Genetic analysis and RAPD markers for drought tolerance in tomato genotypes

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Abstract

Genetic parameters, heritabilities and genetic variability were assessed in five tomato genotypes and their F_1 crosses using phenotypic data and RAPD markers under normal and drought stress conditions. The results showed that mean squares of the genotype by environment (GxE) interaction were found to be highly significant for all studied traits, suggesting a differential response of studied genotypes to drought stress. The magnitudes of σ^2_A were higher in magnitude than those of σ^2_D for most cases indicating that the additive gene action played a major role in the inheritance of these traits. Moreover, the interaction of σ^2_AxE was less than σ^2_DxE for most studied traits, suggesting that the additive gene effect was more stable over the environments than non-additive effect. These results were verified by the estimates of the broad- and narrow-sense heritability obtained for different traits. The RAPDs analysis showed that 31 out of 57 bands were polymorphic. The percentage of polymorphism ranged from 20 (OPP-05) to 100% (OPA-03). The UPGMA cluster analysis based on RAPD markers separated the genotypes into two different clusters, while the dendrogram based on phenotypic data divided into two clusters. The Polymorphism information content (PIC) values varied from 0.10 (OPB-01) to 0.46 (OPA-03).

Introduction

Tomato (*Solanum lycopersicum* L.) is a plant species cultivated worldwide in greenhouses as well as in open fields. Tomato plants belong to the *Solanaceae* family and produce fruit of different sizes and colors. Tomato fruits are popular for their versatile use when consumed fresh as well as their suitability for canning and sauce production. Tomato is a rich source of vitamin A, C and minerals like Ca, P and Fe (Dhaliwal *et al*. 2003). Tomato fruits are major contributors of antioxidants such as carotenoids, phenolics, ascorbic acid and small amounts of vitamin E in daily diets (Rai *et al*. 2012). Drought is one of the most important abiotic constraints in plant production. The most effective way to stabilize and improve tomato production under drought stress conditions is to improve the varieties for drought tolerance. However, breeding for this trait is particularly challenging

because of the variability in the timing, duration and intensity of drought, the genetic complexity of drought tolerance, and the large genotype by environment interactions affect the expression of the trait. Information pertaining to different types of gene action, relative magnitude of genetic variance, and combining ability estimates are important and vital parameters to mould the genetic makeup of tomato crop. This important information could prove an essential strategy to tomato breeders in the screening of better parental combinations for further enhancement. The entire genetic variability observed in the analysis for each trait was partitioned into its components, i.e. general (GCA), specific combining ability (SCA) and reciprocal effects (Sprague 1966; Griffing 1956). They stated that GCA effects were due to additive type of gene action and SCA effects were due to dominant or epistatic gene action. Several studies of combining ability for yield components are available in many species. Khan *et al*. (1991) and Yaqoob *et al*. (1997) found the predominancy of GCA to be more important than that of SCA, while Ortiz (2004) and Biswas *et al*. (2005) suggested that SCA was more important. Thus crossing in a diallel fashion is the only specific and flourishing approach of measurement for the identification and selection of superior genetically recombined material. Molecular markers have opened a new vista to study genetic diversity; these markers have the potential to reveal a large amount of variation with good coverage of the entire genome. One of such techniques is the use of RAPD (Williams *et al.* 1990). The advantages of RAPD are its simplicity, rapidity, requirement for only a small quantity of DNA, and the ability to generate numerous polymorphisms (Cheng *et al.* 1997) with good coverage of the entire genome (Melchinger, 1993). RAPD markers have been widely used in several important plants including Barley (Hoffman *et al*. 2003), Cotton (Dongre and Parkhi, 2005), Sorghum (Jeya *et al.* 2006), Faba bean (Tanttawi *et al.* 2007), Cowpea (Abdelsabour *et al*. 2010), Wheat (Khaled *et al.* 2015) and Tomato (Ezekiel *et al.* 2011; Sharifova *et al.* 2013 and Elsharief *et al*. 2015). The current study was carried out to: 1) evaluate five tomato genotypes on order to ascertain the relative performance regarding combining ability effects for yield and some other traits under normal and drought stress conditions and 2) assess the genetic variability among parental genotypes based on phenotypic data and RAPD markers.

Materials and Methods

Plant materials

Field experiments were conducted at the Experimental Farm of Faculty of Agriculture, Sohag University, Sohag Province, Egypt in two consecutive winter seasons 2013-2014 and 2014- 2015. Five tomato genotypes (Table 1), i.e.,

Super marmande (P_1), Qaha (P_2), Super strain-B (P_3), Castle Rock (P_4) and Cherry (P_5) were used in the study.

Field experiment

In 2013/2014 growing season, a half-diallel mating design was made among the five tomato genotypes to produce 10 F_1 hybrids. In 2014/2015 growing season, seeds of the five parental genotypes and their F_1 hybrids were sown in nursery and after 6 weeks, the seedlings were transplanted in sandy-clay soil of an open field in two experiments. The first experiment was grown under supplemental water applied regularly as recommended (Normal environment ‘‘N’’) while, the second experiment received half of the number of irrigation (drought stress environment ‘‘D’’) compared to the first experiment. Each experiment was evaluated in a randomized complete block design (RCBD) with three replications.

Morphological traits and measurements for each replicate, field data were recorded on five randomly selected plants for: **Earliness (ER),** number of days from seedlings transplanted to the opening of flowers at 50% of plants in each plot; **Plant height (cm),** the plant height was recorded in centimeters (cm) at the end of the growing season; **Number of Fruits per plant (NFR/P),** the average numbers of fruits per plant produced by the five plants in each replicate to the end of harvest; **Fruit diameter (FD, cm),** the average of diameter of all fruits produced by the five plants and **Fruits yield per plant (FY/P, kg),** the average weight of fruits per plant from the first to the end of harvest season.

Statistical analysis

Analysis of variance

Phenotypic data of parental genotypes and their hybrids were subjected to general analysis of variance for the Randomized Complete Block Design (RCBD) according to Steel and Torrie (1980).

General combining ability (GCA) and specific combining ability (SCA) were partitioned from total genetic variance in each experiment according to Griffing (1956) method 2. In addition, the combined analysis over the two environments was calculated to partition the mean squares of genotypes and the genotypes by environments (GxE) interaction into

Mean squares of genotypes and replications for all studied traits were tested for significance according to the F-test. The analysis of variation (S.O.V) was applied according to Cochran and Cox (1957).

Gene action analysis

sources of variations due to GCA, SCA, GCA x E, SCA x E. The genetic components were obtained according to Matzinger and Kempthorne (1956).

Heritability estimates

Estimates of broad-(h^2_B) and narrow-sense (h^2_S) heritability were calculated according to the following equations:

- Four each environment: $h^2_B = [(\sigma^2A + \sigma^2D) / (\sigma^2A + \sigma^2D + \sigma^2e)] \times 100$

$h^2_N = [(\sigma^2A) / (\sigma^2A + \sigma^2D + \sigma^2e)] \times 100$

- Four combined data: $h^2_B = [(\sigma^2A + \sigma^2D) / (\sigma^2A + \sigma^2D + \sigma^2AxE + \sigma^2DxE + \sigma^2e)] \times 100$

$h^2_N = [(\sigma^2A) / (\sigma^2A + \sigma^2D + \sigma^2AxE + \sigma^2DxE + \sigma^2e)] \times 100$

Drought susceptibility index (DSI):

Drought susceptibility index (DSI) was calculated for fruits yield per plant according to the method of Fischer and Maurer (1978). Genotypes with DSI≥1.0 were considered as susceptible to drought. Genotypes with DSI<1.0 were rated as relatively tolerant (less susceptible to drought). Meanwhile, a value of DSI=0 indicates maximum drought tolerance (no effect of drought on yield).

RAPD markers:

DNA extraction

Fresh young leaves of parental tomato plants were harvested and immediately ground in extraction buffer using cetyltrimethyl ammonium bromide (CTAB) protocol as described by Poresbski *et al*. (1997). A total of twenty three varied 10-mer random primers (Metabion International AG, Germany) were scanned across the five parental genotypes.

PCR procedures

Amplification was carried out in a DNA Thermal Cycler (Primus 25, Germany) according to the methods described by Williams *et al*. (1990). The RAPD assay was performed in a 25 µl volume containing 12.5 µl of Go Taq® Green Master Mix (Promega, Madison, USA), 2.5 µl of primer 5 pmol, 7 µl of nuclease-free water and 3 µl of 150 ng genomic DNA templates. PCR amplification was programmed for conditions with an initial denaturation cycle at 94°C for five minutes. The following 35 cycles were composed of: denaturation step at 94°C for 1 min, annealing step at 38°C for 1 min 30 sec and elongation step at 72°C for 2 min 30 sec. The final cycle of polymerization was performed at 72°C for 7 min. The amplification products were electrophoresed in a 1.0% agarose gel stained with 0.2 µl ethidium bromide. The amplified fragments were visualized and photographed using UVP Bio Doc-It imaging system (USA).

Data of RAPDs analysis

The DNA banding patterns generated by RAPDs were analyzed by Gene Profiler software (version 4.03). The presence (1) or absence (0) of each band was recorded for each tomato genotype for all the tested primers. To measure the informativeness of the RAPD markers in differentiating among five tomato genotypes, polymorphism information content (PIC) was calculated according to the formula of Ghislain *et al*. (1999). Genetic similarity estimates for RAPDs were determined using Jaccard's coefficient (Jaccard 1908). A cophenetic matrix was derived from each matrix to test goodness of fit of the clusters by comparing the

two matrices using the Mantel test (Mantel, 1967). Dendrograms were generated with the unweighted pair group method with arithmetic mean (UPGMA) algorithm using the computational package MVSP version 3.1. Finally, the correlation between each distance pair was calculated using NTSYS-pc version 2.2 (Rohlf, 2000).

Results and Discussion

Analysis of variance (ANOVA)

The combined ANOVA (Table 3) showed a highly significant difference ($P<0.01$) between the two environments for number of fruits per plant (NF/P) and fruits yield per plant (FY/P) traits, while there was no significant difference for earliness (ER), plant height (PH) and fruit diameter (FD) traits. Whereas, highly significant differences ($P<0.01$) were found among the genotypes for all studied traits, revealing a large amount of variability among studied genotypes. Moreover, mean squares due to genotype×environment (G×E) interaction were also highly significant ($P<0.01$) for all studied traits except earliness, revealing that genotypes were inconsistent from an environment to another. Generally, the results of this study showed that mean squares of G x E interaction were found to be highly significant for most studied traits. This finding suggested a differential response of the genotypes from an environment to another. Similar results were obtained by Kurian *et al.* (2001); Saleem *et al.* (2009); Ghobary *et al.* (2010) and Saleem *et al.* (2013).

Mean performance

The results cleared that the mean performance of all studied traits for the five parents and their ten F_1 hybrids (Table 3) were varied from normal to drought stress conditions. For earliness trait, the parental genotypes had a number of days to flowering ranged from 34.67 to 53 days for Cherry (P_5) and Qaha (P_2), respectively under normal conditions. Moreover, P_5 was found to be the earliest parent with the mean value of 33.0 days under drought conditions. Concerning F_1 hybrids, the mean performance of ER were narrower than their parents ones under both environments and their combined data. The mean values of plant height trait for the five parents ranged from 43.00 (P_5) to 57.27cm (P_2), from 41.73 (P_5) to 56.07 cm (P_1) and from 42.37 (P_5) to 54.83 cm (P_2), under normal, drought stress conditions and their combined data respectively. The mean performance of F_1 hybrids for plant height trait ranged from 45.72, 46.67 and 45.97 cm and from 76.47, 81.73 and 79.10 cm for the combinations P_1xP_5 and P1xP2 under normal, drought conditions and their combined data, respectively.

The mean performances of number of fruits per plant (NF/P) were displayed by the parental genotype Cherry (P_5), with the mean performances of 93.49, 92.33 and 92.83, under normal, drought stress conditions and from combined data, respectively. On the other hand, the

parental genotype Super strain-B (P_3) had the lowest mean performances of 18.73, 10.67 and 14.70, under normal, drought stress conditions and their combined data, respectively. The hybrids namely P_1xP_5 and P_1xP_2 were the best combinations for NFR/P trait and exhibited the highest mean performances. The mean of fruit diameter (FD) ranged from 2.40 to 5.88, from 2.33 to 5.91 and from 2.36 to 5.89 for parental genotypes P_2 and P_3 under normal, drought stress conditions and their combined data, respectively. Concerning F_1hybrids, the combinations P_3xP_4 and P_1xP_4 recorded the highest values of mean performances (Table 3).

The mean performances of fruits yield per plant (FY/P) were variable from normal and drought stress conditions (Table 3). For the five parental genotypes, it could be noticed that the mean of fruits yield per plant ranged from 4.00 (Cherry, P_5) to 5.06 kg (Super strain-B, P_3) under normal conditions. Whereas, Cherry (P_5) had the highest fruits yield per plant with a mean of 3.94 kg. The mean performances of F_1 hybrids for FY/P trait ranged from 3.94 (P_1xP_5) to 5.33 kg (P_2xP_3), from 3.17 (P_1xP_2) to 3.97 kg (P_3xP_4 and P_3xP_5), and from 3.71 (P_1xP_2) to 4.62 kg (P_2xP_3) under normal, drought stress conditions and their combined data, respectively.

Drought susceptibility index (DSI)

Drought susceptibility index (DSI) values for the parental genotypes ranged from 0.08 (Cherry) to 1.88 (Super strain-B) (Table 3). Regarding to F_1 hybrid the results showed that DSI ranged from 0.20 (P_1xP_5) to 1.44 (P_2xP_3). It could be noticed that the genotypes Cherry (P_5), Super Marmande (P_1), (P_1xP_5) and (P_3xP_5) were relatively tolerant (DSI values < 1) and high grain yield by 3.94, 3.62, 3.79 and 3.97 kg, respectively under drought stress compared to the mean overall studied genotypes. While, genotypes Qaha, Super Strain-B, Castle Rock, (P_1xP_2) and (P_1xP_3) were susceptible to drought (DSI > 1). Drought susceptibility index (DSI) is a measure of yield stability (Ahmad *et al.* 2003). DSI actually provides a measure of yield stability based on minimization of yield loss under stressed, compared to non stressed conditions rather than on yield level under dry conditions per se (Clarke *et al.* 1984; Clarke *et al*. 1992).

General combining ability effects (g_i)

Estimates of general combining ability effects (g_i) of each parent are presented in Table 4. The results showed that the genotype P_5 exhibited negative and highly significant general combining ability effects toward earliness. As for plant height, the genotype P_1 and P_2 were found to be good general combiners toward tallness under all conditions. The genotype P_5 proved to be a good general combiner for number of fruits per plant. Also, the genotypes P_3 and P_4 seemed to be the best general combiners for fruit diameter, while, the other parents were the poorest general combiners for this

trait. Concerning fruit yield per plant trait, the genotype P_3 proved to be a good general combiner under normal condition. It is interesting to notice that the majority of parental genotypes possessed more desirable additive genes for studied traits. These promising genotypes could be utilized in tomato breeding program to improve these traits.

Specific combining ability effects (s_{ij})

The results of specific combining ability effects (Table 5) showed that the crosses P_2xP_3 and P_2xP_4 exhibited desirable and significant SCA effects for earliness under normal condition. While, the crosses P_1xP_3 and P_1xP_4 were the best crosses towards earliness under drought stress condition. The results indicated that three (P_1 x P_2, P $_3$x P_4 and P_3 x P_5) out of the ten crosses were the most promising crosses for tallness. Also, it could be observed that three (P_1 x P_2, P_2 x P_3 and P_3 x P_4) out of the ten crosses were the best hybrids for NF/P. The cross P_1 x P_2 exhibited desirable and significant SCA effects for FD under the two environments, while the cross P_2 x P_5 was the best cross. Regarding FY/P, the crosses P_2 x P_3 and P_3 x P_4 had desirable and significant SCA effects for increasing yield trait. Moreover, the crosses P_1 x P_2 and P_4 x P_5 were the promising hybrids under the normal environment.

It could be observed that the promising tomato hybrids which showed desirable SCA effects revealed as previously mentioned high estimates of heterosis (data not shown). It is also interesting to notice that the best cross combinations were obtained from (good x good), (good x poor) and (poor x poor) generals combiners. Consequently, it is not necessary that parents having high estimates of GCA effects would also give high estimates of SCA effects in their respective cross combinations. These finding are in accordance with those obtained by Kumer *et al.* (2013).

Analysis of combining ability:

Diallel cross mating design is a type of mating system which assists and enables plant breeders to obtain estimates for general combining ability (GCA) and specific combining ability (SCA). These estimates could be translated into additive genetic variance (σ2A) and non additive genetic variance (σ^2D). This information is great important to plant breeders, since the relative magnitudes of each component denote the most suitable breeding programs which could be used. When the total genetic variance for a given trait is mainly additive in nature, it applies that selection would be effective in improving the performances of selective varieties. On the other hand, when non additive genetic variances are the most important components, hybridization and production of F_1 hybrids would be the proper.

Combining ability analysis of variance for all studied traits (Table 6) showed that GCA and SCA mean squares were highly significant under the two environments, confirming the important role of all

types of gene action in the expression of this trait. However, the ratio of GCA/SCA was found to be greater than the unity for all studied traits under the two environments and their combined data. Moreover, the interactions of GCA x E and SCA x E mean squares were highly significant for all traits except FD trait, revealing that the magnitude of all types of gene action fluctuated from normal environment to drought stress conditions. Furthermore, the ratio of GCA x E/ SCA x E was more than one for all studied trait except NF/P, suggesting that the non-additive effect was more stable over the environments than additive one.

In general, the results of combining ability analysis of variance showed the importance of both GAC and SCA mean squares in the inheritance of all studied traits under each environment and for the majority of these traits under combined data. It could be noticed that the interactions of GCA x E and SCA x E mean squares were highly significant for most cases indicating that the genetic behavior of the genotypes under this study was fluctuated from normal environment to drought stress conditions. Our results were in harmony with those previously obtained by Bhatt *et al*. (2001); Hosseny (2002); Hannan *et al*. (2007); Mondal *et al*. (2009); Ghobary *et al*. (2010); Kansouh and Zakher (2011); Kumar *et al*. (2013); Saleem *et al*. (2013) and Figueiredo *et al*. (2015).

Genetic parameters

According to the half diallel cross mating design, the different types of the genetic variances could be translated into genetic parameters with respect to additive genetic variance (σ^2A) and non-additive genetic variance (σ^2D). Therefore, the general combining ability variance (σ^2g) is an indicator for σ^2A. While, the specific combining ability variance (σ^2s) is an estimate for non-additive genetic variance including dominance (σ^2D).

The results of genetic parameters for ER and FD traits (Table 8) showed that the magnitudes of σ^2Awere higher than those of σ^2D, indicating that additive gene action played a major role in the inheritance of earliness trait. Moreover, the interaction of σ^2A x E was less than σ^2D x E suggesting that the additive effect was more stable over the environments than non-additive one for the two traits. The estimates of the heritability in broad sense (h^2_{BS}) were larger in magnitude than those of the heritability in narrow sense (h^2_{NS}) for these traits. This finding ensures the predominance of σ^2Aover the σ^2D for this. Concerning to plant height, the results indicated that the magnitudes of σ^2A were lower than those of σ^2D, revealing the importance of non-additive gene action in the inheritance of this trait. Furthermore, the interaction of σ^2A x E was less than σ^2D x E exhibiting that the additive effect was more stable over the environments than non-additive one. Therefore, the estimates of h^2_{BS} were larger than

those of h^2_{NS} (Table 8). Thus, the σ^2D was important than additive one for inheritance of this trait under each environment and combined data.

The results of NF/P trait cleared that the magnitudes of σ^2A were higher than those ofσ^2D, indicating that additive gene action played a major role in the inheritance of this trait. Whereas, the estimate of σ^2D was higher than those of additive one under normal environment. The interaction of σ^2A x E was less than σ^2D x E suggesting that the additive effect was more stable over the environments than non-additive one. The estimates of h^2_{NB} were larger than those of h^2_{NS} under each environment and combined data. The values of h^2_{BS} were 48.11%, 56.18% and 52.75% under normal, drought stress and their combined data, respectively. These results cleared the importance of σ^2A in the inheritance of this trait.

Concerning the FY/P, the magnitudes of σ^2D were higher than those of σ^2A, revealing that non-additive gene action played a major role in the inheritance of this trait. Whereas, the estimate of σ^2Awas higher than those of non-additive one under normal environment. The interaction of σ^2A x E was higher than σ^2D x E one suggesting that the non-additive effect was more stable over the environments than additive one. The estimates of h^2_b%were larger than those of h^2_n%ones. The values of narrow sense heritability were 56.94%, 12.72% and 10.34% under normal, drought stress and their combined data, respectively.

Generally, it could be regarded that the magnitudes of σ^2A were higher than those of σ^2D for most cases indicating that additive gene action played a major role in the inheritance of these traits under both environments as well as the combined data. Moreover, the interaction of σ^2A x E was less than σ^2D x E for most studied traits, suggesting that the additive effect was more stable over the environments than non-additive gene effect. These results were verified by estimates of the broad- and narrow-sense heritability. In the same direction, the findings of Younis *et al*. (1987) for plant height and Pratta *et al*. (2003) for number of flowers per cluster; illustrated that additive gene effects were found to be more important than non-additive gene effects. Also, Hosseny (2002) illustrated that SCA variance was greater than GCA for plant height and total yield traits. Likely, Dagade *et al*., (2015) showed that the variance due to GCA as indicator for additive gene action was more pronounced for fruit weight per plant. On the other hand, El-Gabry *et al*. (2014) found that the non-additive gene effects played more important roles than additive gene effects in the inheritance of plant height, number of fruits per plant and fruit weight.

RAPD markers analysis

Level of polymorphism based on RAPDs

In the present study, five tomato genotypes were differentiated using 23 RAPD primers, out of them, 10 primers generated different degrees of percentage of polymorphism (%P) (Figures 1). In this study, the number of amplification products per primer varied from 3 to 8, with an average of 5.7 per primer. The number of polymorphic bands ranged from 3 (OPA-10) to 8 (OPA-03, OPA-08 and OPB-09) with an average of approximately 3.10 bands per primer (Table 8). Similarly, many of authors obtained a variant number of RAPD bands which ranged from 3 to 8 (Manoj *et al.* 2006); from 2 to 8 (Thamir *et al.* 2014) and from 2 to 21 per primer (Muhammed *et al.* 2015). The bands size ranged from 350 bp (OPB-01 and OPA-03 primers) to 1500 bp (OPW-13). It was found that fragments sizes in the present study are shorter than those obtained by Rajput *et al*. (2006) and Mansour *et al.* (2010) which ranged from200 to 3000 bp and from 200 to 2000 bp, respectively.

Thirty one out of 57 amplified bands were scored polymorphic. The %Pranged from 20% (OPP-05) to 100% (OPA-03) with an average of 51.44% (Table 8).In this regard, Manoj *et al.*, (2006) obtained percent of polymorphism (%P) of 33.3% between 10 tomato genotypes. Ezekiel *et al.* (2011) recorded a 62.2% level of polymorphism using 74 amplified products.While, Nadra *et al*. (2013) recorded a high level of polymorphic bands (94.168%) using 20 RAPD primers for RAPD analysis applied on 11 tomato varieties. Contrary, Mavromits *et al.* (2013) and Elsharief *et al*. (2015) obtained a low of polymorphic bands of 37.77% and 39%, among 7 and 3 tomato genotypes, respectively.

Correlation between RAPD and morphological markers

Correlation between the two distance matrices generated by morphological traits and RAPD marker was found to be insignificant ($r = 0.025 p = 0.54$). This result supported that the observed relationships using molecular markers may provide information on the history and biology of the cultivars but it does not necessarily reflect what may be observed with respect to agronomic traits (Metais *et al.* 2000). A not significant correlation between phenotypic data and RAPD markers was obtained by Tanttawi *et al.* (2007) and Obiadalla-Ali *et al.* (2015) in faba bean. Genetic markers like RAPDs may accurately assay the degree of genetic change between two genomes, but they may not necessarily reflect the divergence in terms of changes in traits of agronomic importance.

Polymorphism information content (PIC) and Marker index (MI)

The Polymorphism information content (PIC) index has been used extensively in many genetic diversity studies (Tatikonda *et al.* 2009; Talebi *et al.* 2010; Thudi *et al.* 2010). Moreover, the PIC value of markers indicates the usefulness of DNA markers for gene mapping,

molecular breeding and germplasm evaluation (Peng and Lapitan, 2005). In this study, the PIC values for the 10 RAPD primers were varied from 0.10 to 0.46 with an average of 0.24. The lowest and highest PIC indices were recorded for OPB-01 and OPA-03, respectively (Table 8). The Marker index (MI) values ranged from 0.10 to 3.86 for OPB-01 and OPA-03, respectively with an average of 0.96. Our results of PIC and MI are in agreement with those obtained by Khaled *et al.* (2005).

Cluster analysis

The genetic similarity coefficients among the five tomato genotypes were calculated according to the analytical results of electrophoretic band patterns (Table 9, below diagonal) and means of all studied traits (Table 9, above diagonal), and were used for UPGMA cluster analysis. Cluster analysis realized using Nei and Li`s coefficient for the data of RAPD markers revealed similarity coefficient values ranged from 0.69 (Super Marmande and Super Strain-B) to 0.88 (Super Marmande and Qaha) with an average of 0.80%. These results in accordance with the results of Archak *et al.,* (2002) and Comlekcioglu *et al*., (2010), they studied the genetic diversity among some tomato genotypes and showed that the overall high levels of similarity was 0.83 and 0.87, respectively. Contrary, Sharifova *et al*., (2013) obtained different values of similarity, ranged from 0.188 to 1.000. The UPGMA cluster analysis based on the RAPD markers separated the studied genotypes into two different clusters (Figure 2A). The first cluster contained the genotypes Super Marmande and Qaha, branched at high level of similarity of 0.875. The second cluster was sub-divided into two sub-clusters. The first sub-cluster contained the genotypes Cherry and Castle Rock, branched at 0.836 level of similarity, while the genotype Super Strain-B was placed in the second sub-cluster.

Cluster analysis realized using the means of all studied traits revealed similarity coefficients ranged from 49.19% (S. Strain-B and Cherry) to 97.03 (S. Strain-B and Castle Rock) with an average of 75.53%. The dendrogram based on the phenotypic data of the studied traits separated the tomato genotypes into two clusters (Figure 2B). The first cluster contained only one genotype, namely Cherry. The second cluster was sub-divided into two sub-clusters, the genotypes Super Strain-B and Castle Rock were placed in the first sub-cluster, branched at 91.92% with the genotype Qaha.

The second sub-cluster contained Super Marmande which branched at 85.59% with the genotypes belonged to the second cluster. Likely, Elsharief *et al*. (2015**)** showed similar results of genetic relationship among four tomato genotypes. In this study, Cherry tomato belongs to separate group related to the main group that correlates the three other genotypes with a percentage of 55.15%.

Table 1: List and data of the five tomato cultivars used in the study.

Genotypes	Origin	Growth habit	Genotypes
1	Super marmande (P1)	Daehnfeldt, Holland	Semi-determinate
2	Qaha (P2)	Qaha, Qalybia, Egypt	Determinate
3	Super strain-B (P3)	Sun seed, Parma, Idaho USA	Determinate
4	Castle Rock (P4)	Castle Seeds, USA	Determinate
5	Cherry (P5)	Aztec, Mexico	Determinate

Table 2: Analysis of variances and mean squares of the five parents and their F_1 hybrids for studied traits under normal (N), drought (D) conditions and combined data (C).

S.V	D.F		Mean squares														
			ER			PH			NF/P			FD			FY/P		
	S	C	N	D	C	N	D	C	N	D	C	N	D	C	N	D	C
Env.	---	1	---	---	3.21	---	---	1.444	---	---	321.87**	---	---	0.26	---	---	17.09**
Rep.	2	---	0.82	5.09	---	4.64	0.20	---	11.81	2.005	---	0.12	0.09	---	0.0001	0.0022	---
Rep. /Env.	---	4	---	---	2.96	---	---	2.423	---	---	6.91	---	---	0.12	---	---	0.003
Genotypes	14	14	54.76**	71.56**	123.48**	166.31**	217.14**	370.90**	1059.77**	1224.049**	2255.95**	2.58**	2.43**	4.76**	0.64**	0.235 **	0.47**
G x E	---	14	---	---	2.83	---	---	12.55**	---	---	27.87**	---	---	0.23**	---	---	0.40**
Error	28	56	1.61	1.66	1.63	3.59	2.43	3.01	5.58	5.00	5.29	0.05	0.053	0.06	0.03	0.0157	0.02

Significant at 5% and 1% levels of probability, respectively.

S.V, source of variation; D.F, degrees of freedom; S, single environment; ER, earliness; PH, plant height; NF/P, number of fruits per plant; FD, fruit diameter, FY/P, fruits yield per plant and DSI, drought susceptibility index.

Table 3: Mean performance of the five parents and their F_1 hybrids for all studied traits under both conditions as well as the estimates of DSI.

Traits	**ER (day)**			**PH (cm)**			**NFR/P**			**FD (cm)**			**FY/P**			
Genotypes	**N**	**D**	**C**	**N**	**D**	**C**	**N**	**D**	**C**	**N**	**D**	**C**	**N**	**D**	**C**	**DSI**
P_1	52.00	52.67	52.33	50.27	56.07**	53.17	38.27**	28.93	33.60**	4.04	4.05	4.04	4.34	3.62	3.98	0.89
P_2	53.00	51.67	52.33	57.27**	52.40	54.83**	25.67	18.27	21.97	3.20	3.59	3.40	4.38	3.18	3.78	1.48
P_3	50.67**	52.67	51.67	55.07	52.33	53.70	18.73	10.67	14.70	5.88**	5.91**	5.89**	5.06**	3.29	4.17**	1.88
P_4	50.33**	49.67**	50.00*	54.40	51.93	53.17	21.47	11.80	16.63	5.11**	5.24**	5.17**	4.93**	3.46	4.20**	1.61
P_5	34.67**	33.00**	33.83**	43.00	41.73	42.37	93.33**	92.33**	92.83**	2.40	2.33	2.36	4.00	3.94**	3.90	0.08
P_1XP_2	51.33	50.67*	51.00	76.47**	81.73**	79.10**	39.20**	38.00**	38.60**	4.09	4.12	4.11	4.26	3.17	3.71	1.37
P_1XP_3	51.33	50.33**	50.83	52.33	51.33	51.83	23.60	18.13	20.87	4.73**	4.50**	4.62**	4.19	3.31	3.75	1.13
P_1XP_4	51.00*	49.67**	50.33	54.13	53.47	53.80	20.53	17.00	18.77	5.00**	4.77**	4.88**	4.23	3.55	3.89	0.86
P_1XP_5	49.33**	49.33**	49.33**	45.27	46.67	45.97	42.20**	44.73**	43.47**	3.48	3.23	3.35	3.94	3.79**	3.87	0.20
P_2XP_3	50.33**	52.00	51.17	52.33	51.40	51.87	23.60	18.00	20.80	4.09	3.88	3.98	5.33**	3.90**	4.62**	1.44
P_2XP_4	50.33**	50.00**	50.17	54.13	53.47	53.80	20.53	16.87	18.70	4.00	4.13	4.06	4.23	3.54	3.88	0.87
P_2XP_5	47.67**	44.33**	46.00**	50.33	52.07	51.20	23.07	24.07	23.57	3.56	3.48	3.52	4.07	3.54	3.80	0.69
P_3XP_4	50.33**	51.00	50.67	56.00**	55.80**	55.90**	19.60	21.73	20.67	5.36**	5.11**	5.23**	5.22**	3.97**	4.60**	1.29
P_3XP_5	50.00**	49.00**	49.50**	52.07	53.80	52.93	29.73	21.27	25.50	4.78**	3.45	4.12	4.31	3.97**	4.14**	0.42
P_4XP_5	50.00**	50.67*	50.33	48.93	51.60	50.27	26.33	27.33	26.83	3.49	3.82	3.66	4.55	3.60	4.07	1.11
$LSD_{0.05}$	1.224	1.244	0.60	1.829	1.504	0.82	2.281	2.159	1.08	0.217	0.221	0.11	0.159	0.121	0.07	--
$LSD_{0.01}$	1.652	1.678	0.80	2.467	2.029	1.09	3.077	2.912	1.44	0.293	0.298	0.15	0.215	0.163	0.09	--

Table 4: General combining ability effects for studied traits under normal (N), drought (D) conditions and combined data (C).

Genotypes	ER			PH			NF/P			FD			FY/P		
	N	D	C	N	D	C	N	D	C	N	D	C	N	D	C
P_1	1.44	1.52*	5.92**	1.13	3.29**	8.84**	2.25	1.73	7.94**	0.017	0.014	0.05	-0.21*	-0.07	-0.55**
P_2	1.25	0.81	4.11**	3.86	3.02**	13.76**	-4.09**	-4.31**	-16.80**	-0.445*	-0.27*	-1.43**	-0.02	-0.15**	-0.32**
P_3	0.91	1.86**	5.54**	0.30	-0.76	-0.93	-7.48**	-9.03**	-33.01**	0.774**	0.59**	2.73**	0.35**	0.03	0.74**
P_4	0.77	0.86	3.26**	0.17	-0.59	-0.83	-8.06**	-8.16**	-32.44**	0.398*	0.53**	1.84**	0.19	0.01	0.39**
P_5	-4.37**	-5.05**	-18.84**	-5.46**	-4.96**	-20.83**	17.38**	19.77**	74.30**	-0.745**	-0.86**	-3.20**	-0.31**	0.18**	-0.26**
SE(gi)	**0.061**	**0.063**	**0.045**	**0.14**	**0.093**	**0.058**	**0.21**	**0.19**	**0.08**	**0.002**	**0.002**	**0.063**	**0.001**	**0.001**	**0.04**
LSD $_{0.05}$	**1.57**	**1.18**	**0.09**	**2.35**	**1.43**	**0.12**	**2.93**	**2.05**	**0.15**	**0.28**	**0.21**	**0.13**	**0.20**	**0.11**	**0.07**
LSD $_{0.01}$	**2.61**	**1.59**	**0.12**	**3.90**	**1.92**	**0.15**	**4.86**	**2.76**	**0.21**	**0.46**	**0.28**	**0.17**	**0.34**	**0.15**	**0.10**

** ،*Significant at 5% and 1% levels of probability, respectively.

ER, earliness; PH, plant height; NF/P, number of fruits per plant; FD, fruit diameter, FY/P and fruits yield per plant.

Table 5: Specific combining ability effects for studied traits under normal (N), drought (D) conditions and combined data (C).

Traits	ER (day)			PH (cm)			NF/P			FD			FY/P		
Crosses	N	D	C	N	D	C	N	D	C	N	D	C	N	D	C
P_1XP_2	-0.84	-0.78	-0.81**	18.01**	21.71**	19.86**	9.98**	13.31**	11.65**	0.31**	0.28**	0.29	0.03**	-0.21**	-0.09
P_1XP_3	-0.51	-2.16**	-1.33**	-2.56*	-4.91**	-3.74**	-2.23	-1.84	-2.03**	-0.27**	-0.21**	-0.24	-0.41**	-0.24**	-0.33**
P_1XP_4	-0.70	-1.83**	-1.26**	-0.64	-2.95**	-1.80**	-4.71**	-3.84*	-4.28**	0.37**	0.11**	0.25	-0.21**	0.02**	-0.10
P_1XP_5	2.78**	3.75**	3.26**	-3.88**	-5.38**	-4.63**	-8.48**	-4.04**	-6.26**	0.01	-0.03*	-0.02	-0.004	0.09**	0.05
P_2XP_3	-1.32**	0.22	-0.55**	-5.29**	-4.58**	-4.93**	4.11*	4.06**	4.086**	-0.44**	-0.56**	-0.50**	0.54**	0.43**	0.49**
P_2XP_4	-1.18*	-0.78	-0.98**	-3.36**	-2.69**	-3.02**	1.62	2.06	1.84**	-0.17**	-0.24**	-0.20	-0.41**	0.10**	-0.16**
P_2XP_5	1.30**	-0.54	0.38**	-1.53	0.29	-0.62**	-21.28**	-18.67**	-19.98**	0.54**	0.48**	0.51**	-0.07**	-0.08**	-0.07
P_3XP_4	-0.84	-0.83	-0.83**	2.07*	3.43**	2.75**	4.08*	11.64**	7.86**	-0.02	-0.09**	-0.07	0.23**	0.35**	0.29**
P_3XP_5	3.97**	3.08**	3.52**	3.76**	5.80**	4.78**	-11.23**	-16.76**	-13.99**	0.52**	-0.37**	0.07	-0.19**	0.18**	-0.01
P_4XP_5	4.11**	5.75**	4.93**	0.75	3.43**	2.09**	-14.04**	-11.56**	-12.8**	-0.37**	0.02**	-0.17	0.20**	-0.17**	0.02
SE(Sij)	**0.41**	**0.42**	**0.11**	**0.91**	**0.62**	**0.14**	**1.42**	**1.27**	**0.19**	**0.01**	**0.01**	**0.15**	**0.007**	**0.004**	**0.09**
LSD 0.05	**0.91**	**0.94**	**0.21**	**2.03**	**1.37**	**0.28**	**3.16**	**2.83**	**0.37**	**0.03**	**0.03**	**0.30**	**0.016**	**0.009**	**0.18**
LSD 0.01	**1.29**	**1.34**	**0.28**	**2.89**	**1.95**	**0.37**	**4.49**	**4.03**	**0.49**	**0.04**	**0.04**	**0.40**	**0.022**	**0.013**	**0.23**

** ،*Significant at 5% and 1% levels of probability, respectively.

ER, earliness; PH, plant height; NF/P, number of fruits per plant; FD, fruit diameter, FY/P and fruits yield per plant.

Table 6: Combining ability analysis of variance for studied traits under normal (N), drought (D) conditions and combined data (C).

S.V	D.F		Mean squares														
			ER			PH			NF/P			FD			FY/P		
	S	C	N	D	C	N	D	C	N	D	C	N	D	C	N	D	C
GCA	4	4	126.867**	171.357**	98.05*	241.811**	238.667**	153.79**	2334.431**	2943.286**	1751.83**	7.932**	7.542**	5.05**	1.548**	0.310**	0.26
SCA	10	10	25.911**	31.635**	18.40**	136.108**	208.526**	111.57**	549.902**	536.355**	352.04**	0.434*	0.386**	0.20	0.275*	0.2053**	0.12
GCA x E	--	4	----	----	1.36**	----	----	6.37**	----	----	7.40**	----	----	0.09	----	----	0.36**
SCA x E	--	10	----	----	0.78**	----	----	3.31**	----	----	10.04**	----	----	0.07	----	----	0.04
Error	28	56	1.61	1.66	0.03	3.59	2.43	0.05	5.58	5.00	0.09	0.051	0.053	0.06	0.027	0.016	0.02
GCA/SCA	---	---	4.90	5.42	5.33	1.78	1.14	1.38	4.25	5.49	4.98	18.28	19.54	25.11	5.63	1.51	2.21
GCAxE/SCAxE			----	----	1.75	---	----	1.92	----	----	0.74	----	----	1.17	----	----	8.49

** ،*Significant at 5% and 1% levels of probability, respectively.

S.V., source of variation; D.F., degrees of freedom; S, single environment; E, environment; ER, earliness; PH, plant height; NF/P, number of fruits per plant; FD, fruit diameter, FY/P and fruits yield per plant.

Table 7: Estimates of the genetic parameters for studied traits under normal (N), drought (D) conditions and combined data (C).

Items	genetic parameters														
	ER			PH			NF/P			FD			FY/P		
	N	D	C	N	D	C	N	D	Co.	N	D	C	N	D	C
σ^2 A	28.84	39.92	**11.30**	30.20	8.61	**5.60**	509.87	687.69	**200.35**	2.14	2.04	**0.69**	0.36	0.03	**0.03**
σ^2 D	24.30	29.98	**8.81**	132.52	206.10	**54.13**	544.32	531.36	**171.00**	0.38	0.33	**0.06**	0.25	0.19	**0.04**
σ^2 A x E	---	---	**0.33**	---	---	**1.75**	---	---	**1.51**	---	---	**0.01**	---	---	**0.18**
σ^2 D x E	---	---	**0.75**	---	---	**3.26**	---	---	**9.95**	---	---	**0.02**	---	---	**0.02**
h^2_{NS}	52.68	55.79	53.30	18.16	3.97	8.64	48.11	56.18	**52.75**	83.23	84.12	**82.14**	56.94	12.72	**10.34**
h^2_{BS}	97.06	97.68	94.77	97.84	98.89	92.19	99.47	99.59	**96.98**	98.02	97.83	**89.29**	95.56	93.22	**24.14**

** ،*Significant at 5% and 1% levels of probability, respectively.

E, environment; ER, earliness; PH, plant height; NF/P, number of fruits per plant; FD, fruit diameter, FY/P and fruits yield per plant

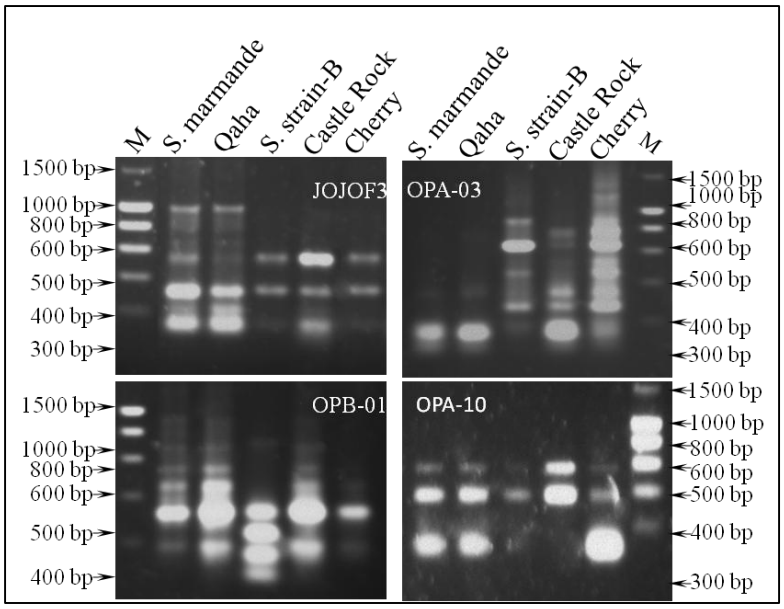

Figure 1: Profile obtained by some studied RAPD primers.

Table 8: Primers used in RAPD analysis, total number of fragments detected by each primer, %P, PIC and MI for five parental tomato genotypes.

Primer Name	Primer Sequence (5'→3')	Amplified bands		%P	PIC	MI
		Total Number of bands	Polymorphic bands			
JOJOF3	GAGGCGTCGC	5	3	60.00	0.29	0.87
OPB-09	TGGGGGACTC	8	7	87.50	0.34	2.38
OPA-03	AGTCAGCCAC	8	8	100.00	0.46	3.68
OPA-10	GTGATCGCAG	3	1	33.33	0.16	0.16
OPW-13	GTTGTTTGCC	4	2	50.00	0.16	0.32
OPG-09	CTGACGTCAC	7	2	28.57	0.14	0.28
OPP-05	CCCCGGTAAC	5	1	20.00	0.10	0.10
OPAD-08	AAGTGCACGG	5	3	60.00	0.22	0.66
OPA-08	GTGACGTAGG	8	2	25.00	0.32	0.64
OPW-08	GACTGCCTCT	4	2	50.00	0.24	0.48
Total		**57**	**31**			
Mean		**5.70**	**3.10**	**51.44**	**0.24**	**0.96**

%P, Percentage of polymorphism; PIC, Polymorphism information content; MI, marker index.

Table 9: Similarity matrix for five tomato parental genotypes obtained from RAPD analysis (below diagonal), and similarity matrix obtained using phenotypic data (above diagonal).

Genotypes	S. Marmande	Qaha	S. Strain-B	Castle Rock	Cherry
S. Marmande	-	88.94	81.38	83.48	64.31
Qaha	0.88	-	91.07	92.78	56
S. Strain-B	0.69	0.73	-	97.03	49.19
Castle Rock	0.82	0.84	0.83	-	51.1
Cherry	0.79	0.71	0.84	0.85	-

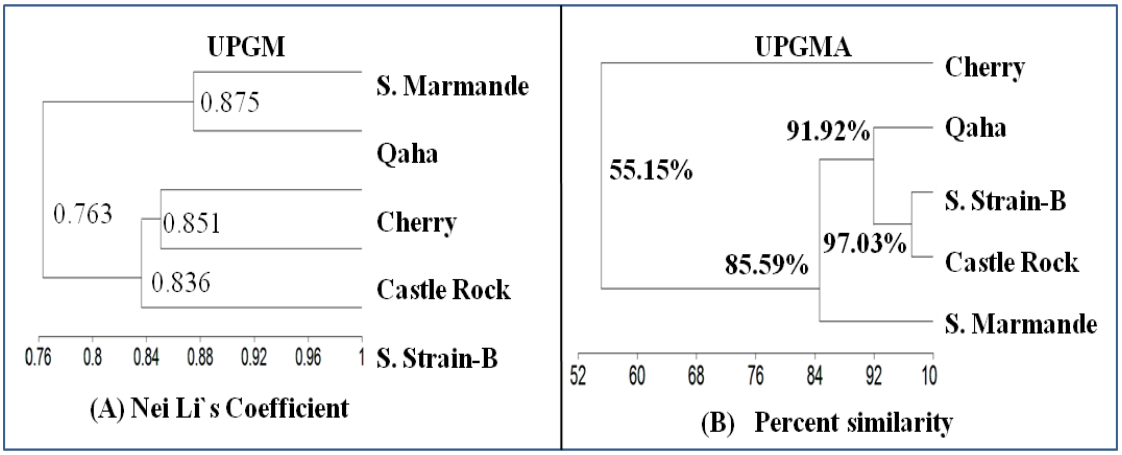

Figure 2: Phylogenetic tree of five tomato genotypes obtained using 57 bands of RAPD markers (A) and phenotypic data (B).

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