant treatment followed by KL-45 (83.93). Regarding number of clusters per plant, the number of clusters ranged from 8.00 to 24.00 clusters per plant. Whereas, the KL-41 was the best genotype at untreated and treated cases which had the same and greatest mean value (24.00). For average fruit weight, also, there was a great variability between the tomatoes genotypes ranged from 45.00 to 124.30 gm. Since this trait represent one of the main stable characters for any variety

of tomato. Hence, comparison between treated and untreated plants for each genotype would be more reliable. Significant differences were observed among Edkawy, Floradade, S. marmande, castle rock and NC1 CELBR at treatment and their corresponding untreated plant. The genotypes S. marmande and NC1 CELBR showed the best response to antioxidant treatment regarding average fruit weight estimated by 122.30 and 124.30 g, respectively.

Table 4. Mean performance of tomato genotypes for some yield and its components traits

Genotypes		Fruit set%		No. of clusters/ Plant		Average fruit weight (gm)		Total yield/ plant	
		Untreated.	Treat.	Untreated.	Treat.	Untreated.	Treat.	Untreated.	Treat.
Edkawy		60.33	72.63	19.00	21.33	84.67	95.67	2.36	3.64
Floradade		58.67	60.83	7.33	8.00	70.00	81.33	2.06	2.49
Castle Rock		59.67	73.33	14.67	14.67	83.33	96.67	3.35	3.70
S. marmande		60.33	74.70	12.00	12.33	101.30	122.30	3.73	5.54
KL-45		80.00	83.93	12.33	14.33	88.33	89.00	2.43	3.49
KL-41		64.53	65.13	24.00	24.00	74.67	77.00	2.17	2.65
KL- 46		69.84	81.40	13.67	14.33	74.67	85.00	3.31	3.84
KL- 47		65.26	71.80	14.33	16.00	72.33	69.67	2.83	3.47
KL-03		73.80	86.23	17.00	16.33	45.00	55.00	2.02	2.48
KL-52		54.60	57.33	18.67	20.00	75.67	77.00	2.45	3.27
NC 1 CELBR		77.37	81.33	9.67	9.00	94.67	124.30	2.79	3.26
LSD	0.05	9.11	3.78	2.36	2.53	13.55	7.93	0.46	0.39
	0.01	12.43	5.16	3.75	3.44	18.47	10.81	0.63	0.53

According to Figure 2, the effect of exogenous treatment across the tomato genotypes was significant and the response was high for fruit set percentage since relative increase over untreated plants ranged from 0.93 to 23.82%.

The highest response under chilling conditions was recorded by S. Marmande followed by Castle Rock, Edkawy and KL-03. Also, moderate changes were observed in number of clusters per plant over untreated plant which ranged from -6.93 to 16.22%. This could be interpreted as the same behavior of the quantitative traits of yield components that the physiological treatments cannot affect the phenotypic performance easily. The current results showed that there was a significant increase in average fruit weight for the majority of studied tomato genotypes under chilling conditions except the line KL-47. This increase ranged from 0.76 to 31.30% over untreated plant. The best response for the exogenous treatment was recorded by NC1 CELBR which gave a relative increase estimated by 31.30% over untreated plant followed by KL-03 and S. Marmande with 22.22 and 20.73%, respectively. These findings confirmed the fact that we could improve the yield under chilling stress through improving the average fruit weight by the application of different exogenous treatments including ATP and glycine betaine. A study on the effect of climate factors on the development of tomato plants showed that high solar irradiance and excessively high air temperature restricting the development of the tomato growth, productivity and fruit quality (Rajasekar *et al*., 2013; Akindele *et al*., 2011).

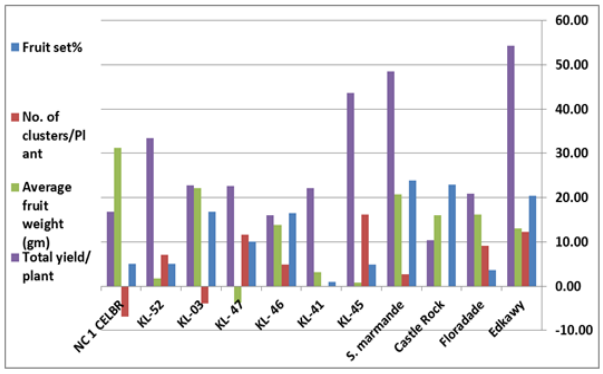


Fig .2. Relative increase over untreated plants for some yield and its components traits

Genetic parameters:

The relative magnitudes of genetic parameters were estimated for all studied traits from the combined data and the obtained results are shown in Table 5. The results revealed that the genetic variation were high and positive for all studied traits except for ascorbic acid which was low. This finding is emphasized by the heritability values, which were more than 86% for all studied traits except for ascorbic acid (43.00). In addition, the values of genetic by treatments interaction variations were positive in all studied traits except for Plant height, TSS and No. of clusters/Plant. These result in agreement with Kerketta and Bahadur (2019).

Table 5. Estimates of relative magnitudes of different genetic parameters for some vegetative, fruit quality and yield components traits in tomato genotypes

Genetic parameters	Plant height	TSS. %	Firmness	Lycopene	Ascorbic acid
σ^2g	238.06	0.45	2.51	7.44	0.190
σ^2gt	-8.89	-0.01	0.06	0.16	0.234
σ^2e	87.82	0.10	0.18	0.21	0.404
σ^2ph	262.88	0.48	2.59	7.59	0.442
H_b%	89.05	91.99	96.51	98.02	43.00
Genetic parameters	Fruit set %	No. of clusters/Plant	Average fruit weight	Total yield/plant	
σ^2g	65.47	22.42	282.99	0.42	
σ^2gt	8.09	-0.16	30.99	0.08	
σ^2e	16.78	2.06	42.45	0.06	
σ^2ph	75.11	23.03	312.64	0.48	
H_b%	87.17	97.03	90.52	87.12	

The Nile Delta region, typically characterized as highly fertile and productive agricultural land in Egypt.

Many kinds of crops and vegetables are grown in this region during summer or winter season including tomato which grows above 10 ºC. Under strong variation in temperature and precipitation conditions, the Edkawy cultivar had the most useful yield components parameters followed by S. Marmande and the line KL-45. Besides its performance under chilling conditions, the cultivar Edkawy is considered as tolerant to salinity. Moreover, this cultivar was characterized by a large number of flowers and fruits per inflorescence and average fruit weight around 90 grams. The S. Marmande cultivar had similar yield components, but its fruits could form high levels of lycopene under chilling conditions. Finally, information on the sensitivity of cultivars to weather conditions is very important for breeders for new cultivars release and also for producers selecting cultivars to grow specifically for both fresh market or for processing. Furthermore, the selection of a suitable cultivar for specific environmental and climatic conditions is of great significance for sustainable production of tomatoes.

From phenotypic data, distances among the eleven tomato genotypes were calculated using Euclidian method.

The results of Euclidian Distances (ED) matrix in Table 6 exhibited that the distances ranged from 12.88 to 58.20. The highest ED value was between the S. marmande and KL-03, whereas the lowest ED value was between the Edkawy and KL-41. Also, from ED matrix, the dendrogram of cluster analysis was performed (Figure 3). This dendrogram is divided into four groups according to the truncated line at a coefficient of dissimilarity = 27.34. The first group contained KL-03, the second group contained KL-45 and KL-46 and the third group contained S. Marmande and NC1 CELBR. The fourth group contained the other genotypes.

Table 6. Euclidian distances (ED) between 11 tomato genotypes based on dissimilarity index obtained from phenotypic data.

	Edkawy	Floradade	Castle Rock	S. marmande	KL-45	KL-41	KL-46	KL-47	KL-03	KL-52
Floradade	19.30									
Castle Rock	27.37	23.41								
S. marmande	28.12	32.47	18.23							
KL-45	46.51	42.02	25.81	31.38						
KL-41	12.88	21.28	33.62	38.74	50.82					
KL- 46	40.66	31.69	19.04	32.73	17.48	43.06				
KL- 47	26.36	16.35	13.86	28.96	28.67	28.06	16.72			
KL-03	43.72	31.46	43.48	58.20	52.28	36.03	39.66	30.51		
KL-52	23.55	18.31	14.96	27.12	35.81	27.05	23.64	13.47	37.79	
NC1 CELBR	21.84	28.26	29.99	25.09	38.00	28.53	39.05	29.96	49.65	34.00

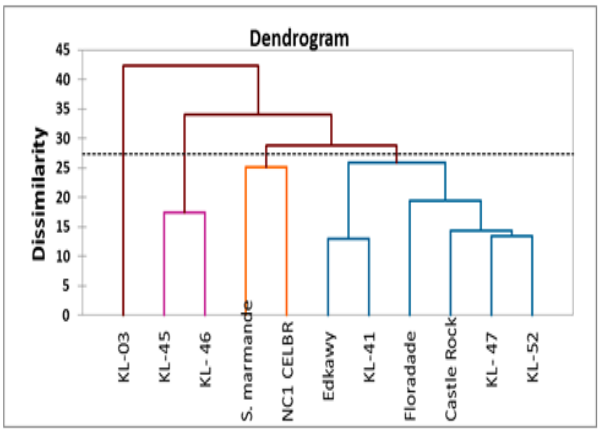


Fig .3. Agglomerative hierarchical clustering (AHC) derived by UPGMA method using Euclidean distance approach for phenotype data of eleven tomatoes genotype.

Legend: TL represents truncated line at a coefficient of Dissimilarity = 25.48

Molecular diversity assessment:

Eight SCoT primers were used to study the genetic diversity among 11 genotypes of tomato (Edkawy, Floradade, Castle Rock, Super marmande, NC-1 CELBR, KL-45, KL-41, KL- 46, KL- 47, KL-03, and KL-52) in this study. As can be seen from Table 7 and Figuer 4 the SCoT primers produced a total of 94 bands (amplicons) with an average of 11.75 bands per primer ranged from seven bands (SCoT-6 and SCoT-9) to 22 bands (SCoT-1). Among them 84 (89.4%) bands were polymorphic; of which 14 (14.9%) were unique markers (+ or -). The primer SCoT-1 showed the maximum number of 21 polymorphic bands from a total of 22 amplified bands. The overall size of amplified products ranged from 87 (SCoT-11) to 2598 (SCoT-3). Percent of polymorphism ranged from 80 to 100 %. The resolving power (Rp) provides a modest indication of the ability of SCoT primers to distinguish among genotypes. Thus, the Rp of each primer was estimated in order to determine the most informative ones for the eight primers ranged from 1.638 for primer SCoT-11 to 10.192 for primer SCoT-1 with a mean value of (4.98). Three of the SCoT primers (SCoT-1, SCoT-3 and SCoT-4) possessed high Rp values (10.19, 7.28 and 6.01, respectively) and are the most efficient for surveying genetic diversity. The highest number of unique markers was generated by primer SCoT-11 (five positive unique markers). These results were in agreement with Shahlaei *et al*. (2014). They used 10 selected SCoT primers to study the genetic diversity for ten genotypes of tomato. The SCoT primers produced a total of 83 bands and the average Rp value was 1.88.

Table 7. SCoT markers amplicons types, total number of amplicons, percentage of polymorphism and resolving power obtained by analyzing different tomato genotypes

Primer		Amplicons							
				Polymorphic					
Name	Sequence (5′→3`)	Molecular size range (bp)	Monomorphic	Polymorphic without unique	Unique +	Unique -	Total	Polymorphism %	Resolving power Rp
SCoT-1	CAACAATGGCTACCACCA	149:1091	1	20	-	1	22	95.45 %	10.19
SCoT-3	CAACAATGGCTACCACCG	91:2598	3	12	-	-	15	80.00%	7.28
SCoT-4	CAACAATGGCTACCACCT	210: 621	-	11	-	-	11	100 %	6.01
SCoT-6	CAACAATGGCTACCACGC	317: 610	-	7	-	-	7	100 %	4.37
SCoT-8	CAACAATGGCTACCACGT	293: 722	1	7	3	-	11	90.90%	4.19
SCoT-9	CAACAATGGCTACCAGCA	426:1217	1	3	2	1	7	85.71 %	2.55
SCoT-10	CAACAATGGCTACCAGCC	264:1805	2	7	1	1	11	81.82 %	3.64
SCoT-11	AAGCAATGGCTACCACCA	87: 506	2	3	5	-	10	80.00 %	1.64
Total			10	70	11	3	94		

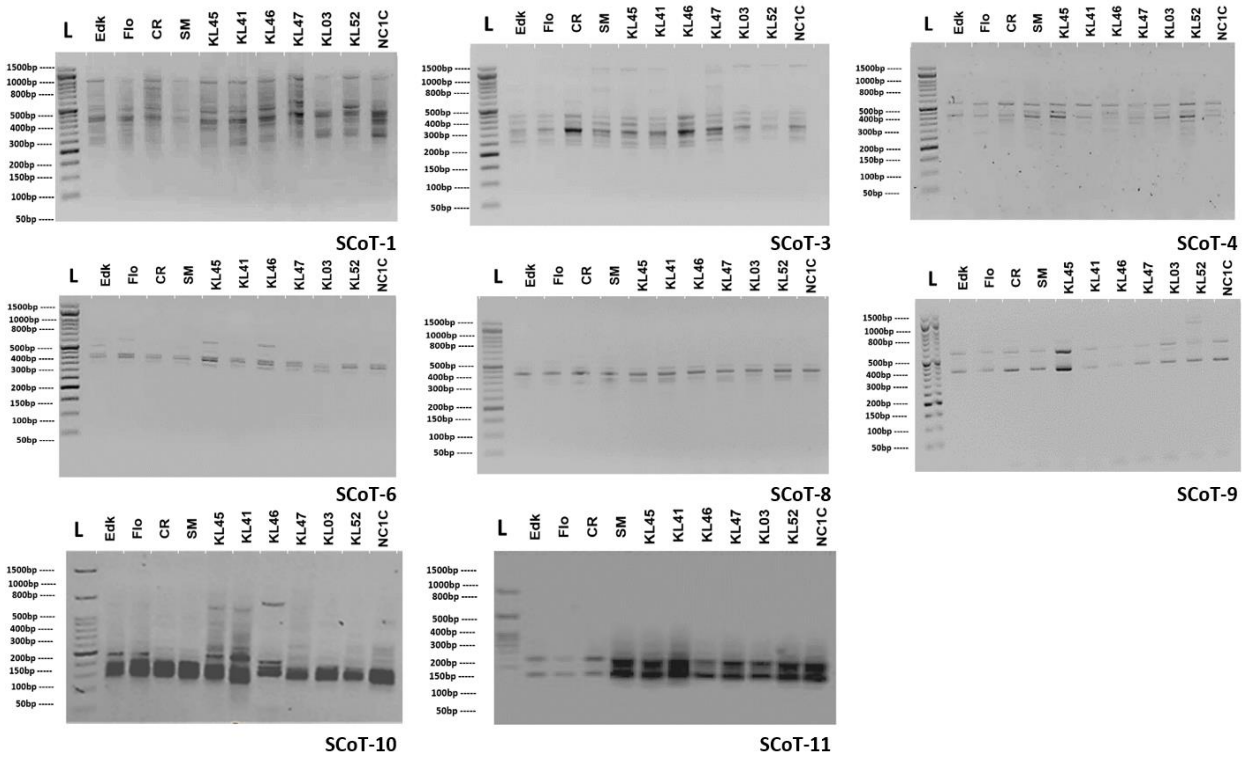


Fig. 4. Banding patterns of SCoT -PCR products for genotypes of tomato produced with eight SCoT primers. M, 1.5 kb ladder and lanes 2 to 12 represent the 11 genotypes.

Where: Edk:Edkawy, Flo: Floradade, CR: Castle Rock, SM: Super marmande, KL-45, KL- 47, KL-41, KL-03, KL-46, KL-52, and NC1C: NC1 CELBR.

Finally, it became clear that this molecular technique was efficient in terms of assessing genetic diversity among the tomato genotypes, suggesting the possibility of using results of this technique in signing genetic fingerprinting for these genotypes. Therefore, these genetic fingerprints for all genotypes of tomato were performed as DNA-profile diagram (Figure 5) based on 94 amplicons obtained using SCoT technique. This profile showed that the amplicons per lines were variously ranged from 32 (for KL-03) to 57 (for KL-45). In addition, among the 11 genotypes, seven (Edkawy, KL-45, KL-41, KL-46, KL-47, KL-52 and NC1 CELBR) were characterized by 14 unique markers (11 positive and 3 negative). Edkawy, KL-46 and NC1 CELBR are characterized by the negative markers, while KL-41 and KL-52 had the highest numbers of positive markers (three for each one). These markers were spread over these lines variously differentiate each line from the other. These unique amplicons may be useful as unique markers as explained by Abd El-Aziz *et al*. (2016) in tomato, Abd El-Aziz and Habiba (2016) in canola and Abd El-Hadi *et al*. (2017) in squash. These results indicated that DNA-profiling diagram also is a useful tool for molecular identification for these studied genotypes. So, it was deduced that SCoT primers used in this study were with high degree of confidence for the molecular identification.

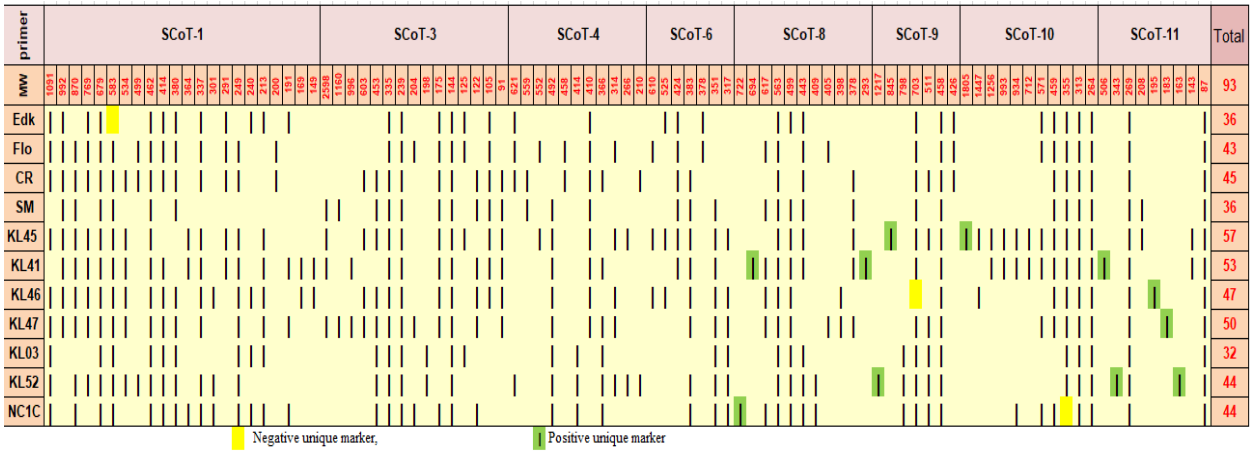


Fig. 5. DNA-profile representation of SCoT fingerprints of 11 tomato genotypes.

Where: Edk:Edkawy, Flo: Floradade, CR: Castle Rock, SM: Super marmande, KL-45, KL- 47, KL-41, KL-03, KL-46, KL-52, and NC1C: NC1 CELBR

According to Table 8, the molecular distance (MD) between all studied genotypes based on SCoT ranged from 0.215 to 0.482. The highest molecular distance (MD) was among KL-41 and KL-03 (0.482). While the lowest MD according to the same data was between Floradade and Edkawy (0.215) followed by KL-46 and KL-47 (0.216). This means that KL-41 and KL-03 were the best genotypes that can be used in breeding programs.

Table 8: Molecular distances (MD) matrix for tomato genotypes based on SCoT markers

Similarity	Edk	Flo	CR	SM	KL45	KL41	KL46	KL47	KL03	KL52
Flo	0.215									
CR	0.309	0.227								
SM	0.389	0.329	0.284							
KL45	0.376	0.360	0.333	0.333						
KL41	0.371	0.369	0.327	0.303	0.236					
KL46	0.373	0.378	0.326	0.325	0.288	0.320				
KL47	0.395	0.355	0.284	0.279	0.290	0.262	0.216			
KL03	0.382	0.467	0.429	0.412	0.438	0.482	0.392	0.366		
KL52	0.475	0.425	0.326	0.450	0.406	0.464	0.363	0.340	0.237	
NC1C	0.400	0.425	0.393	0.375	0.386	0.340	0.319	0.255	0.237	0.273

Where: Edk:Edkawy, Flo: Floradade, CR: Castle Rock, SM: Super marmande, KL-45, KL- 47, KL-41, KL-03, KL-46, KL-52, and NC1C: NC1 CELBR

Based on the UPGMA clustering algorithm from SCoT markers, the 11 genotypes of tomato were grouped into three major clusters (Fig. 6) according to the truncated line at a coefficient of dissimilarity = 0.333. The first cluster was divided into two sub-clusters. The first sub-cluster consisted of one genotype (NC1 CELBR.) and the second sub-cluster consisted of two genotypes (KL-03 and KL-52). While, the second major cluster was divided into two sub-clusters. The first sub-cluster consisted of one genotype (Castle Rock) and the second sub-cluster consisted of two genotypes (Edkawy and Floradade). The third major cluster was divided into three sub-clusters, the first consisted of one genotype (Super marmande), the second sub-cluster consisted of two genotypes (KL-46 and KL-47) and the third sub-cluster consisted of two genotypes (KL-45 and KL-41).

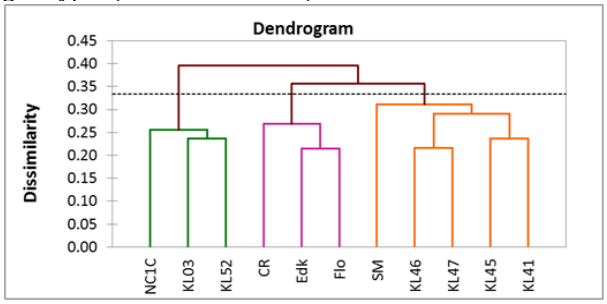


Fig. 6. Dendrogram derived by UPGMA method using Dice-dissimilarity coefficient for binary data of SCoT technique for eleven tomato genotypes.

Legend: TL represents truncated line at a coefficient of Dissimilarity = 0.333

Relationship between phenotypic distances and molecular distances:

by calculating the correlation coefficients among Euclidian distances based on phenotypic data and molecular distances based on SCoT, insignificant positive correlation (r = 0.131) was found between them. In harmony with this result, a poor correlation between phenotypic and molecular distances was found by (Sant *et al*., 1999; Yadav *et al*., 2010 and Abd El-Aziz *et al*., 2016). The poor correlation among two types of genetic distances can be explicated by the fact that all studied genotypes had been evaluated at a one location apart from evaluated under different climatic conditions.

In conclusion, the molecular analysis of the tested tomato genotypes has shown clear differences at the molecular level among these genotypes. Also, these differences were demonstrated by the performance of these genotypes under chilling stress and untreated plants. Also, the genetic analyses using SCoT markers would be more useful for tomato improvement programs,

REFERENCES

Abd El-Aziz M. H; S. Y. Mohamed and Hadeer E. Magwaid (2019). Molecular and phytochemical assessment for some seedy strains of Alamar apricot rootstock under salinity stress. Egyptian Journal of Basic and Applied Sciences, 6 (1): 173–186.

Abd El-Aziz M.H. and Rehab M.M. Habiba (2016). Molecular assessment of genetic diversity in some canola homozygous lines. Egypt J Genet Cytol., 45: 129 –145.

Abd El-Aziz M.H.; SM. Farid and A.A., Sara (2016). Evaluation of molecular and phenotypic diversity in relation to heterosis in some tomato lines under different climatic conditions. J Agric Chem Biotechn., 7(5): 141–151. Mansoura Univ.

Abd El-Hadi A.H.; M.H. Abd El-Aziz and M.A. Abd Alla (2017). Molecular and phenotypic evaluation of some summer squash inbred lines. J Agric. Chem. Biotechn., 8 (12): 581–587. Mansoura Univ.

Adhikari S.; S. Saha and TK. Bandyopadhyay (2015). Efficiency of ISSR marker for characterization of Cymbopogon germplasm and their suitability in molecular barcoding. Plant Syst. Evaluation. 301:439 – 450

Akindele A.J.; D.J. Ogunniyan and E.O. Ajayi (2011). Phenotypic relationship among agronomic characters of commercial tomato (Lycopersicum esculentum) hybrids. American-Eurasian Journal of Agronomy 4 (1): 17-22

Atherton J.G. and J. Rudich (1986). The Tomato Crop: A Scientific Basis for Improvement. Chapman and Hall, London and New York, Pp 661.

Bita, C.E. and T. Gerats (2013). Plant tolerance to high temperature in a changing environment: Scientific fundamentals and production of heat stress tolerant crops. Plant Sci. Vol 4, article 273:1-18

Collard B.C.Y. and D.J. Mackill (2009). Start Codon Targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mole. Biol. Repo., 27(1): 86 – 93

Hoda I. Ahmed ; Abeer I. Shabana and A. Y. Elsayed (2016). Chilling tolerance enhancement in tomato (*Solanum lycopersicum*) plant by spraying of some anti-stress compounds. Journal of Plant Production, 7 (2):185 - 195.

Kaur S.; A. K. Singh; Sreshti Bagati; Mamta Sharma and Satish Sharma (2018). Morphological Markers based Assessment of Genetic Diversity in Cultivated Tomato (*Solanum Lycopersicon* L.) Genotypes. International Journal of Environment, Agriculture and Biotechnology (IJEAB), 3(2): 567 - 573

Kerketta A. and V. Bahadur (2019). Genetic Variability, Heritability and Genetic Advance for Yield and Yield Contributing Characters in Tomato (*Solanum lycopersicum* L.) Genotypes. *Int. J. Pure App. Biosci.*, 7 (3): 577-582.

Li, Y.; Wang, H. and Y. Zhang, (2018). Can the world's favorite fruit, tomato, provide an effective biosynthetic chassis for high-value metabolites? Plant Cell Rep. https://doi.org/10.1007/s00299-018-2283-8

Nahar, K. and S.M. Ullah (2012). Morphological and physiological characters of tomato(*Lycopersicon esculentum* Mill) cultivars under water stress. Bangladesh J. Agr. Res., 37(2):355–360.

Nei M. and WH. Li (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci, USA.; 76: 5269–5273

Prevost A. and M.J. Wilkinson (1999). A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theo. App. Genet.; 98:107–112.

Rajasekar M.; T. Arumugam and S. Ramesh Kumar (2013). Influence of weather and growing environment on vegetable growth and field. Journal of Horticulture and Forestry 5 (10): 160-167. DOI: 10.5897/JHF2013.0317

Sant, V. J.; A. G. Patankar; N. D. Sarode; L. B. Mhase; M. N. Sainani; R. B. Deshmukh; P. K. Ranjekar and V. S. Gupta (1999). Potential of DNA markers in detecting divergence and in analysing heterosis in Indian elite chickpea cultivars. Theor.Appl.Genet., 98:1217-1225.

Shahlaei A.; S. Torabi and M. Khosroshahli (2014). Efficiency of SCoT and ISSR markers in assessment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. International Journal of Biosciences, 5(2): 14-22.

Steel R.G.D. and J.H. Torrie (1960). Principles and Procedures of Statistics with Special Reference to the Biological Sciences. McGraw Hill, New York, 187-287.

Talebi R; R. Fayaz; M. Mardi; SM. Pirsyedi and AM. Naji (2008). Genetic relationships among chickpea (Cicer arietinum) elite lines based on RAPD and agronomic markers. Int J Agric Biol 8:301–305.

Tester, M. and P. Langridge, (2010). Breeding technologies to increase crop production in a chworld. *Science* 327, 818-822.

Yadav, K.; B. D. Singh; C. P. Srivastava; R. Chand and A. Yadav (2010). Analysis of genetic divergence in pea (*Pisum sativum* L.) using quantitative traits and RAPD markers.IndianJournalofGenetics,70 (4): 363-369

تحسين تحمل البرودة وكفاءة استخدام تكنيك SCoT فى التقييم الجزيئى في بعض التراكيب الوراثية للطماطم.

رحاب محمد محمد حبيبة [1] ، احمد يوسف السيد [2] و حسناء احمد صالح

[1]قسم الوراثة ـ كلية الزراعة ـ جامعة المنصورة ـ مصر

[2]قسم بحوث الخضر- معهد بحوث البساتين-مركز البحوث الزراعية مصر.

يحدث انخفاض كبير في محصول الطماطم وجودة الثمار سنويًا في مصر خلال موسم الشتاء وأوائل الصيف نتيجة لإجهاد البرودة. في هذه الدراسة ، تم تقييم مجموعة من سلالات وأصناف الطماطم لاستجابتها لكل من ATP و glycine betaine لتحمل البرودة و ذلك لانتخاب التراكيب الوراثية الواعدة لمزيد من برامج التربية. أجريت التجربة الحقلية بمزرعة قسم الوراثة بكلية الزراعة جامعة المنصورة اثناء موسم الشتاء فى تصميم القطاعات كاملة العشوائية بثلاث مكررات. وتم تسجيل البيانات على بعض الصفات الخضرية و جودة الثمار ومكونات المحصول. وأظهرت النتائج أن الصنف Edkawy كان أكثر تأثرا و فاعلية للمعاملة فى صفات مكونات المحصول يليه الصنف S. Marmande والصنف KL- 45 . كما تميز هذا الصنف بوجود عدد كبير من الثمار في كل نورة وكان متوسط وزن الثمرة حوالي 90 جرام .تم إجراء التقييم الجزيئى باستخدام تقنية SCoT لإحدى عشر تركيب وراثى من الطماطم. أنتجت بادئات SCoT المستخدمة 94 حزمة رئيسية بمتوسط 11.75 حزمة/ بادىء , تراوح عددها من سبعة لكل من SCoT-6 و SCoT-9 إلى 22 للبادىء SCoT-1 . وكانت أكبر مسافة جزيئية (MD) موجودة بين KL-03 و KL-41 (0.482) بينما كانت أقل مسافة جزيئية (MD) بين Floradade و Edkawy (0.215) يليها بين KL- 47 و KL- 46 (0.216) . كما اعطى الأحدى عشر تركيب وراثى 14 حزمة (marker loci), هذه الـ marker موزعة على هذه التراكيب الوراثية ومختلفة من تركيب وراثى لاخر.