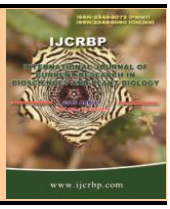
EXCELLENT PUBLISHERS

International Journal of Current Research in Biosciences and Plant Biology

ISSN: 2349-8080 Volume 2 Number 4 (April-2015) pp. 128-142

www.ijcrbp.com



Original Research Article

Horticultural and Genetical Characteristics of Tomato Somaclones under Salt and Heat Stresses

A.N. El-Banna*, S.A. Dora, A.A. Aboshosha and Nada A. El-Morsy

Genetics Department, Faculty of Agriculture, Kafrelsheikh University, 33516, Egypt

**Corresponding author.*

Abstract

Tissue culturability response of tomato cv. Super Strain B and Riogrand were analyzed *in vitro* to investigate the effect of different plant growth regulators and explant type on callus induction and plant regeneration characters Moreover, horticultural and fruit quality, biochemical (protein) and molecular (SSR) characterization of Super Strain B-induced somaclonal variants under salt and heat stresses were studied. The percentage of callus induction varied depending on the genotype and plant growth regulators. Highly significant differences between the tested genotypes were observed. The genotype Riogrand exhibited higher percentage of callus formation (79.51%) over Super Strain B (68.66%). The percentage of plant regeneration varied according to the explant types and plant growth regulators. The cotyledonary explants showed the highest value (2.31) followed by leaves (1.56) and hypocotyls (0.33). The majority of somaclones represented significant values over their parental genotype for total yield, number of branches, early yield, average fruit weight, fruit set, fruit firmness, total soluble sugar content and vitamin C content. Molecular and biochemical analysis using SSR and protein markers confirmed the existence of genetic variation between the produced somaclones and their parental cultivar. The percentages of polymorphism were 100 and 88.8% for SSR and protein markers, respectively. Furthermore, some somaclones possessed some bands which were absent in the parent and could be related to heat and salt stress. The majority of these somaclonal variants had some desirable traits and could be used to assist breeders in selecting heat and salt tolerant cultivars and nutritional quality.

Keywords

Salt and heat tolerance

Somaclonal variation

SSR markers

Tomato

Yield component

Introduction

Tomato (*Solanum lycopersicum* Mill.), 2n=2x=24; is the most important vegetable crop in the world after potato, being cultivated in many regions of the world. In terms of medical, science it encompass antioxidant lycopene (alkaloids) a health promoting compounds whose consumption reduces the incidence of many types of cancer (Rao et al., 2000; Pohar et al., 2003). It is one of the most important protective foods as it

possesses appreciable quantities of vitamins and minerals and sometimes rightly referred to as poor man's orange (Devi et al., 2008). Tomato was also used as bioreactor in biopharming for the production and oral delivery of vaccines (Jiang et al., 2007) and as functional food for cancer prevention (Buttelli et al., 2008). Tomato plant growth was shown to be moderately sensitive or moderately tolerant to salinity depending on cultivar or growth stage (Santa-Cruz et al., 2002). Soil salinization is becoming a serious threat to world agriculture (He et al., 2009). Salt stress affects many aspects of plant metabolism and as a result, growth and yields are reduced (Munns, 2005). In addition, heat stress is the rate limiting abiotic factor responsible for reducing tomato yield in Mediterranean and tropical countries. Tomato production under high temperature conditions, such as the summer in Egypt, sharply reduces quality and yield. For instance, low fruit setting, reduction in the flower fertilization rate, decrease in the lycopene content and high level of evaporation are all affiliated with high temperature stress (Hall and Ziska, 2000; Hall, 2001). Selection for heat tolerance under field conditions provides general data to identify potential tolerant germplasm (Hall, 2001). In this investigation using tissue culture for obtaining genetic variations and evaluating obtained plants under salinity and heat conditions is one of its aims for improvement tomato production.

In vitro techniques are important tools for modern plant improvement programs to produce virus free plants (Moghaleb et al., 1999), to introduce new traits into selected plants, to multiply elite selections, and to develop suitable cultivars in the minimum time (Taji et al., 2002). The occurrence of genetic variation among plants regenerated from *in vitro* culture has been referred to as somaclonal variation (Rani and Raina, 2000). Variation in chromosome numbers and structures, and chromosome irregularities (such as breaks, acentric and centric fragments, ring chromosomes, deletions and inversions) are observed during *in vitro* differentiation and among regenerated somaclones (Mujib et al., 2007).

In vitro plant regeneration has been found to depend on many factors, of which most important are: composition of the basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels and vessel covers (Bhatia et al., 2004). During the last four decades, significant advances have been made in the development of *in vitro* culture techniques, which have been extensively applied to different crop species (Harish et al., 2010). Targets of somaclonal variation production include enhancement in productivity by increasing resistance to abiotic and biotic stresses as well as fundamental studies such as identification and characterization of key regulatory genes. Any biotechnological approaches have been focused on the improvement of tomato crop, which can grow in different agro climatic zones to meet the demands (Mandel and Sheeja, 2003). Molecular techniques such as microsatellites or simple sequence repeats (SSRs) are often favored over traditional phenotypic or cytological measurements, and generally assess even small variations of the genome. Microsatellites or simple sequence repeats (SSRs) have played an important role in genome evolution (Toth et al., 2000). SSRs have become a popular type of co-dominant molecular marker in genetic analysis and plant breeding application (Cho et al., 2000) and provided breeders and geneticists with efficient tool to link phenotypic and genotypic variations (Temnykh et al., 2001). One of the biochemical methods extensively used in taxonomic and assessment of genetic diversity studies is the electrophoretic analysis of the proteins. These proteins are physiologically stable and easy to handle (Ladizinsky and Hymowitz, 1979). They operate at the level of gene product where the environment has very little influence (Feldman and Sears, 1981). The aim of this study was to increase somatic variations of two tomato cultivars and detection of these variations using SSR and protein markers. This variation can be used as a valuable tool for selection of plants for desirable traits such as salt and heat tolerance in tomato breeding programs.

Materials and methods

Seed germination

Seeds of the fresh market cultivars of tomato, Riogrande and Super Strain B" were washed with continuously running tap water for 15 min. Under laminar flow cabinet, seeds were surface sterilized for 1 min in 70% (v/v) ethanol, followed by immersing seed for 20 min in 20% solution of commercial bleach (Clorox) containing 5.25% sodium hypochlorite and then washed five times with sterile distilled water. Ten seeds were directly transferred to each glass gar with 25 ml of half-strength MS medium (Murashige and Skoog, 1962) supplemented with 1.5% sucrose and

incubated at 25°C in the dark till full germination and later transferred to a 16 h photoperiod at 25 ± 2°C.

Somaclonal variants production

Callus induction and plant regeneration

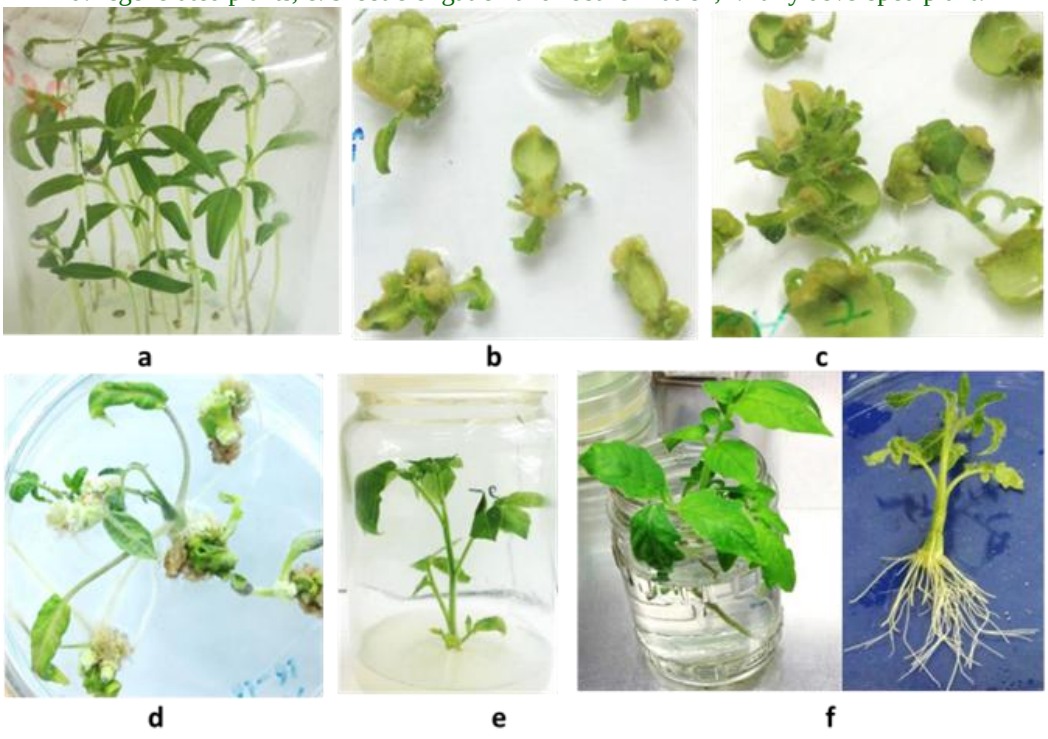
Cotyledons from 10-12 days old seedlings, leaf explants (0.5 cm^2) and hypocotyls (0.5 to 1.0 cm long) from 15-17 days old seedlings were aseptically excised. Cotyledons and leaf explants were placed in abaxial orientation (downside of the leaf touching the medium) and hypocotyls were placed horizontally on the surface of the medium. The Petri dishes were incubated at 25±2ºC under 16/8 h photoperiodic regime for four to five weeks. Nine MS media (MS_1 , MS_2 , MS_3 , MS_4 , MS_5 , MS_6 ,MS_7 , MS_8 and MS_9) varied according to their combination and concentration of plant growth regulators were used in this study (Table 1). All of the used culture media were solidified with 0.8% agar and their pH was adjusted to 5.7-5.8 before autoclaving at 121ºC. After four to five weeks of incubation, the mean percentage of formed calli, and mean numbers of regenerated shoots/explant which have been developed on the nine MS media were recorded. Shoots from callus were separated; callus was removed and planted again on full MS medium for shoot elongation and rooting (Fig. 1).

Table 1. Media used for *in vitro* culture of tomato and their content of plant growth regulators.

Medium code	Composition
MS1	MS + 1 mg/l BAP + 0.1 mg/l NAA
MS2	MS + 2 mg/l BAP + 0.2 mg/l NAA
MS3	MS + 4 mg/l BAP + 0.4 mg/l NAA
MS4	MS + 1 mg/l BAP + 1 mg/l kin
MS5	MS + 2 mg/l kin + 0.1 mg/l NAA
MS6	MS + 5 mg/l BAP + 0.2 mg/l IAA
MS7	MS + 2.5 mg/l BAP + 0.2 mg/l IAA
MS8	MS + 2 mg/l BAP + 0.5 mg/l IAA
MS9	MS + 1 mg/l zeatin + 0.1 mg/l IAA

Fig.1: Steps of callus induction and plant regeneration.

a: 10-12 days old seedling, b: callus induction and shoot forming from explants, c: shoot growth and multiplication, d: regenerated plants, e: shoot elongation and root formation, f: fully developed plant.



Root formation and acclimatization

Shoots were separated and transferred to the rooting medium that consisted of MS basal medium without plant growth regulators. Rooted shoots were removed from the jars and washed thoroughly to remove any traces of agar and then transferred to pots containing sterile vermiculite, sand and soil (1:1:1). After sprinkling water, the pots were covered with clear plastic bags to avoid evaporation as well as transpiration that were removed after making holes each day till six days. Acclimatized plants (Fig. 2) were transferred to greenhouse conditions and fruits were harvested from the good performance 19 plants.

Fig.2: Acclimatization stage. a: package plants, b: tomato plants after removing polyethylene bags.

Field evaluation of tomato somaclones

Nineteen somaclone families were evaluated along with their original cultivar (Super Strain B) in the summer season from May to September, 2014 at Experimental Farm of the Horticulture Department Faculty of Agriculture, Kafrelsheikh University. Temperature and relative humidity were measured by EASYLOG USB-2 (RH/Temp data logger -35ºC to 80ºC, humidity). Soil surface sample (0-25cm) was analyzed (chemical) following the procedure detailed by USDA (2004). The experiment was arranged in randomized complete blocks design with three replications. The seeds of genotypes were sown in nursery in seedling trays on April. The seedlings were transplanted on May. The agricultural practices were executed according to the recommendations for a commercial tomato crop under the same region (Fig. 3). Five plants from each experimental unit were randomly chosen for horticultural traits analysis.

Genomic DNA extraction

For DNA extraction, approximately 150 mg fresh leaves of eighteen randomly selected Super Strain B regenerants and their corresponding parent were grounded using pestle and mortar with liquid nitrogen and total genomic DNA was extracted according to modification of (Walbot and Warren, 1988). The genomic DNA samples were adjusted to 30 ng/µl concentration with ddH_2O and subjected to PCR analysis.

PCR amplification conditions

Five specific SSR primers were used for the analysis of genetic similarity between the original cultivar (Super Strain B) and the 18th somaclonal variants. Amplification reactions were performed in MJ Mini Bio RAD, thermal cycler. PCR reactions were carried out in a total volume of 20µl, the PCR reaction mixture

consisted of 1µl (30 ng) template DNA, 2 µl (10x PCR buffer), 2 µl (dNTPs, 2mM), 1.6 µl (MgCl2, 25mM) 1µl (10 ng of SSR primers), 0.125 (*Taq* polymerase, 5U/µl). Template DNA was initially denatured at 95°C for 5 min, followed by 35 cycles under the following parameters, denaturation for 1 min at 95°C, annealing at 52°C for 30 sec and primer extension for 1 min. at 72°C. A final incubation for 7 min at 72°C was performed to ensure that the primer extension reaction was preceded to completion.

Fig. 3: Field experiment.

a: plants in vegetative stage, b: plants in fruiting stage, c: individual plants from different somaclones in fruiting stage.

SSR data analysis

The PCR amplified products were separated by electrophoresis on 2% agarose gels using 0.5 X TBE buffer. The gels were stained with ethidium bromide and photographed by gel documentation system (UVITEC, UK). The experiment was repeated for 3 times and reproducible SSR bands were used for further analysis. The amplified bands were scored for each SSR marker based on the presence or absence of

bands, generating a binary data matrix of 1 and 0 for each marker system. Matrix was then analyzed using the PAST, ver. 1.90 (Hammer et al., 2001). Data matrix was used to calculate genetic similarity based on Jaccard's similarity coefficients (Jaccard, 1908).

Biochemical analysis

Protein extracts were prepared from young leaves. Homogenates were obtained by mechanically grinding with sucrose 20%. Protein extracts were centrifuged at 14,000 rpm for 10 min at 4ºC and apply in 12% polyacrylamide gels according to Laemmli (1970), and stained by Coomassie blue dye.

Statistical analysis

The analysis of variance (ANOVA) was used to test the differences between the performances of genotypes, explants and different media (Snedecor, 1962).

Results and discussion

Callus induction and plant regeneration

Cotyledons from 10-12 days old seedlings, leaf explants and hypocotyls from 15-17 days old seedlings were cultured on MS medium supplemented with various combinations of plant growth regulators (Table 1). Mean squares for the percentages of callus formation for two tomato genotypes and three types of explants on nine different media are presented in Table 2. Results revealed highly significant differences between the genotypes and the used media. The data also revealed that there were highly significant differences in the interaction between genotypes and explant types, genotypes and media used and between genotypes, explant types and the used media. No significant differences were found between explants and in the interaction between explant types and the used media.

Table 2. Mean squares for the percentages of callus formation (C%) and number of shoots per explant for two genotypes and three explants cultured on nine different culture media.

Source of variation	D.F.	MS	
		Callus formation %	No. Shoots/explant
Genotypes (G)	1	4762.12092**	4.11526^{ns}
Explants (E)	2	404.03^{ns}	54.06716**
Media (M)	8	10184.4**	25.87269**
G × E	2	1633.44222**	0.51336^{ns}
G × M	8	2520.02647**	5.47150**
E × M	16	301.94314^{ns}	7.09773**
G × E x M	16	2116.32277**	1.21603^{ns}

**, * and ns indicate significant differences at $p<0.01$, $p<0.05$ and not significant, respectively, according to F test.

Table 3. Mean percentages of callus formation for two tomato genotypes and three types of explants cultured on nine different media.

Media	Supper Strain B			Riogrand			Mean M
	Explant						
	Cotyledons	Leaves	Hypocotyls	Cotyledons	Leaves	Hypocotyls	
1	75.56 a-e	95.00 ab	90.00 abc	100.00 a	100.00 a	100.00 a	93.43 a
2	100.00 a	100.00 a	100.00 a	100.00 a	93.33 abc	86.67 a-d	96.67 a
3	100.00 a	100.00 a	100.00 a	93.33 abc	83.33 a-d	100.00 a	96.11 a
4	0.00 m	6.667 lm	23.33 i-m	63.33 d-g	86.67 a-d	13.33 k-m	32.22 d
5	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a	100.00 a
6	30.00 h-l	20.0 j-m	30.00 h-l	93.33 abc	86.67 a-d	53.33 e-h	52.22 c
7	33.33 h-k	26.67 i-l	90.00 abc	96.67 ab	80.00 a-d	40.00 g-j	61.11 bc
8	70.00 b-f	66.67 c-f	80.00 a-d	73.33 a-e	80.00 a-d	36.67 h-k	67.78 b
9	93.33 abc	90.00 abc	33.33 h-k	40.00 g-j	46.67 f-i	100.00 a	67.22 b
G × E	66.91	67.22	71.85	84.44	84.07	70.00	
Mean G	68.66 b			79.51 a			
Mean E	75.68		75.65		70.93		

G-Genotypes; E-Explants; M-Media; Values having the same alphabetical letter within each factor are not significantly different at the 5% level, according to Duncan's test.

For plant regeneration, the results in Table 2 revealed highly significant differences between explant types and the used media. Moreover, highly significant differences in the interaction between explant types and the used media as well as genotype and media used. While no significant differences were found between genotypes, the interaction between genotypes and explant types and the interaction between genotypes, explant types and the used media. The percentages of plant regeneration varied according to the explant types and plant growth regulators. The genotypes Riogrand and Super Strain B gave values of 1.56 and 1.24 shoots per explant, respectively (Table 4). The cotyledonary explants showed the highest value of 2.31 followed by leaves (1.56) and hypocotyls (0.29). MS9 medium gave the highest mean for no. of shoots per explant value (3.37) followed by the values of MS8, MS7, MS4 and MS6 media (2.47, 2.12, 1.88 and 1.78, respectively), while MS5, MS3, MS2 and MS1media gave the lowest values (0.00, 0.10, 0.22 and 0.64), respectively. The best response for regeneration was given when cotyledonary explants from Riogrand genotype were cultured on MS9 medium (6.43). These findings were in agreement with those of Mirghis et al. (1995) and Jabeen et al. (2005), who reported that the *in vitro* reactions of genotypes for regeneration ability were dependent on the culture medium. A combination of phytohormones (cytokinins and auxins) has been reported to determine the course of morphogenesis, including shoot organogenesis in tomato cultivars (Chaudhry et al., 2004 and 2010; Sheeja et al., 2004; Afroz et al., 2009). The cytokinin BA promotes cell division, shoot multiplication and auxiliary bud formation (Sutter, 1996). The necessity of cytokinin for shoot initiation is well established (Osman et al., 2010). Many reports suggested the use of zeatin as a primordial phytohormone for regeneration, alone or in combination with auxin. Cotyledons gave better shoot regeneration than hypocotyl explants, indicating that the cotyledonary tissue of tomato is an excellent explant for plant regeneration. Previous studies demonstrated that the cotyledons of tomato were superior to other sources of explants, including hypocotyls, stems and leaves for promoting shoot organogenesis in tomato (Hamza and Chupeau, 1993 and Osman et al., 2010).

Table 4. Mean number of shoots / explant for two tomato genotypes and three types of explants cultured on nine different media.

Media	Genotype						Mean M
	Supper Strain B			Riogrand			
	Explant						
	Cotyledons	Leaves	Hypocotyls	Cotyledons	Leaves	Hypocotyls	
1	2.53	0.40	0.00	0.93	0.00	0.00	0.64 c
2	0.47	0.60	0.00	0.00	0.27	0.00	0.22 c
3	0.47	0.00	0.00	0.00	0.13	0.00	0.10 c
4	2.40	4.10	0.43	2.05	2.00	0.27	1.88 b
5	0.00	0.00	0.00	0.00	0.00	0.00	0.00 c
6	1.72	3.32	0.00	2.25	2.97	0.40	1.78 b
7	4.57	0.83	0.20	5.07	2.07	0.00	2.12 b
8	3.98	0.87	0.40	6.22	3.13	0.20	2.47 ab
9	2.47	2.23	1.43	6.43	5.10	2.53	3.37 a
G x E	2.07 ab	1.37 b	0.20 c	2.55 a	1.74 b	0.38 c	
Mean G	1.24			1.56			
Mean E	2.31 a		1.56 b		0.29 c		

G-Genotypes; E-Explants; M-Media; Values having the same alphabetical letter within each factor are not significantly different at the 5% level, according to Duncan's test.

Horticultural and fruit quality characters

Horticultural characters and fruit quality characters for nineteen somaclones were evaluated along with their original cultivar (Super Strain B) in the summer season from May to August, 2014. The mean temperature and relative humidity for the agriculture region in this period recorded very high temperature in this summer season (Table 5). Soil chemical analysis showed that salinity value was 9.87 dSm^{-1} in soil past, which was considered as very high saline soil (Table 6). This salinity stress affected tomato growth and yield. Data in Table 7 illustrated that, all somaclones had low number of nodes below the first cluster per plant

compared to the parent. The lowest value (5.8) was obtained from somaclones 4 and 15. Regarding plant height, significant variations were observed among somaclones. The highest value (84.6 cm) was obtained from somaclone 8, while the lowest values (63.2 cm and 63.8 cm) were obtained by somaclone 4 and the original cultivar, respectively. Regarding number of branches per plant, significant variations were observed among the genotypes. The highest values (8.6 and 8.4) were obtained by somaclones 17 and 14, respectively. Differences in number of branching in the mutants may be due to its genetic potential. The results concerning plant height and number of branches per plant, as vegetative growth characters, are in a harmony with earlier reports of genotypic variations with regard to both traits by (Mehta and Asati, 2008; Singh and Asati, 2011; Solieman et al., 2013) who found significant differences among tomato genotypes for both traits.

Table 5. The mean maximum temperature (MAX T.), minimum temperature (MINI T.), maximum relative humidity (MAX RH) and minimum relative humidity (MINI RH) in the experimental farm region from May to August, 2014.

Month (2014)	MAX T. (^{o}C)	MINI T. (^{o}C)	MAX RH (%)	MINI RH (%)
May	37.56	17.32	86.74	31.40
June	36.70	20.11	90.83	39.85
July	37.59	21.53	92.72	45.50
August	38.75	22.59	92.17	45.22

Table 6. Soil physic-chemical characteristics of the cultivation area.

pH (1:1)	EC dSm^{-1} Soil past	*SAR	meqL^{-1}							
			Na	Mg	Ca	K	CO_3	HCO_3	Cl	SO_4
6.89	9.87	10.55	50.06	22.87	21.99	3.78	0.0	3.34	39.07	56.29
*SAR-Sodium absorption ratio.										

Pollen fertility percentage recorded highly significant differences among the tested genotypes. The highest value (88.9%) was obtained from somaclone 10; in contrast, the lowest value (11.1 %) was obtained from somaclone 4, while the original cultivar gave 58.3% (Table 7 and Fig. 4). Regarding fruit set percentage, significant variations were observed among the genotypes. The highest values (91.6 and 91.5%) were obtained from somaclones 2 and 12, respectively, while the lowest value (47.9%) was recorded by somaclone 4. Significant variations were observed among all genotypes for early yield. The highest value (971.8 g/plant**)** was obtained from somaclone 13, while the original cultivar recorded 267.4 g/plant. Regarding total yield, highly significant variations were observed among all genotypes. Except somaclones 1 and 4, all somaclones surpassed the original parent by at least two folds for this trait. The highest value (1445.1 g/plant) was obtained from somaclone 2. Many investigators reported genetic variations among tomato cultivars and hybrids in early and total yield among them (Shalaby, 2012; Shalaby and El-Banna, 2013 and Solieman, et al., 2013).

High temperatures affect several physiological and biochemical processes dealing finally with yield reduction (Dinar and Rudich, 1985). Ashafuzzaman et al. (2010) studied the effect of heat stress and reported that significant differences were found among genotypes and seasons. The highest values in all traits such as early and total yield were observed in winter season, while the lowest values were observed in summer season, which agree with our results. Results of some fruit quality characters are summarized in Table 8. Highly significant differences were obtained in fruit diameter, length and weight among somaclones and the original cultivar. For fruit diameter, the highest value (6.0 cm) was obtained from somaclone 8. For fruit length, the highest values (6.9 cm and 6.8 cm) were obtained from somaclone 3 and the original cultivar, respectively. Regarding fruit weight, the highest value (102 g) was obtained from somaclone 2, while the lowest values (50.2 and 50.3 g) were obtained from somaclones 4 and 1, respectively. The present result confirmed the earlier reports of (Wagh et al., 2004; Mansour et al., 2009) who reported genotypic variations in tomato yield and average fruit weight and size.

Table 7. Horticultural characteristics of tomato cultivar Super Strain B and its somaclones.

Genotypes	No. of nodes below the first cluster	Plant height (cm)	No. of branches/ plant	Pollen fertility (%)	Fruit set (%)	Early yield (g/plant)	Total yield (g/plant)
Parent	7.7 a	63.8 d	4.6 e	58.3 de	64.1 c	267.4 bc	523.1 cd
s1	6.0 cd	64.8 cd	4.9 de	35.7 g	81.0 ab	276.2 bc	456.2 d
s2	7.1 ab	80.3 ab	6.4 ab	74.4 ab	91.6 a	840.6 a	1445.1 a
s3	7.0 ab	68.2 bc	5.1 cd	81.0 ab	84.4 ab	675.8 a	1169.7 ab
s4	5.8 d	63.2 d	5.2 bc	11.1 h	47.9 d	167.6 c	377.3 d
s5	7.1 ab	73.3 ab	7.1 ab	60.7 cd	87.9 ab	737.8 a	1054.8 ab
s6	7.6 ab	78.9 ab	7.6 a	66.6 bc	79.5 ab	804.1 a	1198.8 ab
s7	6.7 ab	75.6 ab	7.4 a	82.2 ab	79.6 ab	785.7 a	1156.8 ab
s8	7.2 ab	84.6 a	7.7 a	81.0 ab	72.4 bc	906.6 a	1195.6 ab
s9	7.4 ab	75.1 ab	7.9 a	56.1 de	74.6 ab	710.5 a	896.0 ab
s10	6.6 ab	75.2 ab	7.6 a	88.9 a	79.7 ab	919.4 a	1316.3 ab
s11	6.9 ab	82.2 a	7.3 ab	56.8 de	78.6 ab	734.0 a	1013.6 ab
s12	6.8 ab	68.0 bc	7.7 a	68.8 ab	91.5 a	709.4 a	1116.1 ab
s13	7.6 ab	82.2 a	6.6 ab	63.0 bc	75.6 ab	971.8 a	1294.3 ab
s14	7.2 ab	79.3 ab	8.4 a	63.3 bc	74.9 ab	646.9 ab	1054.5 ab
s15	5.8 d	64.2 d	7.3 ab	62.1 bc	84.7 ab	930.9 a	1383.2 ab
s16	6.3 bc	74.1 ab	6.9 ab	73.1 ab	77.7 ab	924.8 a	1249.7 ab
s17	6.6 ab	78.3 ab	8.6 a	53.3 ef	85.8 ab	619.7 ab	842.4 bc
s18	6.3 bc	78.0 ab	7.9 a	43.6 fg	81.3 ab	862.9 a	1285.1 ab
s19	6.9 ab	76.6 ab	6.8 ab	75.7 ab	85.1 ab	684.0 a	1116.9 ab

Mean values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Table 8. Mean values of some fruit characters of tomato cultivar Super Strain B and its somaclones.

Genotypes	Fruit Diameter (cm)	Fruit length (cm)	Fruit weight (g)	No. of locules/fruit	Fruit firmness (Lbf)	TSS (%)	VC (mg/100g)	Acidity
Parent	5.2 ab	6.9 a	57.0 de	4.2 de	391.9 ab	5.5	30.4 d	0.28
s1	5.8 ab	5.3 bc	50.3 e	6.4 ab	349.8 ab	5.8	38.0 ab	0.24
s2	5.7 ab	6.4 ab	102.0 a	4.6 cd	356.2 ab	5.5	34.1 bc	0.20
s3	5.1 bc	6.8 a	75.3 bc	3.3 ef	424.8 ab	5.5	30.2 d	0.25
s4	4.8 c	5.6 bc	50.2 e	5.4 ab	439.4 a	5.5	35.0 ab	0.26
s5	5.3 ab	4.9 c	71.9 cd	4.9 bc	308.1 bc	5.7	34.8 ab	0.28
s6	5.7 ab	5.0 c	82.8 ab	5.8 ab	354.4 ab	5.9	31.8 cd	0.30
s7	5.4 ab	5.1 c	67.1 cd	4.9 bc	313.1 bc	5.4	33.8 bc	0.25
s8	6.0 a	5.4 bc	101.0 ab	7.3 ab	366.8 ab	5.4	37.3 ab	0.25
s9	5.7 ab	5.3 bc	86.6 ab	8.1 a	330.7 ab	5.7	36.3 ab	0.26
s10	5.7 ab	5.1 c	79.7 ab	5.6 ab	373.7 ab	5.3	38.6 ab	0.24
s11	5.5 ab	5.3 bc	79.6 ab	5.0 bc	294.1 de	5.7	34.2 bc	0.27
s12	5.7 ab	5.7 bc	60.4 cd	5.0 bc	377.0 ab	5.7	35.7 ab	0.23
s13	5.8 ab	5.4 bc	75.4 bc	7.8 ab	328.1 ab	5.7	35.4 ab	0.30
s14	5.1 bc	4.9 c	68.1 cd	4.4 cd	292.2 de	5.7	41.3 a	0.19
s15	4.7 c	5.9 ab	66.6 cd	2.1 g	377.8 ab	5.3	32.2 bc	0.22
s16	4.7 c	5.9 ab	65.1 cd	3.0 fg	419.3 ab	5.7	33.0 bc	0.24
s17	5.3 ab	5.1 c	72.5 cd	4.3 de	274.8 de	5.3	35.0 ab	0.26
s18	5.6 ab	4.9 c	67.0 cd	6.0 ab	246.3 e	5.3	41.4 a	0.31
s19	5.7 ab	5.2 bc	84.9 ab	4.9 bc	302.4 cd	5.9	37.5 ab	0.22

P-Super strain B; TSS-total soluble sugar; VC-Vitamin C; Values having the same alphabetical letter within each column are not significantly different at the 5% level, according to Duncan's test.

Fig. 4: Pollen fertility. a: The highest somaclone (no.10), b: the original cultivar and c: the lowest somaclone (no.4).

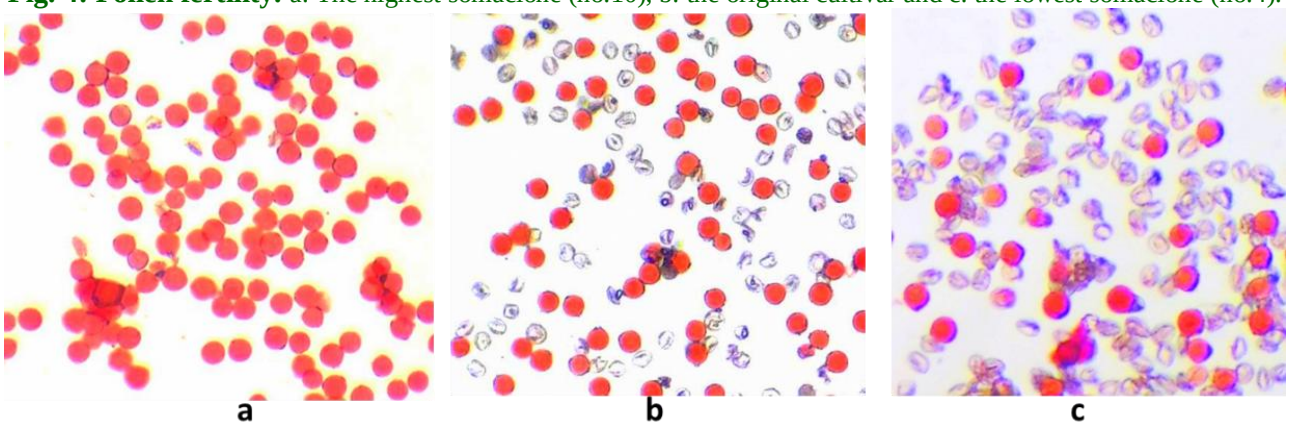
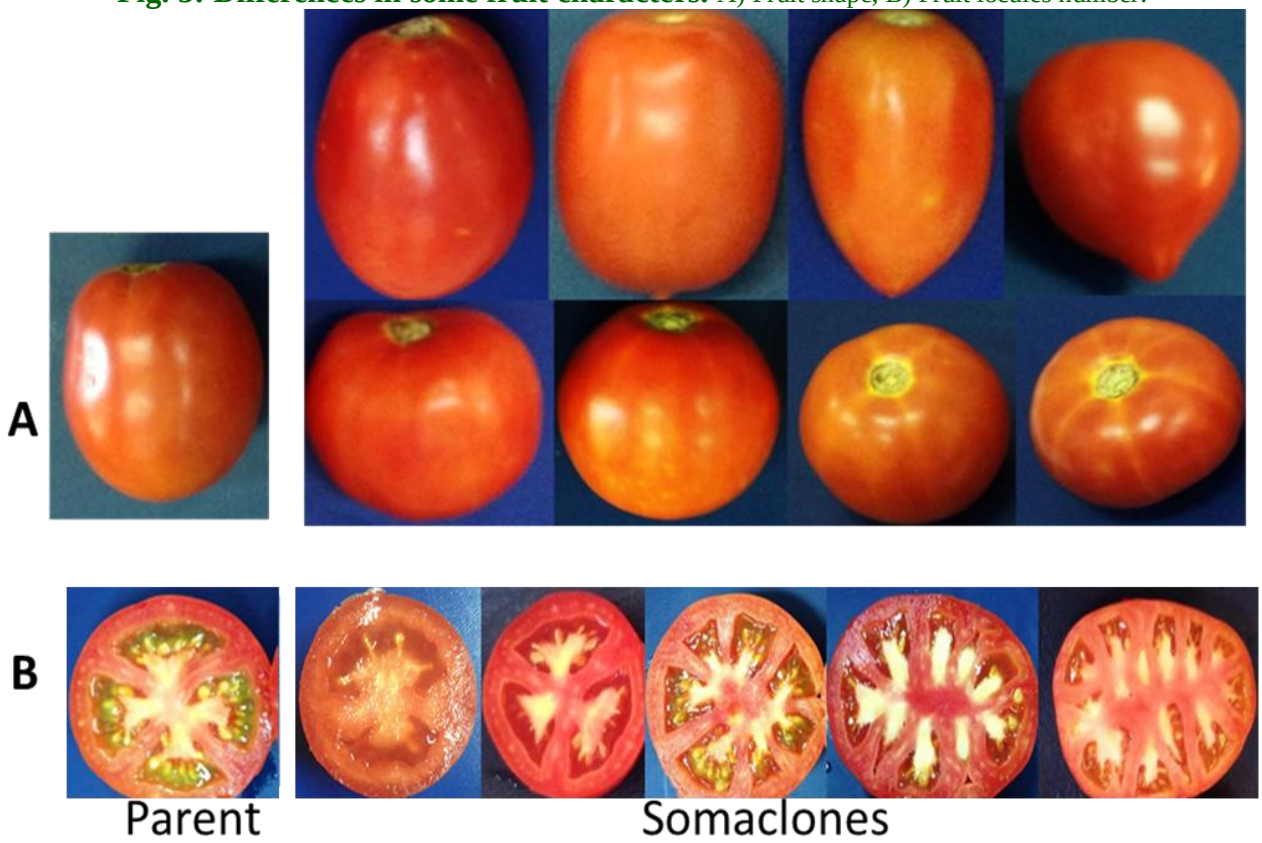


Fig. 5: Differences in some fruit characters. A) Fruit shape, B) Fruit locules number.



The ratio between fruit diameter and length results in fruit different shapes as showed in Fig. 5. High significant differences were obtained in the number of locules/fruit; fruit firmness and vitamin C content among somaclones and the original cultivar, but no significant differences were found in the percentage of TSS and acidity. For the number of locules/fruit, the highest value (8.1) was obtained from somaclone 9, while the lowest value 2.1 was obtained from somaclone 15 (Table 8 and Fig. 5). Regarding fruit firmness, the highest value (439.4) was obtained from somaclone 4, while the lowest value (246.3) was obtained from somaclone 18. Significant differences in fruit firmness were found among tomato cultivars and hybrids (El-Gazar et al., 2002). For TSS and acidity, all somaclones and the original cultivar observed closed values from 5.3 to 5.9% for TSS and from 0.19 to 0.31 for acidity. Regarding ascorbic acid (VC) the highest value (41.4 mg/100g) was obtained from somaclone 18, while the lowest values (30.2 and 30.4mg/100g) were obtained from somaclone 3 and the original cultivar, respectively. Previous studies

reported also significant differences for this trait among tomato cultivars and hybrids (Dawa et al., 2007; Kansouh and Masoud, 2007). The highly significant differences between the somaclones and the original cultivar in the most traits indicate that these regenerated plants genetically differed in one or more genes from their original cultivar. Genotypic variation had a significant effect on all studied traits. Such results are in consonance with those of (Mansour et al., 2009; Soleiman et al., 2013 and Shalaby and El-Banna, 2013) who found that the studied genotypes showed relatively significant wide ranges of genetic variability among tomato cultivars for the most studied traits.

SSR and protein markers

Molecular assay of somaclonal variants using 5 SSR primers was clearly distinguished between the studied genotypes. The polymorphism percentages in the amplified DNA pattern were 100% (Table 9) SSR primers produced 20 bands in all the genotypes studied, out which seven bands were unique. These bands could be used as a genetic fingerprint for those genotypes. SSR (Le tat002) produced the highest number of bands (6), out of them, three bands with molecular size of 1100, 950 and 200bp were unique in the parent and somaclone 19, respectively (Table 10 and Fig. 6). While alleles with size of 1200 and 1000bp were absent in the parent and present in the majority of somaclones. Le caa001 and Le ga003 produced the lowest number of alleles (3), alleles with size of 110 and 100 bp were present in some promising somaclones and absent in the others beside the parent (Table 10). SSR primer (Le ta023) amplified four polymorphic alleles, two of them (170 and 160bp) were unique for somaclones 17 and 15, respectively, whereas the allele (190bp) was present only in some somaclones and absent in the parent. Primer (Le Hmg2a) amplified polymorphic non parental two unique bands with size of 230 and 190 bp in the somaclones 9 and 19, respectively. Similarity indices were developed for 18 genotypes and indicate low genetic similarity ranging from 18 to 62% between the parent and its somaclones (data not shown).

Table 9. Names, sequences and polymorphism percentages of the five SSR primers.

Primers	F/R Primer (5`-3`)	Polymorphic/total bands	Polymorphism %	Unique bands
Le caa001	F –AGAAGGCGTGAGAGGCAAC R –CTTAGCACTTGATGTTGATTGG	3/3	100	0
Le tat002	F –ACGCTTGGCTGCCTCGGA R –AACTTTATTATTGCCACGTAGTCATGA	6/6	100	3
Le ga003	F- ACCCTAAAACTAACGACATTCAACG R –TTCGTGGACTAATGTGAAGTGTACC	3/3	100	0
Le ta023	F –ATTGCTCATACATAACCCCC R –GGGACAAAATGGTAATCCAT	4/4	100	2
Le Hmg2a	F –ATCTGAAGAGCCTGTTTATCC R –AAAGCGTAACGACATGTAAAG	4/4	100	2
Total		20		7

In this study we detected genetic variation in somaclonal variant using SSR markers that have a number of advantages over other molecular markers including, multiple SSR alleles may be detected at a single locus using a simple PCR-based screen; SSRs are evenly distributed all over the genome; they are co-dominant; very small quantities of DNA are required for screening; and analysis may be semi-automated all of these primers could reveal polymorphic DNA banding pattern reached to 100%. The finding here also are in agreement with earlier reports on application of RAPD and SSR in describing genetic polymorphism among regenerated plants in several other plants, *viz.*, rice (Khai and Lang, 2005), oil palm (Sanputawong and Te-chato, 2011) and in tomato (Shalaby and El-Banna, 2013).

The protein banding pattern produced 198 bands which were distributed in all genotypes with molecular size ranging from 22 to 125 KDa. 16 out of 18 were polymorphic (88.8%). All studied genotypes were clearly identifiable (Table 11). Protein pattern showed that almost of bands were differ in their intensity among studied genotypes. Furthermore, some genotypes possessed some bands which were absent in the parent that could be related to heat and salt stress.

Table 10. Distribution of SSR markers among the studied genotypes.

Primers	MW	P	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	s14	s15	s16	s17	s18	s19
le caa 001	120	1	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	1	1
	110	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0
	100	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0
le ga 003	250	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	240	0	1	0	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1
	220	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0
le ta023	180	1	0	0	1	0	1	1	1	1	1	1	0	0	0	0	0	0	1	0
	190	0	1	1	0	1	0	0	0	0	0	0	1	1	1	0	1	0	0	1
	170	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
	160	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
le Hmg2a	250	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	0	0
	230	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
	200	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0
	190	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
le tat 002	1200	0	1	0	0	1	0	0	1	1	0	1	1	1	0	1	1	1	0	0
	1100	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	1000	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
	950	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
	220	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
	200	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

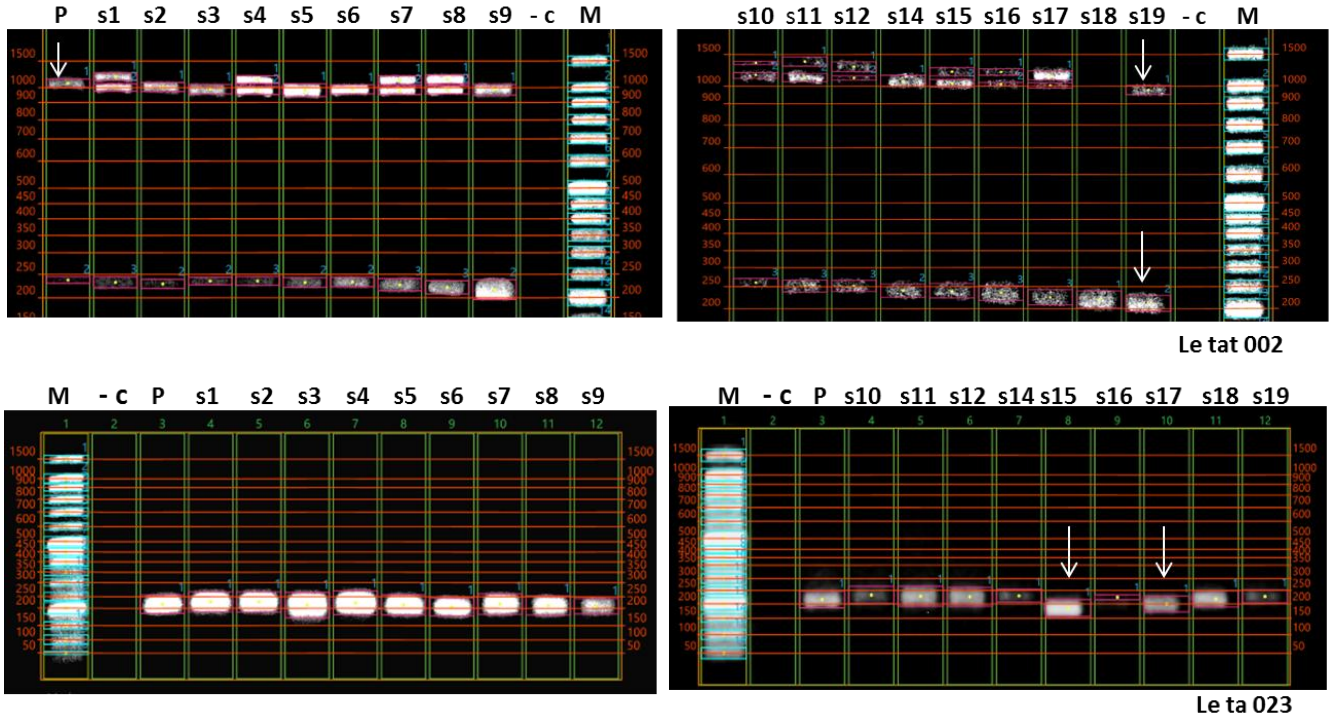
Table 11. Description of total soluble protein of tomato cultivar Super Strain B and their nineten somaclones.

No	KDa	P	s1	s2	s3	s4	s5	s6	s7	s8	s9	s10	s11	s12	s13	s14	s15	s16	s17	s18	s19
1	125	-	-	3	-	-	-	-	-	-	3	-	-	2	-	2	4	2	-	-	2
2	105	-	-	2	3	2	1	1	2	2	3	-	2	2	-	2	3	2	-	-	3
3	80	2	1	4	3	-	1	2	4	-	4	2	3	3	1	3	4	3	2	2	2
4	75	3	-	-	-	-	3	1	2	2	2	3	2	2	2	-	-	-	-	-	-
5	70	-	2	3	3	3	-	-	-	-	-	-	-	-	-	3	2	3	2	1	2
6	65	4	-	-	-	-	4	3	-	-	-	3	-	-	3	4	-	4	4	-	-
7	60	-	3	4	4	2	-	-	3	3	4	-	4	4	-	-	4	-	-	3	2
8	55	1	-	3	3	2	3	2	3	2	-	2	3	3	1	3	3	3	2	-	-
9	52	-	1	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	3	-
10	50	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	43	2	1	3	2	2	2	1	2	2	3	2	3	3	2	3	3	3	1	-	3
12	39	2	-	2	2	2	2	1	2	2	-	2	-	-	-	2	-	-	-	-	-
13	36	-	-	-	-	-	-	-	-	-	2	-	2	3	2	-	2	2	1	-	2
14	30	2	-	2	2	1	2	2	2	1	2	2	1	3	1	2	2	3	2	1	2
15	27	2	-	1	2	1	2	2	2	-	2	1	1	3	1	2	3	-	1	1	3
16	25	-	-	2	2	-	3	-	-	2	2	1	1	-	-	2	2	-	1	-	-
17	23	4	4	-	3	3	-	4	2	-	-	-	-	-	-	2	-	3	-	-	-
18	22	-	-	-	-	-	-	-	-	3	2	3	4	3	4	-	3	-	-	3	-
Total		9	6	11	11	10	10	10	10	9	12	10	11	11	9	12	12	10	9	7	9

'-' absent; 1- weak; 2-light; 3-medium; 4-dark.

Fig. 6: Banding pattern for all genotypes amplified by the two SSR primers.

M: 50bp DNA ladder. P, parental cultivar, 1- 19, somaclonal variants. Images were analyzed using My Image Analysis v2.0, thermo scientific software.



Bands with molecular size of 105, 70, 60, 36, 25 and 22 were found only in some somaclones that have good horticultural and fruit quality characters, these bands could be linked to heat and or salt tolerance. Protein markers are highly polymorphic and environmental influence on their electrophoretic pattern is limited (Gepts et al., 1986). Protein markers have been used to assess genetic diversity of some potato cultivars (El-Bannna and Khattab, 2013) and rice somaclones (El-Bannna and Khattab, 2012). It could be concluded that the results of protein could differentiate the studied genotypes producing some specific bands that could be used to distinguish such genotypes from each other and could be linked to heat and or salt tolerance.

References

Afroz, A., Chaudhry, Z., Khan, R., Rashid, H., Khan, S. A., 2009. Effect of GA3 on regeneration response of three tomato cultivars (*Lycopersicon esculentum*). Pak. J. Bot. 41, 143–151.

Ashrafuzzman, M., Haque, M.A., Ismail, M. R., Islam, M.T., Shahidullah, S.M., 2010. Genotypic and seasonal variation in plant development and yield attributes in tomato (*Lycopersicon esculentum* Mill) cultivars. Int. J. Bot. 6, 41-46.

Bhatia, P., Ashwath, N., David, M., 2005. Effect of genotype, explants orientation and wounding on shoot regeneration in tomato. *In Vitro* Cell Dev. Biol. Plant. 41, 457- 464.

Butelli, E., Titta, L., Giorgio, M., Mock, H.P., Matros, A., Peterek, S., Schijlen, Hall, R.D., Bovy, A.G., Luo, J., Martin, C., 2008. Enrichment of tomato fruit, with health-promoting anthocyanins by expression of select transcription factors. Nature Biotechnol. 26, 1301–1308.

Chaudhry, Z., Abbas, S., Yasmin, A., Rashid, H., Ahmed, H., Anjum, A.M., 2010. Tissue culture studies in tomato (*Lycopersicon esculentum*) var. Moneymaker. Pak. J. Bot. 42, 155–163.

Chaudhry, Z., Habib, D., Rashid, H., Qurashi, A.S., 2004. Regeneration from various explants of in vitro seedling of tomato (*Lycopersicon esculentum* L., cv. Roma). Pak. J. Biol. Sci. 7, 269–272.

Cho, Y.G., Ishii, T., Temnykh, S., Chen, X., Lipovich, L., Park, W.D., Ayres, N., Cartinhour, S., McCouch, S.R., 2000. Diversity of microsatellites derived from genomic libraries and GenBank sequences in rice (*Oryza sativa* L.). Theor. Appl. Genet. 100, 713-722.

Dawa, K.K., El-Ghareeb, I.M., Abd E-Haj, I., 2007. The utilization of heterosis in the breeding of tomato. J. Agric. Sci. Mansoura Univ. 32, 2405-2114.

Devi, M., Dhaliwal, M.S., Kaur, A., Gosal, S.S., 2008. Effect of growth regulators on *in vitro* morphogenetic response of tomato. Indian J. Biotechnol. 7, 526- 530.

Dinar, M., Rudich, J., 1985. Effect of heat stress on assimilate partition in tomato. Ann. Bot. 56, 239-248.

El-Banna, A., Khattab, I., 2012. Biochemical characterization of rice somaclones resistant to blast. Curr. Res. J. Biol. Sci. 4,137-142.

El-Banna, A., Khattab, I., 2013. Assessing genetic diversity of some potato (*Solanum tuberosum* L.) cultivars by protein and RAPD markers. Egyptian J. Genet. Cytol. 42, 89-101.

El-Gazar, T., El-Sayed, H., Zanata, O., 2002. Inheritance of vegetative and fruit quality of some tomato crosses in late summer season. J. Agric. Sci. Mansoura Univ., 27(8), 5473-5484.

Feldman, M., Sears, E.R., 1981. The wild gene resources of wheat. Sci. Amer. 244, 102-112.

Gepts P., Osborne, T., Rashka, K., Bliss, F.A., 1986. Phaseoline protein of Wild form variability in and landraces of the common beans (*Phaseolus vulgare*): Evidence for multiple centres of domestication. Econ. Bot. 40, 451–46.

Hall, A.E., 2001. Crop responses to the environment, CRC Press LLC, Boca Raton, FLA. 232p.

Hall, A.E., Ziska, L.H., 2000. Crop breeding strategies for the 21st century. In: Climate Change and Global Crop Productivity (Eds.: Reddy, K.R., Hodges, H.F.). CABI Publishing, New York, USA. pp.407-423.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Palenotological statistics software package for education and data analysis. Palenotol. Electron. 4(1), 9.

Hamza, S., Chupeau, Y., 1993. Re-evaluation of conditions for plant regeneration and *Agrobacterium* mediated transformation from tomato (*Lycopersicon esculentum* Mill.). J. Exp. Bot. 44, 1837–1845.

Harish, M.C., Rajeevkumar, S., Sathishkumar, R., 2010. Efficient in vitro callus induction and regeneration of different tomato cultivars of India. Asian J. Biotechnol. 2, 178 184.

He, S.Z., Han, Y.F., Wang, Y.P., Zhai, H., Liu, Q.C., 2009. *In vitro* selection and identification of sweet potato (*Ipomoea batatas* (L.) Lam.) plants tolerant to NaCl. Plant Cell Tissue Organ. Cult. 96, 69–74.

Jabeen, N., Chaudhry, Z., Rashid, H., Mirza, B., 2005. Effect of genotype and explants type on *in vitro* shoot regeneration of tomato (*Lycopersicon esculentum*). Pak. J. Bot. 37, 899–903.

Jaccard, B., 1908. Nouvelles recherches sur la distribution florale. Bull. Sco. Vaud. Sci. Nat. 44, 223-270.

Jiang, X.L., He, Z.M., Peng, Z.Q., Qi, Y., Chen, Q., Yu, S.Y. 2007. Cholera toxin B protein in transgenic tomato fruit induces systemic immune response in mice. Transgenic Res. 16, 169-175.

Kansouh, A.M., Masoud, A.M., 2007. Manifestation of heterosis in tomato (*Lycopersicon esculentum* Mill.) by line x tester analysis. Alex. J. Agric Res. 52, 75-90.

Khai, T.H., Lang, T.N., 2005. Using SSR markers to identify allele variation of somaclonal mutants in indica rice. Omonrice 13, 121-12.

Ladizinsky, G., Hymowitz, T. 1979. Seed protein electrophoresis in taxonomic and evolutionary studies, Theor. Appl. Genet. 54, 145-151.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227, 680-685.

Mandel, A.B., Sheeja, T.E., 2003. In vitro flowering and fruiting in tomato (*Lycopersicon esculentum* Mill). Asia Pac. J. Mol. Biol. Biotechnol. 11, 37-42.

Mansour, A., Ismail, H., Ramadan, M., Gyulai, G., 2009. Comparative genotypic and phenotypic analysis of tomato (*Lycopesicon esculentum* Mill) cultivars grown under tow different seasons in Egypt. Afr. J. Plant Sci. Biotechnol. 3, 73-79.

Mehta, N., Asati, B. S., 2008. Genetic relationship of growth and development traits with fruit yield in tomato (*Lycopesicon esculentum* Mill). Karnataka J. Agric. Sci., 21, 92-96.

Mirghis, E., Mirghis, R., Lacatus, V., Fernandez, M. R., Cuartero, J., Gomez, G. M. L., 1995. Analysis of tomato cultivars and hybrids for in vitro callus formation and regeneration. First International Symposium on Solanaceae for Fresh Market. Acta Horticult. 412, 111–116.

Moghaleb, R.E.A., Saneoka, H., Fujita, K., 1999. Plant regeneration from hypocotyls and cotyledon explants of tomato (*Lycopersicon esculentum*). Soil Sci. Plant Nutr. 45, 639-646.

Mujib, A., Banerjee, S., Ghosh, P.D., 2007. Callus induction, somatic embryogenesis and

chromosomal instability in tissue culture raised hippeastrum (*Hippeastrum hybridum* cv. United Nations). Propag. Ornament. Plants 7, 169-174.

Munns, R., 2005. Genes and salt tolerance: bringing them together. New Phytol. 167, 645-663.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassys with tobacco tissue culture. Physiol Plant. 15, 473-497.

Osman, G. M., Elhadi, A.E., Khalafalla, M.M., 2010. Callus formation and organogenesis of tomato (*Lycopersicon esculentum* Mill, cv. Omdurman) induced by thidiazuron. A. J. of Biotechnol., 9, 4407–4413.

Pohar, K.S., Gong, M.C. Bahnson, R., Miller, E.C., Clinton, S.K., 2003. Mutation induction and tissue culture in improving fruits. Plant Cell Tissue Organ Cult. 64, 185–210.

Rani, V., Raina, S., 2000. Genetic fidelity of organized meristem derived micropropagated plants: a critical reappraisal. In Vitro Cell. Devlop. Biol. Plant. 36, 319-330.

Rao, A., Agarwal, S., 2000. Role of antioxidant lycopene in cancer and heart disease. J. Amer. College Nutr. 19, 563-569.

Sanputawong, S., Te-chato, S., 2011. Analysis of somaclonal variation of callus, somatic embryo and plant regeneration of *in vitro* oil palm (*Elaeis guineensis* Jacq.) J. Agric. Technol. 7, 531-545.

Santa-Cruz, A., Martinez-Rodriguez, M., Perez-Alfocea, F., Romero-Aranda, R. Bolarin, M.C., 2002. The roostock effect on the tomato salinity response depends on the shoot genotype. Plant Sci. 162, 825-831.

Snedecor, G. W., 1962. Statistical Methods. The Iowa State College Press, Cornell Univ. Ithaca N.Y. Nat. Acad. Sci. 891, 413-436.

Shalaby, T.A., 2012. Line x tester analysis for combining ability and heterosis in tomato under late summer season conditions. J. Plant Prod. Mansoura Univ. 3, 2857-2865.

Shalaby, T.A., El-Banna A.S., 2013. Molecular and horticultural characteristics of *in vitro* induced tomato mutants. J. Agric. Sci. 5, 155-163.

Sheeja, T., Mondal, E.A., Rathore, R.K., 2004. Efficient plantlet regeneration in tomato (*Lycopersicon esculentum* Mill.). Plant Tissue Cult. 14, 45–53.

Singh, A.K., Asati, B.S., 2011. Combining ability and heterosis studies in tomato under bacterial wilt condition. Bangladesh J. Agric. Res. 36, 313-318.

Solieman, T.H.I., El-Gabry, M.A.H., Abido, A.I., 2013. Heterosis, potence ratio and correlation of some important characters in tomato (*Solanum lycopersicum* L.). Sci. Hort. 150, 25-30.

Sutter, E.G., 1996. General laboratory requirements, media and sterilization methods. In: Plant Tissue Culture Concepts and Laboratory Exercises (Eds.: Trigiano, R.N., Gray, D.J.). CRC Press, New York, USA. pp.11-25.

Taji, A., Kumar, P.P., Lakshmanan, P., 2002. *In Vitro* Plant Breeding. Food Products Press, New York. 167p.

Temnykh, S., DeClerck. G., Lukashova, A., Lipovich, L., Cartinhour, S., McCouch, S.R., 2001. Computational and experimental analysis of microsatellites in rice (*Oryza sativa* L.): frequency, length variation, transposon associations, and genetics. Genome Res. 11, 1441-1452.

Toth, G., Gaspari, Z., Jurka, J., 2000. Microsatellites in different eukaryotic genomes: survey and analysis. Genome Res. 10, 667-981.

USDA, 2004. Soil survey laboratory methods manual soil survey investigation report No. 42 version 4.0.

Wagh, R.S., Bharud, R.W., More, S.R., 2004. Stability differences among tomato genotypes for yield and yield attributes. Ann. Plant Physiol. 18, 176-181.

Walbot, V., Warren, C., 1988. Regulation of *Mu* element copy number in maize lines with an active or inactive Mutator transposable element system. Mol. Genet. Genomics 211, 27-34.