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# Molecular and Horticultural Characteristics of *In vitro* Induced Tomato Mutants

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**Abstract**

In addition to the traditional breeding approaches, genetic variability in tomato can be induced also by means of mutagenesis. The objective of this study was to develop an *In vitro* technique suitable for mutation induction on tomato and characterize them by RAPD and SSR markers as well as horticultural characteristics. The influence of various concentrations (0, 0.07, 0.14 and 0.25%) of the chemical mutagen, Ethyl Methane Sulfonate (EMS), on the *in vitro* shoot formation from cotyledon explants of two tomato cultivars was studied. The percentage of responding explants ranged from 45.2 to 95% in dependence on genotype and EMS concentrations. Two PCR-based techniques, RAPD and SSR, were used for analysis of genetic variations in regenerated plants from *in vitro* cultures combined with EMS treatment (0.25%). The percentage of polymorphism detected by RAPD and SSR primers reached 25.64%. Grouping of the original cultivar and their mutants indicated the genetic distinctness as they are placed in different clusters/groups far from each other. Mutants regenerated from the wide cultivated cultivar in Egypt (Super strain B) were evaluated with their origin cultivar in a field experiment for yield potential and fruit quality. The results revealed that the mutants were differed in number of branches, early and total yield, average fruit weight, fruit firmness and TSS content. Moreover, mutant lines S1, S3, S6 and S13 had some desirable horticultural traits and could be used in improving tomato crop by breeding programs or they could be considered as new breeding lines.

**Keywords:** tomato mutants, RAPD, SSR, *In vitro*, yield components

## 1. Introduction

Tomato (Solanum lycopersicum *L*.) is a worldwide cultivated crop, which is used both as a fresh and processed product. In the last few years, its global production has increased approximately 10% since for many countries it is a significant source of vitamins and minerals. Furthermore, it has also been recently demonstrated that it is the main source of the carotenoid lycopene, which has antioxidant properties and may help to protect against diseases, such as cancer and cardiovascular disease (Giovannucci, 1999).

In Egypt, tomato is important vegetable crop which ranks first among the vegetable crops based on cultivated area (about 252 thousand hectares) and production (10.3 million tons). Tomato is suitable plant material for physiological and cytological studies due to its ease of culturing and genetic uniformity resulting from autogamy (Rick, 1980). Plant tissue culture is an important tool of biotechnology, which can be used to improve productivity of a crop via rapid availability of superior planting stock. Somaclonal variation can pose a severe threat to the genomic integrity of generated plants, which is particularly required during the genetic transformation experiments and to achieve genetic uniformity of the propagules (Soniya et al., 2001). Mutation can bring the changes at the DNA level. However, for most at the micro-propagated crops, 5% somaclonal variation is permitted (Evans, 1989). 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; Rashid et al., 2010). The most frequently used method of regeneration in tomato is *via* shoot organogenesis from callus of leaf or cotyledon explants (Compton & Veilleux, 1991). The regeneration ability of a number of tomato cultivars have been tested for their ability to produce callus and shoot induction in earlier studies (Costa et al., 2000; Harish et al., 2010). Many biotechnological approaches have been focused on the improvement of tomato crop, which can grow in different agro climatic zones to meet the demands (Mandel & Sheeja, 2003). Molecular marker such as

microsatellites or simple sequence repeats (SSR) and random amplified polymorphic DNA (RAPD) are often favored over traditional phenotypic or cytological measurements, and generally assess even small variations of the genome. The use of the PCR-based RAPD technique has been applied successfully for determination of genetic diversity in regenerated plants of tomato (Soniya et al., 2001; Chen et al., 2009). The aim of this study was to increase genetic variations in two tomato cultivars by using ethyl methane sulfonate (EMS) and detection of induced genetic variations by using RAPD-PCR and SSR marker. We also attempt to evaluate the variations with respect to yield components and fruit quality traits.

## 2. Materials and Methods

### *2.1 Seed Germination*

This study was conducted at the Departments of Genetics and Horticulture at Faculty of Agriculture, Kafrelsheikh University, Egypt during 2010-2012. Seeds of tomato cultivars "Rio grande and super strain B" were washed with continuously running tap water for 15 min. Under laminar flow cabinet, seeds were disinfected with 20% of Clorex (Sodium hypochlorite 5.25%) for 15 min, and then rinsed 4-5 times with sterile distilled water. After surface sterilization, the seeds were inoculated on half-strength MS (Murashige & Skoog, 1962) medium and incubated at 25°C in the dark till full germination and later transferred to a 16/8 h photoperiod at 25 ± 2°C.

### *2.2 Plant Regeneration*

Cotyledonary explants, that were obtained from aseptically grown one week old seedlings, were placed on the surface of MS medium supplemented with 1 $mgl^{-1}$ from each of benzyl adenine (BA) and kinetin and different concentration (0, 0.07, 0.14 and 0.25%) of Ethyl Methane Sulfonate (EMS). For each EMS treatment, the lethal dose (LD) was determined by calculating the survival rate of the plants: the number of survival explants in each EMS treatment was divided by the number of untreated explants. The dishes (7 cm) containing 15 ml of previous described medium were incubated at 25 ± 2°C with a photoperiod of 16/8 h for 2 days, then the explants were transferred to fresh medium without EMS. After four weeks the number of responding explants was recorded. Regenerated shoots (Figure 1a) were transferred to jars containing the same medium for multiplication. Cultures remained on the multiplication medium for other four weeks, and then the number of normal and abnormal plantlets was recorded. Shoots were separated and transferred to the rooting medium that consisted of MS basal medium without plant growth regulators. Rooted shoots (Figure 1b) were removed from the jars, thoroughly washed to remove any traces of agar and then transferred to pots containing autoclaved peat moss and vermiculite (1:1) and 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 and acclimatized plants (Figure 1C) were transferred to green house conditions and fruits were harvested from the good performance 15 plants.

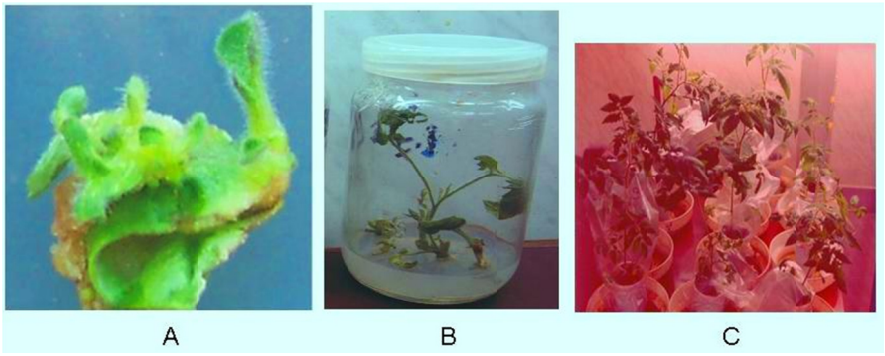


Figure 1. Different stages of tomato organogenesis in tomato Super Strain B cultivar. A: shoots regeneration from cotyledon explants B: shoot elongation and C: acclimatized plants.

### *2.3 DNA Extraction and Quantification*

For DNA extraction, approximately 150 mg fresh leaves of $M_2$ plants regenerated from Super Strain B (0.25% EMS) and their corresponding mother plant were grounded using pestle and mortar with liquid nitrogen and then extracted using cetyl trimethyl ammonium bromide (CTAB) buffer according to Doyle and Doyle (1990). The quality and quantity of DNA samples were measured by a Nano-Drop ND-100 UV-Vis spectrophotometer which

enabled highly accurate analysis of extremely small samples (2 µl DNA) with remarkable re-productivity. The genomic DNA samples were adjusted to 30 ng/µl concentration with distilled $H_2O$ and subjected to PCR analysis.

*2.4 Screening of Primers*

Fifteen decamer oligonucleotide RAPD and five specific SSR primers (Eurofins MWG Operon, Germany) were used for analysis the genetic similarity between the original cultivar (Super Strain B) and the mutants produced by treated with 0.25% of EMS (Table 2).

*2.5 PCR Amplification Conditions*

Amplification reactions were performed in an MJ Mini Bio RAD, thermal cycler. PCR reactions were carried out in a total volume of 20 µl, the PCR reaction mixture consisted of 1 µl (30 ng) template DNA, 1 µl (5 x Promega Go *taq* PCR buffer), 2 µl (dNTPs, 2 mM), 1.6 µl ($MgCl_2$, 25 mM) 1.5 µl (10 ng of 10 mer RAPD primers and SSR primers), 0.125 µl (Go*Taq* polymerase, 5 U/µl). Template DNA was initially denatured at 95°C for 5 min, followed by 40 cycles under the following parameters: denaturation for 1 min at 95°C, appropriate annealing temperature for 1 min 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 proceeded to completion

*2.6 Gel Scoring and Data Analysis*

The PCR amplified products were separated by electrophoresis on a 1.5% agarose gels using 0.5 X TBE buffer (44.5 mMTris/Borate, 0.5 mM EDTA, pH 8). The gels were stained with ethidium bromide and photographed by gel documentation system (UVITEC, UK).The experiment was repeated for 3 times and reproducible RAPD and SSR bands were used for further analysis. The bands obtained were treated as binary characters and coded accordingly (presence = 1, absence = 0). Jaccard similarity (Jaccard, 1908) was determined among the genotypes studied to be used in clustering. The genotypes showing similarity in their RAPD and SSR characteristics were grouped by using UPGMA (Unweighted Paired Group with Arithmetic Average). SPSS-10 package was used for statistical analysis

*2.7 Field Evaluation of Induced Mutants*

Regenerated plants were acclimatized and transplanted under green house conditions. At the harvesting stage seeds were collected from the best 15 plants and stored separately till sown time. Mutants were evaluated along with their original cultivar (Super Strain B) in summer season of 2011. The experiment was arranged in randomized complete block design with 3 replications. The seeds of genotypes were sown in nursery in seedling trays on February 13$^{th}$. The seedlings were transplanted on April 6th. Each plot consisted of two ridges, 1m wide and 6 m long and 40 cm a part between plants. During the experiment, the growing practices were executed according to the recommendations for a commercial tomato crop under the same region.

Data were recorded for plant height (cm) and number of branches per plant after 60 days from transplant. Early yield (g/plant) as the yield of the first three pickings and total yield as the total weight (g/plant) of all harvested fruits were recorded. Average fruit weight (g) was calculated. The percentage of total soluble solids (TSS % brix) content in fruit juice was determined by a hand refractometer. Fruit firmness (g/cm$^2$) was measured using a needle type pocket penetrometer.

*2.8 Statistical Analysis*

Data were processed by analysis of variance and Duncan's multiple range test of the SPSS program version 10 was used for the comparison among treatment means.

## 3. Results and Discussion

*3.1 Induction of Mutation Using In vitro EMS Treatments*

Mutation is a possible source of variation in plant breeding. In addition to the traditional breeding approaches, genetic variability in tomato can be induced also by mutagenesis and tissue culture technique. The effect of various concentrations of the chemical mutagen (EMS) on the *In vitro* shoot formation from cotyledon explants of two tomato cultivars was studied. The percentage of direct regeneration from cotyledon explants after four weeks of culture is presented in Table 1.

Table 1. Effect of interaction between cultivar and ethyl methane sulfonate (EMS) on shoot induction from cotyledon explants of tomato

| Treatments | | Responding explants (%) | Number of Shoots/explants | Abnormal shoots % |
|---|---|---|---|---|
| cultivar | EMS% | | | |
| Rio Grande | 0 | 95.0 a | 4.5 b | 18.2 d |
| | 0.07 | 94.7 a | 4.2 d | 23.8 c |
| | 0.14 | 81.3 b | 3.3c | 33.3 b |
| | 0.25 | 80.6 b | 1.7 d | 47.1 a |
| **Mean** | | **87.9 A** | **3.4 A** | **30.6 A** |
| Super Strain B | 0 | 62.1 c | 5.5 a | 25.5 c |
| | 0.07 | 57.1 cd | 2.3 cd | 26.1 c |
| | 0.14 | 52.2 d | 2.2 d | 31.8 bc |
| | 0.25 | 45.2e | 1.6 d | 37.5 b |
| **Mean** | | **54.1 B** | **2.9 B** | **30.2 A** |

Means followed by the same letters are not significant at 0.05 level according to Duncan's test.

Data revealed that the percentage of responding explants ranged from 45.2 to 95% in dependence on genotype and EMS concentrations. Responding percentage deceased with increase the concentration of EMS from 0.07 to 0.25% in both cultivars. The average percentage of responding explants overall EMS concentrations for the cultivar "Rio grande" was higher than that for the "super strain B cultivar". The results revealed that genotypical differences were found between cultivars. Previous reports indicated that genotypic variation was observed among tomato cultivars in terms of callus and regeneration responses (Bhatia et al., 2005). A significant decline in shoot formation and increase in abnormal percentage were observed with the increment of EMS concentration in the medium indicating their inhibitory response on shoot induction. Moustafa et al. (1989) also obtained dependence between applied doses of gamma irradiation and N-nitose-N′-Ethyl Urea (ENU) on cultured maize callus growth and plant regeneration. Svetleva and Crino (2005) reported that ENU inhibited callus growth more than EMS.

*3.2 RAPD and SSR Analysis*

The regenerated plants from untreated explants (0% EMS) were compared to the original cultivar (Super Strain B) by using 30 RAPD and 10 SSR primers to estimate the genetic stability of plant regenerated from untreated explants and also to detect the consistency of the plant regeneration technique in maintaining the genetic stability of the regenerated plants. This comparison showed no genetic variability between the regenerated plants and their original cultivar (data not shown). This result allowed us to use the original cultivar as a control. Fifteen mutants were selected from $M_2$ plants. Fourteen out of fifteen RAPD primers and 5 out of 5 SSR primers could reveal some polymorphism in the amplified DNA pattern (Figures 2 and 3).

RAPD and SSR primers produced 78 bands in all the studied genotypes, out which 20 bands (25.64%) were polymorphic while 58 (74.35%) were common in the original cultivar and the selected mutants. Among the primers used, OPE-02 and LE caa001 produced the highest number of bands (9 and 3 respectively), while RAPD primers, OPA-05, OPA-10 and OPX-18 produced equal number of bands (3) and SSR primers, LEtat002 as well as LE 21085 produced only one monomorphic band (Table 2).

Table 2. Distribution of RAPD and SSR markers among the original cultivar (Super strain B) and its mutants

| Primer Name | Sequence (5′to 3′) | No. of bands | Polymorphic band | Polymorphism % |
|---|---|---|---|---|
| **RAPD** | | | | |
| OPA-04 | AATCGGGCTG | 5 | 0 | 0 |
| OPA-05 | AGGGGTCTTG | 3 | 1 | 33.33 |
| OPA-06 | GGTCCCTGAC | 4 | 0 | 0 |
| OPA-10 | GTGATCGCAG | 3 | 2 | 66.66 |
| OPA-16 | AGCCAGCGAA | 5 | 1 | 20 |
| OPB-01 | GTTTCGCTCC | 5 | 0 | 0 |
| OPB-02 | TGATCCCTGG | 4 | 2 | 50 |
| OPB-03 | CATCCCCCTG | 4 | 2 | 50 |
| OPE-02 | GGTGCGGGAA | 9 | 2 | 22.22 |
| OPK-11 | AATGCCCCAG | 4 | 0 | 0 |
| OPQ-14 | GGACGCTTCA | 8 | 1 | 12.5 |
| OPV-02 | AGTCACTCCC | 4 | 1 | 25 |
| OPV-07 | GAAGCCAGCC | 8 | 0 | 0 |
| OPX-18 | GAAGCCAGCC | 3 | 1 | 33.33 |
| **SSR** | | | | |
| LEtat002 (F) | ACGCTTGGCTGCCTCGGA | 1 | 0 | 0 |
| LEtat002 (R) | AACTTTATTATTGCCACGTAGTCATGA | | | |
| LEag003 (F) | ACCCTAAAACTAACGACATTCAACG | 2 | 2 | 100 |
| LEag003 (R) | TTCGTGGACTAATGTATGAAGTGTACC | | | |
| LEat002 (F) | ACTGCATTTCAGGTACATACTCTC | 2 | 2 | 100 |
| LEat002 (R) | ATAAACTCGTAGACCATACCCTC | | | |
| LEcaa001(F) | AGAAGGCGTGAGAGGCAAC | 3 | 3 | 100 |
| LEcaa001 (R) | CTT AGCACTTGATGTTGATTGG | | | |
| LE 21085 (F) | CATTTTATCATTTATTTGTGTCTT | 1 | 0 | 0 |
| LE 21085 (R) | ACAAAAAAAGGTGACGATACA | | | |
| Total | | 78 | 20 | 25.64 |

OPA 16 and OPA05 amplified polymorphic parental band with approximate size 3050 and 2980bp, respectively, in all tested mutants except S14 (Figure 2). On the other hand, OPV 02 primer produced one polymorphic non parental band (approximate size 300bp) except the mutant, S13. Similarly, some parental SSR bands occurred in some mutants and were absent in the others (Figure 3).

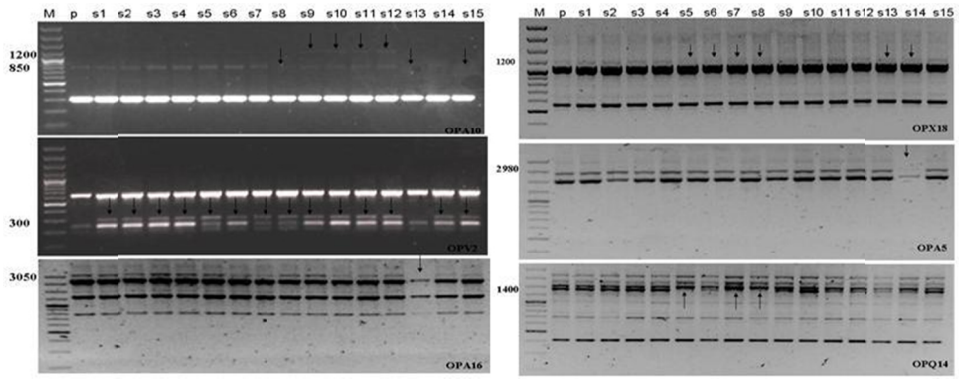


Figure 2. RAPD Pattern generated by some RAPD primers from genomic DNA of the original cultivar (lane p) and mutants S1 to S15 (lane, 3-17). Lane M contains 100 bp DNA Ladder. Arrowheads indicate polymorphic bands

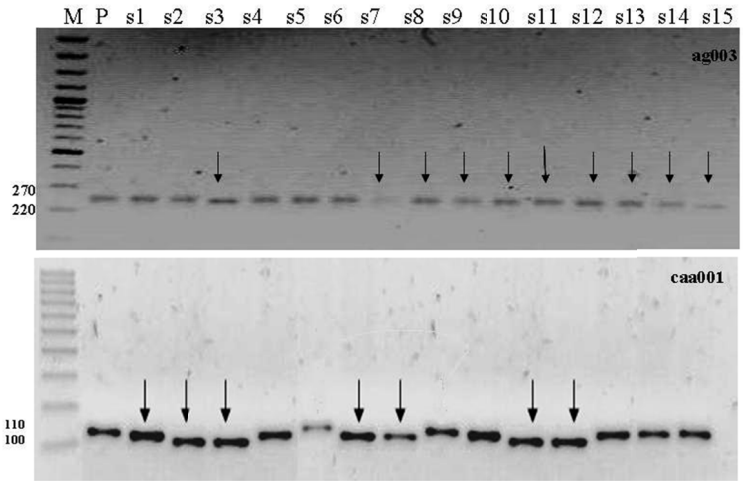


Figure 3. SSR Pattern generated by some SSR primers from genomic DNA of the original cultivar (lane p) and mutants S1 to S15 (lane, 3-17). Lane M contains 100 bp DNA Ladder. Arrowheads indicate polymorphic bands

### *3.3 Combined Genetic Relationships*

The similarity matrices resulting from the combined RAPD and SSR markers data were used to generate genetic relationships among the plants studied. No genetic difference was detected among the mutants S1, S2, S3, S4, S10, S11 and S12. The highest percentage of similarity between the original cultivar and its mutants was detected for S7, S8 (98.4%) followed by 99.7% for S1, S2, S3 and S6. On the other hand, the lowest similarity matrix (82.2%) was obtained for S14 followed by 87% for S13 and 91% for S15. The dendrogram built on the basis of combined data from RAPD and SSR analysis represents the genetic distances among the 15 mutants (Figure 4).

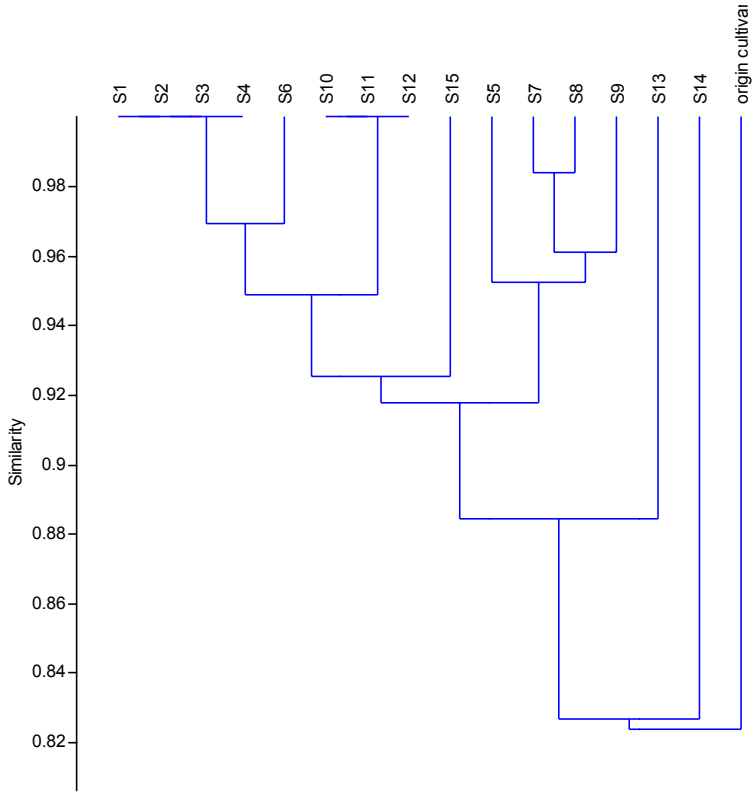


Figure 4. Combined (RAPD, SSR )-based dendrograms of fifteen mutants and their original super strain B cultivar constructed using unweighted pair-group arithmetic average (UPGMA) and similarity matrices computed according to Jaccard coefficient

The dendrogram includes three clusters, the first containing the original cultivar and the second cluster contains S14 while the third cluster contains two sub clusters. The first sub cluster contains S13 and the second is divided into two main groups which contain S5, S7, S8 and S9 and the remaining mutants in the last group. Some of these primers could reveal additional DNA bands, for example OPA10, OPV02 and SSR, ag003 and caa003 were able to detect the extra DNA band while OPX18, OPA 05, OPA 16 and OPQ 14 detected missing DNA bands in some mutants. Similar results in potato callus using RAPD-PCR have been reported by Bordallo et al. (2004). They observed genetic variations after different treatments with plant growth regulators in potato callus based on additional or missing bands detected in the pattern of DNA using some random primers. The finding here also are in the agreements with earlier reports on application of RAPD and SSR in describing genetic polymorphism among regenerated plants in several other plants, viz *Apium* species, *Prunus* species and rice (Isabel et al., 1993; Yang & Quiros, 1993; Khai & Lang, 2005).

In our study, additional or missing DNA bands were detected due to EMS treatments and possibly consequent base substitutions. It has also been documented that if the callus phase is not long enough during plant regeneration, less variation can be expected. For example, Soniya et al. (2001) found that during plant regeneration from tomato plant, more than 90% of regenerated plants had no variation using RAPD marker. In our study, the duration of callus proliferation was not too long, but EMS treatments of the explants could increase the occurrence of mutations.

### *3.4 Field Performance of the Tomato Mutants*

Evaluation was conducted based on morphological traits including plant height, number of branches per plant, average fruit weight, early and total yield, fruit firmness and TSS percentage. Data in Table 3 illustrated that, plant height differed significantly among mutants. The tallest plant was obtained from mutant line S9 with 97 cm. In contrast, the shortest plant was observed in mutant line S13 with 42 cm. Regarding number of branches per plant, significant variation was observed among tomato mutants (Table 3). The highest number of branches per plant was observed in mutants S4, S7, S9 and S13. 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, 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 3. Horticultural characters of tomato mutants and their original cultivar

| Mutants | No. of branches | Plant height (cm) | Early yield (g) | Total yield (g) | Average fruit weight (g) | Fruit firmness ($g/cm^3$) | TSS (%) |
|---|---|---|---|---|---|---|---|
| SSB [z] | 7.0 de | 67.3 e | 323 bcd | 1995 b-f | 88 de | 733.3 a-d | 5.3 ab |
| S1 | 5.3 de | 61.0 fg | 404 a-d | 3662 a | 101 c | 666.7 a-d | 3.7 e |
| S2 | 6.7 de | 66.4 e | 509 a-d | 2660 a-e | 117.3 b | 953.9 a | 3.9 de |
| S.3 | 14.7 ab | 71.4 d | 683 ab | 3243 ab | 102.3 c | 650.0 a-d | 4.1 de |
| S.4 | 16.3 a | 46.6 h | 507 a-d | 1225 f | 64 g | 550.0 b-e | 3.8 de |
| S.5 | 8.7 cd | 62.4 f | 594 abc | 2474 a-f | 59 h | 730.0 a-d | 4.4 cde |
| S.6 | 4.0 e | 61.0 fg | 784 a | 2999 abc | 84 e | 833.3 ab | 5.5 a |
| S.7 | 18.7 a | 71.7 d | 259 cd | 1786 c-f | 46 j | 573.3 bcd | 5.0 abc |
| S.8 | 11.7 bc | 50.0 h | 110 d | 1564 def | 46 j | 510.0 b-e | 4.0 de |
| S.9 | 17.8 a | 97.0 a | 166 d | 2680 a-e | 70 f | 523.3 b-e | 3.9 de |
| S.10 | 8.0 cde | 78.5c | 329 bcd | 2610 a-f | 58 h | 450.0 de | 4.0 de |
| S.11 | 9.0 cd | 57.5 g | 246 cd | 1293 ef | 92 d | 703.3 a-d | 4.5 bcd |
| S.12 | 7.3 cde | 83.2 b | 400 a-d | 2800 a-d | 120 b | 823.3 abc | 5.0 abc |
| S.13 | 17.3 a | 42.0 i | 445 a-d | 3058 abc | 166 a | 650.0 a-d | 4.1 de |
| S.14 | 8.0 cde | 78.4 c | 461 a-d | 2858 a-d | 92 d | 203.3 e | 5.3 ab |
| S.15 | 15.7 ab | 86.3 b | 387 bcd | 1739 c-f | 52 i | 456.7 cde | 4.6 bcd |

Values having common letter in a column do not differ significantly at 5% level as per DMRT.

[z] SSB: Super Strain B cultivar.

Regarding early and total yield, differences among mutants were highly significant for early and total yield (Table 3). The mutant S6 gave the highest early yield. Concerning total yield, fruit yield per plant was significantly influenced by differences in genotypes (Table 3). Mutant S1 gave the highest total yield per plant followed by mutant S3, S13 and S6. Many investigators reported genetic variations among tomato cultivars and hybrids in early and total yield among them Shalaby (2008), Shalaby (2012) and Solieman et al. (2013).

The fruit qualities measured in this study were average fruit weight, fruit firmness and TSS content. Differences among mutants were highly significant for the three characters (Table 3). Mutant S13 produced the heaviest fruit weight while mutant S7 and S8 produced the smallest ones. The present result confirmed the earlier reports of Wagh et al. (2004) where they reported genotypic variations in tomato yield and average fruit weight. The mutant S2 had the highest fruit firmness value and the mutant S14 had the lowest value. Firmness is the most important factors for determination of tomato quality (Burton, 1982), and firmness may be the final index by which the consumers decide to purchase a given batch of tomatoes (Gormley & Egan, 1978). TSS percentage ranged from 3.7 for the mutant S1 to 5.5 for mutant S6.

The highly significant differences between the mutants and the original cultivars in the most traits indicates 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 Mansour et al. (2009), Soleiman et al. (2013) who found that the mean values of cultivars showed relatively significant wide ranges of genetic variability among tomato cultivars for the most studied traits.

## 4. Conclusion

On the basis of the conducted investigations, we can conclude that the treatment of cotyledon explants by ethyl methane sulfonate (EMS) influenced plantlets formation. Genetic variations were observed among regenerated plants through somatic embryogenesis. The study also shows the use of RAPD and SSR markers in revealing variations in tomato and the occurrence of specific bands/ loci in the mutants may be used in the genetic identification of the mutant lines which were morphologically superior and physiologically efficient. The results of field evaluation indicated that the mutant S1, S6, S3 and S13 had some desirable horticultural traits like early and total yield and mutant S2 for fruit firmness and could be used in improving tomato crop by breeding programs or they could be considered as new breeding lines.

## References

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. http://dx.doi.org/10.1079/IVP2005649

Bordallo, P. N., Silva, D. H., Maria, J., Cruz, C. D., & Fontes, E. P. (2004). Somaclonal variation on *in vitro* callus culture potato cultivars. *Hortic Bras, 22*, 300-304. http://dx.doi.org/10.1590/S0102-05362004000200027

Burton, W. G. (1982). Ripening and senescence of fruits. In W. G. Burton (Ed.), *Post-Harvest Physiology of Food Crops* (pp. 181-198). Logman Group Ltd.

Chen, J., Wang, H., Shen, H., Chai, M., Li, J., Qi, M., & Yang, W. (2009). Genetic variation in tomato populations from four breeding programs revealed by single nucleotide polymorghism and simple sequence repeat markers. *Scientia Hortic, 122*, 6-16. http://dx.doi.org/10.1016/j.scienta.2009.03.025

Compton, M. E., & Veilleux, R. E. (1991). Shoot, root and flower morphogenesis on tomato inflorescence explants. *Plant Cell Tiss. Org. Cult., 24*, 223-231. http://dx.doi.org/10.1007/BF00033481

Costa, M. G., Nogueira, F. T., Figueira, M. L., Otoni, W. C., Brommonschenkel, S. H., & Cecon, P. R. (2000). Influence of the antibiotic timentin on plant regeneration of tomato (*Lycopersicon esculentum* Mill.) cultivars. *Plant Cell Rep., 19*, 327-332. http://dx.doi.org/10.1007/s002990050021

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus, 12*, 13-15.

Evans, D. A. (1989). Somaclonal variation-genetic basis and breeding applications. *Trends Genet, 5*, 46-50. http://dx.doi.org/10.1016/0168-9525(89)90021-8

Gormley, L., & Egan, S. (1978). Firmness and colour of fruit at some tomato cultivars from various sources during storage. *J. of Science of Food and Agric., 29*, 534-538. http://dx.doi.org/10.1002/jsfa.2740290607

Giovannucci, E. (1999). Nutritional factors in human cancers. *Adv. Exp. Med. Biol., 472*, 29-42. http://dx.doi.org/10.1007/978-1-4757-3230-6_3

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. http://dx.doi.org/10.3923/ajbkr.2010.178.184

Isabel, N., Tremblay, L., Michaud, M., Tremblay, F. M., & Bousquet, I. (1993). RAPDs as an aid to evaluate the genetic integrity of somatic embryogenesis- derived populations of *Picea mariana* (Mill) BSP. *Theor Appl Genet., 86*, 81-87. http://dx.doi.org/10.1007/BF00223811

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

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

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. *African J. Plant Sci. and 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.

Moustafa, R. A., Duncan, D. R., & Widholm, J. M. (1989). The effect of gamma radiation and N-ethyl-Nnitrosourea on cultured maize callus growth and plant regeneration. *Plant Cell Tiss Org Cult., 17*, 121-132. http://dx.doi.org/10.1007/BF00046857

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and Bioassys with tobacco tissue culture. *Physiol Plant, 15*, 473-497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

Rashid, R., Bal, S. S., Kaur, A., Gosal, S. S., Itoo, H., & Bhat, J. A. (2010). Effect of growth regulators in direct shoot regeneration in tomato. *Int. J Biotech and Biochem., 6*, 81-86.

Rick, C. M. (1980). *Tomato: In: Hybridization of Crop Plant*. Am Soc Argon 667 S. Segoe road, Madison 669-680.

Shalaby, T. A. (2008). Combining ability and heterosis in a half diallel cross of tomato in north Egypt. *J. Agric. Res. Kafrelsheikh Univ., 34*, 1110-1125.

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

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. http://dx.doi.org/10.3329/bjar.v36i2.9259

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.). *Scientia Hort., 150*, 25-30. http://dx.doi.org/10.1016/j.scienta.2012.10.024

Soniya, E. V., Banerjee, N. S., & Das, M. R. (2001). Genetic analysis of somaclonal variation among callus derived plants of tomato. *Curr. Sci., 80*, 1213-1215.

Svetleva, D. L., & Crino, P. (2005). Effect of Ethyl Methaesulfonate (EMS) and N-nitose-N´-Ethyl Urea (ENU) on callus growth of common bean. *J. of Central European Agric., 6*, 59-64.

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.

Yang, X., & Quiros, C. (1993). Identification and classification of celery cultivars with RAPD markers. *Theor Appl Genet., 86*, 205-21.