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In vitro evaluation of six tomato genotypes for water stress

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Abstract: Abiotic stress factors are the main limitation to plant growth and yield in agriculture. Six genotypes of tomato (*Lycopersicon esculentum* Mill.) were screened for water stress tolerance by measuring growth parameters and biochemical characteristics *in vitro*. Three treatments of water stress was evaluated by adding 20, 40 and 60g/l of PEG to Murashige- Skoog (MS) medium and compared to MS medium without PEG for the control. Osmotic pressure (MPa) of media ranged from −0.181 to −0.037 MPa. Genotypes differed for their responses. Plant length and plant diameter, leafs number and area, roots number, length and diameter and plant fresh and dry weights were measured, plus to the content of chlorophyll *a* and *b*, content of carotenoid and prolin in plants. Overall, the growth and most of biochemical parameters decreased with PEG concentrations increasing. Grouping genotypes by cluster analysis, based on the studied parameters response to water stress, resulted in three distinct groups: : (1) drought tolerant group consisting of one genotype: G3; a moderately drought tolerant group consisting of three genotypes: G2, G4 and G5; (3) a drought susceptible group consisting of two genotypes: G1 and G6 (Fig. 1).
The variation in germplasm indicated that tomato genotypes can be developed for production under some levels of PEG.
Keywords : Screening, Tomato, PEG, Water stress, *In vitro.*

Introduction

Salinity stress has become an important problem regarding agricultural production in many regions of the world especially in arid and semi-arid regions[1].Low water potential induces oxidative bursts leading to elevated levels of antioxidant enzymatic activities and high solute concentration and protein accumulations[2]. The onset of stress may initially cause a loss of cell turgor which in turn reduces gas exchange and leaf elongation since both are turgor-dependent processes. The result is a decrease in growth rate since this is a function of transpiration rate and leaf area[3]. Evapotranspiration (ET) has been positively correlated with yield of many crops since it is a direct measure of crop water loss. Thus, there has been a growing use of ET data for irrigation scheduling studies. Water stress causes a decrease intranspiration, an increase in foliage temperature and closure of stomata[4].Various effects of water stress have been reported in different crops such as tomato [5], soybean [6&7] and corn [8].

Tomato is a major vegetable crop that has achieved tremendous popularity over the last century. It is grown inpractically every country in the world, in outdoor fields, greenhouses and net houses [9].Tomato plants need a controlled supply of water throughout the growing period for optimal quality and higher yield[10]. Tomato is considered as being very sensitive to drought during and immediately after transplanting, at flowering and

during fruit development[11].[12] showed that earlier water stress (20 days after sowing) is inhibitory compared to the later one (30 day after sowing).

Water stress reduces leaf area in tomato[13]which in turn results in negatively affected on shoot lengths [14]and affects the quality of fruits and discourages crop yield[15].Changes in the amount of chlorophyll in plant is expected in this kind of stress[10],because of destroy of the chloroplast and disappearing thylakoid structures[16], it is also affects some solutes like carbohydrate, soluble protein and ions content (Na^{+}, K, Ca^{+2}, and Mg^{+2}) [17],and increases proline contents in plants[18].Prolin accumulation is believed to play adaptive roles in plant stress tolerance [19].Accumulation of proline has been advocated as a parameter of selection for stress tolerance [20].Moreover, the role of praline as an osmotic factor is already established [21].Root traits are considered an important character in drought-tolerant genotypes[22].

For developing drought-tolerant genotypes, improvement in root traits is considered to be important [22].An *in vitro* method could be a possible alternative to overcome the problems associated with field evaluation of tomato, since the root traits in field-grown plants are exhausting and time-consuming [23].

PEG is found to reduce cell water potential and to induce osmotic stress [24]. An increase in concentration of PEG-6000, resulted a decrease in germination rate, root length, shoot length and seed vigor in certain crop plants[8].

Although tomato is lower tolerant for water stress than other crops, it is generally accepted that there are differences in susceptibility to water stress among cultivars [25].Thus, the purpose of the present study is to study the effect of *in vitro* water stress on some growth and biochemical parameters, and then to classify six tomato genotypes for water stress tolerance.

Materials and Methods

Plant material and culture conditions

The study was carried out in 2014 at the National Commission for Biotechnology (Damascus, Syria).Six genotypes of tomatoes were collected from two Syrians regions and were numbered from 1 to 7 according to their order in the National Commission for Biotechnology bank as shown in table (1)

Table 1: Tomato genotypes and their place of collection.

Symbol of genotype	Genotype Cod	Collection region
G1	G0714	The south region of Syria
G2	G0788	The south region of Syria
G3	G0899	The south region of Syria
G4	G0757	The west region of Syria
G5	G0257	The west region of Syria
G6	G0357	The west region of Syria

Tomato seeds were sterilized with 70 % ethanol for 1 minute and then with mercuric chloride (0.5%) for 10 minutes and thoroughly washed with sterile distilled water for three times. The seeds inoculated onto autoclaved media [26] (basal media). Seedlings were maintained under optimum culture conditions (light intensity of 30 $\mu Mm^{-2}S^{-1}$, 16 photoperiod and22±2 ºC). After 45 days, plants were divided into (1-1.5)cm explants with one bud and leaf. *In vitro* grown plants were propagated in the same medium (MS) with a 4-week intervalin order to obtain enough plant material. At the 4thculture, in order to assess the *in vitro* screening of tomato genotypes for water stress tolerance, three PEG concentrations (20,40 and 60 g/l),in addition to the control (without PEG), were used. Osmotic pressure of MS medium containing different PEG concentrations, were measured using an osmometer (OM 815, VOGLEL, Löser).

Three replicates per treatment and ten plants per every replicate were used. The treatments will be refereed T0 for the control, T1 for 20g/l, T2 for 40g/l and T3 for 60g/l of PEG. After 45days of stress application, *in vitro* grown morphological and biochemical parameters associated with water stress tolerance were taken.

Measurements

After 45 days of stress application plants were rinsed in sterilized water, and separated into leaves, stems and roots. Number of leaves and roots were recorded. Leaf areas were measured with a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE).Length and thickness of both the roots and the stem were measured. Plant fresh and dry weights (oven-dried at 70 °C for atleast 72 h) were determined [27].In order to assess the chemical analysis, three plants per treatments were frozen in liquid nitrogen and ground. The resulting powder stored at -80°C.

Chemical analysis

To determine the leaf chlorophyll content (*a*&*b*),0.2 g of powder was homogenized by adding 7 ml of acetone (80 %). The mixture was centrifuged at 3400 rpm for20 minutes at 4 °C (Tabletop model, IEC 215, USA). The resulting supernatant was used to determine the absorbance at 664 and 647 nm using the spectrophotometer (ShimadzuMini-1240 UV–Vis, USA). If the absorbance is greater than1, the resulting supernatant is diluted by 10 % using acetone (80 %). The leaves chlorophyll concentration was determined according to [28]:

[Chlorophyll *a*] =-1.93 × Abs_{647}+11.93 × Abs_{664}

[Chlorophyll *b*] = 20.36 × Abs_{647}- 5.50 × Abs_{664}

Where: A_{647} and A_{664} are the absorbance at 647 and 664 nm, respectively.

The carotene concentration was analyzed by high performance liquid chromatography (HPLC, Hamilton, UK) [29]. The samples were placed in the sample tray cooled to 6 °C and covered by aluminum foil to minimize light. Samples (20 µl) were injected onto a reverse phase column[30]. Separation was performed at35 °C with a mobile phase of methanol, acetonitrile and chloroform (42.5/42.5/15 v/v). The flow rate was maintained at 1.2 ml/min. Peaks were monitored at 450 nm. Standard solutions of β-carotene with concentration from 0.5 to 10 µg/ml were used to obtain a standard curve.

The free proline content was determined according to [31]. Frozen leaf tissue (0.5 g) was homogenized with 2 mL of 3% sulfosalicylic acid. The suspension was centrifuged at 10000 rpm for 10 min. The volume was completed to 5 ml using 3% sulfosalicylic acid. The extract was filtered with Whatman No.2 filter paper. In a test tube, 2 mL of filtrate, 2 mL of acid-ninhydrin, and 2 mL of glacial acetic acid were mixed and incubated at 100 °C for 1 h. Thereaction was terminated on ice, and the reaction mixture was then extracted with 4 mL of toluene. The chromophore-containing toluene was separated fromthe hydrated phase. The absorbance at 520 nm was determined by spectrophotometer with toluene as the blank. The praline concentration was calculated based on a standard curve and was expressed as µmol proline g^{-1} FW.

Experimental design and statistical analysis

The experiment was designed as completely randomized design with ten replications. Using the R−Project statistical software, data were subjected to ANOVA analysis. Cluster analysis was assessed according to the genotypes response to stress based on the sum of relative values of all measurements as compared to the control.

Results

Growth parameters

Plant length:

Plant length is decreased as a response to decrease of PEG concentrations increasing (table 2). There was not significant differences between studied genotypes, since we can notice that G6have the highest mean for plant length with3.427cm, while the lowest mean was for G5with1.979cm. In the other hand, significant differences were observed between the controls and PEG treatments in some of genotypes such as G2, G3and G4. For example, plant length of G2decreased significantly from 5.500cm for the control to 0.925cm for T1. While, an increase of plant length from 2.575 cm for the control to 3.125 cm for T1, then a decrease to 2.5 and 1cm for the T2 and T3, respectively, was observed in G1.

Table 2: The effect of different drought stress treatments on plant length for six genotypes.

Plant length (cm)						
Means for	**LSD 1% for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
2.300^{A}	2.940	1.000^{a}	2.500^{a}	3.125^{a}	2.575^{a}	G1
2.375^{A}	2.249	1.200^{b}	1.875^{b}	0.925^{b}	5.500^{a}	G2
2.648^{A}	4.378	1.100^{b}	0.950^{b}	1.125^{b}	7.412^{a}	G3
3.271^{A}	5.366	1.839^{b}	1.000^{b}	2.250^{b}	8.339^{a}	G4
1.979^{A}	2.373	0.500^{b}	0.750^{b}	3.733^{a}	2.750^{ab}	G5
3.427^{A}	4.961	1.509^{a}	2.134^{a}	4.875^{a}	5.625^{a}	G6
2.477						LSD 1% for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Plant diameter:

Plant diameter varies due to PEG depended on the tomato genotypes (table 3).These variations ranged between 2.252and 1.273mm for G3 and G4, respectively. As a general trend, the plant diameter decreased with the increase of the PEG concentration, although these decrease was not significant. However, some of genotypes showed various changes following the PEG treatment. Plant diameter reduced significantly from 2.685 mm for the control to 1.223 mm for T13in G1.

Table 3: The effect of different drought stress treatments on plant diameter for six genotypes.

Plant diameter(mm)						
Means for	**LSD 1% for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
1.981^{AB}	1.098	1.223^{b}	1.793^{ab}	2.225^{ab}	2.685^{a}	G1
1.924^{AB}	1.26	1.375^{b}	1.635^{b}	1.735^{ab}	2.953^{a}	G2
2.252^{A}	2.166	1.218^{a}	2.515^{a}	2.935^{a}	2.458^{a}	G3
1.273^{C}	0.684	0.953^{a}	1.005^{a}	1.299^{a}	1.526^{a}	G4
1.427^{BC}	0.783	0.740^{b}	1.253^{ab}	1.608^{a}	2.005^{a}	G5
2.019^{AB}	1.345	1.710^{a}	1.830^{a}	2.035^{a}	2.620^{a}	G6
0.702						LSD 1% for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Number of leaves:

Number of leaves was changed according to genotype. The highest value (3.375 leaf/plant) was recorded for G1 and the lowest value (2.040 leaf/plant) was recorded for G3.In most of genotypes, number of leaves was affected negatively by increase of PEG concentrations, where it was reduced compared to the control (table 4). No significant differences were observed in genotypes between the control and treatments, where the number of leaves reduced from 4leaf/plant for control to 3leaf/plant, 2.75 leaf/plant and 2.5 leaf/plant forT1, T2 and T3, respectively in G2.

Table 4: The effect of different drought stress treatments on number of leaves for six genotypes.

Number of leaves						
Means for	**LSD 1% for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
3.375^{A}	1.817	3.250^{a}	3.750^{a}	3.000^{a}	3.500^{a}	G1
3.063^{AB}	2.312	2.500^{a}	2.750^{a}	3.000^{a}	4.000^{a}	G2
2.040^{B}	2.645	1.000^{b}	1.500^{ab}	1.500^{ab}	4.000^{a}	G3
2.290^{AB}	2.293	1.153^{b}	1.500^{b}	2.500^{ab}	3.810^{a}	G4
2.415^{AB}	1.765	1.500^{a}	2.000^{a}	2.850^{a}	3.250^{a}	G5
2.642^{AB}	1.764	1.661^{b}	2.161^{ab}	3.250^{ab}	3.500^{a}	G6
1.1301						LSD 1% for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*)**

Leaf area:

G5 had the highest leaf area with 2729.596 mm^2. Leaf area of control plants ranged between 439.034 mm^2 for G4 and 2729.596 mm^2 for G5 (table5). The PEG treatments decreased leaf area in all genotypes except G3. For example in G6, leaf area decreasedby31,75 and 83%for T1, T2 andT3, respectively, compared to control.

Table 5: The effect of different drought stress treatments on leaf area for six genotypes.

Leaf area$(mm)^2$						
Means for	**LSD 1% for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
744.889^{B}	527.699	342.650^{b}	393.725^{b}	1090.214^{a}	1152.967^{a}	G1
604.833^{B}	538.364	110.647^{b}	203.540^{b}	169.660^{b}	1935.485^{a}	G2
706.706^{B}	1130.94	136.841^{b}	151.218^{b}	257.781^{b}	2078.310^{a}	G3
439.034^{B}	682.631	69.006^{b}	100.730^{b}	181.114^{b}	1049.656^{a}	G4
2729.596	2048.475	1402.120^{b}	2139.870^{b}	2503.750^{b}	5317.500^{a}	G5
1143.264	1472.509	381.104^{b}	552.938^{b}	1500.181^{ab}	2185.620^{a}	G6
958.351						LSD 1% for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Fresh weight of plant:

The plant fresh weight varied among the six genotypes studied (table 6). G3 had the highest fresh weight (0.979g), while G6 had the lowest fresh weight (0.363g)as compared to other genotypes. As a general trend, the plant fresh weight decreased with increasing of PEG concentration. For example, the plant fresh weight was considerably reduced with the highest PEG concentration (T3) to 0.331, 0.213, 0.123,0.122, 0.134 and 0.393 g for the genotypesG1, G2, G3, G4, G5 and G6, respectively, as compared to the control 1.240, 1.255, 1.329, 0.887, 1.024 and 1.888 g, respectively.

Table 6: The effect of different drought stress treatments on fresh weight of plant for six genotypes.

Fresh weight						
Means for	**LSD $_{1\%}$ for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
0.765^{AB}	0.646	0.331^{b}	0.894^{ab}	0.593^{ab}	1.240^{a}	G1
0.564^{AB}	0.555	0.213^{b}	0.336^{b}	0.452^{b}	1.255^{a}	G2
0.502^{AB}	0.793	0.123^{b}	0.225^{b}	0.300^{b}	1.329^{a}	G3
0.363^{B}	0.479	0.122^{b}	0.073^{b}	0.225^{b}	0.887^{a}	G4
0.523^{AB}	0.386	0.134^{b}	0.183^{b}	0.727^{a}	1.024^{a}	G5
0.979^{A}	0.827	0.393^{c}	0.593^{bc}	1.250^{ab}	1.888^{a}	G6
0.484						LSD $_{1\%}$ for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Dry weight of plant:

Concerning the plant dry weight, No significant differences between genotypes for fresh and dry weight (table 7). Dry weight generally reduced by PEG treatments in all genotypes (table 7). For example, the plant dry weight was considerably reduced with the highest PEG concentration (T3) by 66, 65, 76, 79, 78 and 72 % as compared to the control in G1, G2, G3, G4, G5 and G6, respectively.

Table7: The effect of different drought stress treatments on dry weight of plant length for six genotypes.

Dry weight (g)						
Means for genotypes	**LSD $_{1\%}$ for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
0.097^{A}	0.196	0.056^{a}	0.072^{a}	0.106^{a}	0.163^{a}	G1
0.060^{A}	0.042	0.037^{b}	0.046^{b}	0.052^{b}	0.105^{a}	G2
0.048^{A}	0.053	0.023^{b}	0.031^{b}	0.035^{b}	0.097^{a}	G3
0.039^{A}	0.041	0.015^{b}	0.013^{b}	0.037^{ab}	0.073^{a}	G4
0.103^{A}	0.438	0.023^{a}	0.021^{a}	0.273^{a}	0.105^{a}	G5
0.077^{A}	0.072	0.036^{b}	0.052^{b}	0.092^{ab}	0.133^{a}	G6
0.092						LSD $_{1\%}$ for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Number of roots:

Significant differences were observed between genotypes for number of roots the highest number of roots recorded for G6 with 7.092 root/plant but the lowest was for G4 with 2.217 root/plant.

All the genotypes showed a regular reduction of roots number as compared to the control, for example in G2 the number of roots decreased from 7 root/plant for control to 2, 2.5 and 0.5 root/plant for T1, T2 and T3 respectively (table 8).

Table 8: The effect of different drought stress treatments onnumber of roots for six genotypes.

Number of roots (root/plant)						
Means for genotypes	**LSD 1% for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
4.625^{AB}	4.474	3.000^{a}	4.250^{a}	5.250^{a}	6.000^{a}	G1
2.938^{B}	3.284	0.500^{b}	2.250^{b}	2.000^{b}	7.000^{a}	G2
2.296^{B}	5.527	0.250^{b}	1.000^{ab}	1.250^{ab}	6.300^{a}	G3
2.217^{B}	3.609	0.500^{b}	0.500^{b}	1.750^{ab}	5.250^{a}	G4
3.233^{B}	3.286	1.500^{b}	3.250^{ab}	3.050^{ab}	5.000^{a}	G5
7.092^{A}	5.226	2.893^{b}	4.143^{b}	10.000^{a}	13.250^{a}	G6
2.946						LSD 1% for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Roots length:

Significant difference was observed between studied genotypes, the highest length of roots noticed in G1(6.7cm), while the lowest was in G3(2.038cm). The roots length was reduced with PEG treatments in all genotypes except a little bit increase for T2 in G1. For example, the roots length was considerably reduced from 6.313cm for the control to 1.875,0.250 and 0.653cm in T1, T2 and T3, respectively, in G4, (table 9).

Table 9: The effect of different drought stress treatments onroot length for six genotypes.

Root length (cm)						
Means for	**LSD 1% for**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
6.700^{A}	6.086	4.000^{a}	8.125^{a}	6.625^{a}	8.050^{a}	G1
3.881^{ABC}	6.046	0.250^{b}	4.025^{b}	0.875^{b}	10.375^{a}	G2
2.038^{C}	4.847	0.500^{a}	0.900^{a}	1.750^{a}	4.477^{a}	G3
2.469^{BC}	4.426	0.563^{b}	0.250^{b}	1.875^{b}	6.313^{a}	G4
3.688^{ABC}	2.825	0.750^{c}	1.875^{bc}	4.417^{b}	7.625^{a}	G5
5.251^{AB}	4.487	2.857^{a}	5.732^{a}	6.125^{a}	7.000^{a}	G6
3.055						LSD 1% for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Roots diameter:

Roots diameter were significantly varied between genotypes (table9). The roots diameter reduced with PEG concentration following the genotype. For example, in G1, the root diameter decreased from 0.653mm to 0.438, 0.340 and 0.1 mm for T1, T2 and T3 respectively (table10).

Table10: The effect of different drought stress treatments onroot diameter for six genotypes.

Root diameter (mm)						
Means for	LSD $_{1\%}$ for	Treatments				Genotype
		T3	T2	T1	T0	
0.383^{AB}	0.528	0.100^{b}	0.340^{ab}	0.438^{ab}	0.653^{a}	G1
0.334^{B}	0.681	0.080^{a}	0.268^{a}	0.460^{a}	0.528^{a}	G2
0.295^{B}	0.660	0.150^{a}	0.155^{a}	0.180^{a}	0.646^{a}	G3
0.577^{AB}	0.591	0.165^{b}	0.548^{ab}	0.758^{a}	0.898^{a}	G4
0.570^{AB}	0.471	0.505^{ab}	0.685^{ab}	0.284^{b}	0.830^{a}	G5
0.685^{A}	0.875	0.249^{b}	0.524^{ab}	0.830^{ab}	1.258^{a}	G6
0.348						LSD $_{1\%}$ for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Biochemical parameters

Chlorophyll *a* content

The results of chlorophyll *a* were varied according to the genotypes and treatments (table 11).Significant differences between the genotypes were recorded. The highest leaf chlorophyll *a* content was recorded in G1(103.363µg/g) and the lowest one was in G4 (11.369µg/g). Within the genotype, differences between treatments were observed. In some genotypes, such as G4, the content of chlorophyll *a* decreased significantly from 10.434for control to 8.543 for T1, and then increased to 10.567 and 15.932µg/gin T2 and T3, respectively. In other genotypes, such as G1,G2 and G6this parameter decreased significantly from 207.576µg/gto 101.972, 81.114 and 22.79 for G1 and from 36.443 µg/g to 26.888, 25.229 and 8.932µg/gfor G2, while it decreased from 98.297µg/g to 96.811µg/g, 51.977µg/g and 45.969 µg/g for G6in T1, T2 and T3, respectively. While this parameter varied in the rest genotypes (G1, G4 and G5) according to PEG concentration.

Table 11: The effect of different drought stress treatments on chlorophyll *a* for studied genotypes.

Chlorophyll *a* (µg/g)						
Means for genotypes	LSD $_{1\%}$ for treatments	Treatments				Genotype
		T3	T2	T1	T0	
103.363^{A}	0.753	22.790^{d}	81.114^{c}	101.972^{b}	207.576^{a}	G1
24.394^{B}	0.733	8.932^{d}	25.229^{c}	26.888^{b}	36.443^{a}	G2
13.974^{B}	0.751	9.361^{d}	11.456^{c}	13.162^{b}	21.913^{a}	G3
11.369^{B}	0.143	15.932^{a}	10.567^{b}	8.543^{d}	10.434^{c}	G4
68.476^{A}	0.706	8.043^{d}	53.131^{b}	21.444^{c}	191.285^{a}	G5
73.264^{A}	0.729	45.969^{d}	51.977^{c}	96.811^{b}	98.297^{a}	G6
40.161						LSD $_{1\%}$ for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Chlorophyll *b* content

Significant differences between genotypes were observed. Plants of G1had significantly the highest value of chlorophyll *b* content (50.472µg/g) as compared to all genotypes, while G4 had the lowest with (5.204µg/g).In all genotypes, the content of chlorophyll *b* was decreased with increasing of PEG concentration (table 12). This parameter decreased significantly from 44.135µg/g in the control to 27.452µg /g in T1, 16.802 in T2 and 7.073 in T3.

Table 12: The effect of different drought stress treatments on some chlorophyll *b* for studied genotypes.

Chlorophyll *b*(µg/g)						
Means for	**LSD $_{1\%}$ for treatments**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
50.472^{A}	1.362	9.905^{d}	46.050^{b}	37.362^{c}	108.568^{a}	G1
9.550^{CD}	0.460	3.643^{d}	9.577^{c}	11.089^{b}	13.919^{a}	G2
9.124^{CD}	1.835	3.677^{b}	4.584^{b}	5.414^{b}	22.822^{a}	G3
5.204^{D}	0.258	3.207^{d}	4.826^{c}	6.069^{b}	6.713^{a}	G4
23.866^{BC}	0.275	7.073^{d}	16.802^{c}	27.452^{b}	44.135^{a}	G5
38.788^{AB}	0.442	26.638^{c}	22.849^{d}	42.551^{b}	63.110^{a}	G6
16.747						LSD $_{1\%}$ for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Carotenoid content

Significant differences were observed in the carotenoid content according to the genotype. The highest content was observed in the genotype G6 (21.915µg /g).

A significant decrease of this parameter was observed according to the treatments in most genotypes. For example,in G4 the carotenoid content decreased from 4.983 µg /g for the control to3.343,2.785 and 2.435µg /g for T1, T2 and T3, respectively. In genotype G6, this parameter increased significantly from 24.899µg /g in the control to 30.370 µg /g for T1 to decrease again to 17.002 in T2 and 15.190 µg /g in T3(table 13).

Table 13: The effect of different drought stress treatments on content of carotenoid for studied genotypes.

Carotenoid(µg/g)						
Means for	**LSD $_{1\%}$ for treatments**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
29.261^{A}	0.398	7.295^{d}	30.227^{b}	23.676^{c}	55.843^{a}	G1
6.543^{B}	0.130	3.144^{c}	2.604^{d}	8.638^{b}	11.784^{a}	G2
3.831^{B}	0.538	3.955^{ab}	3.352^{c}	3.6918^{bc}	4.324^{a}	G3
3.387^{B}	0.076	2.435^{d}	2.785^{c}	3.343^{b}	4.983^{a}	G4
6.275^{B}	0.077	6.860^{c}	3.682^{d}	6.990^{b}	7.569^{a}	G5
21.915^{A}	0.125	15.190^{d}	17.002^{c}	30.370^{a}	24.899^{b}	G6
7.445						LSD $_{1\%}$ for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Proline content

In general, proline content was increased with the increase of drought intensity. The highest proline content was observed in G3with 66.005µg /g follow by G5, G1, G4, G2 then G6 (65.685,32.799,30.468,29.114 and 28.507µg /g, respectively).The drought stress mediated by PEG conducted in most genotypes to proline accumulations in plant. For example, it increased by 118,160 and 184%as compared to the control, for T1,T2 and T3, respectively in genotype G2. While, it increased by 111%, 116% and 529% for T1, T2 and T3 respectively as compared to the control in G4.

Table 14: The effect of different drought stress treatments on content of for studied genotypes.

Prolin content(µg/g)						
Means for	**LSD $_{1\%}$ for treatments**	**Treatments**				**Genotype**
		T3	**T2**	**T1**	**T0**	
32.799^{B}	0.182	67.214^{a}	38.285^{b}	20.800^{c}	4.971^{d}	G1
29.114^{B}	0.036	38.171^{a}	33.114^{b}	24.457^{c}	20.714^{d}	G2
66.005^{A}	5.036	119.050^{a}	112.257^{b}	18.885^{c}	13.828^{d}	G3
30.468^{B}	0.033	75.371^{a}	16.485^{b}	15.771^{c}	14.242^{d}	G4
65.685^{A}	0.036	60.371^{b}	31.742^{d}	117.285^{a}	53.342^{c}	G5
28.507^{B}	0.221	96.114^{a}	8.142^{b}	6.285^{c}	3.485^{d}	G6
31.020						LSD $_{1\%}$ for genotypes

***Different small letters within row indicate significant differences between treatments, and capital letters indicate significant differences between genotypes determined by the Fisher test (*P<0.01*).**

Osmotic pressure

Medium osmotic pressure (MPa) was decreased with PEG concentration increasing, as shown in the table (15). The osmotic pressure attainted -0.181MPa for the higher PEG concentration.

Table 15: PEG concentrations (g/l) and osmotic pressures (MPa) of MS medium.

Treatments	PEG concentration (g/l)	Medium osmotic pressure (MPa)
T0	0	0
T1	20	-0.037
T2	40	-0.098
T3	60	-0.181

Cluster analyses

The cluster analysis, based on the sum of relative values of the differences between the control and stressed plants for growth and chemical parameters, resulted in three distinct groups: (1) drought tolerant group consisting of one genotype: G3; (2) a moderately drought tolerant group consisting of three genotypes: G2, G4 and G5; (3) a drought susceptible group consisting of two genotypes: G1 and G6 (Fig. 1).

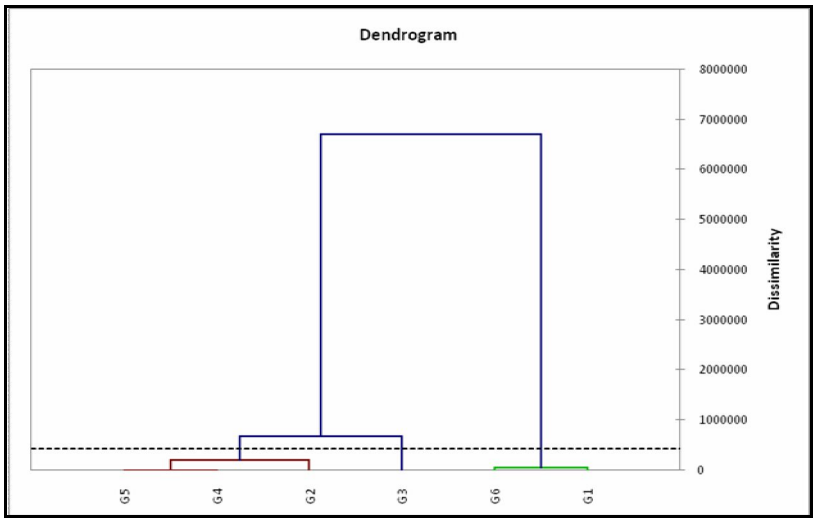

Fig. 1: Dendrogram based on relative values of growth parameters of six tomato genotypes under different drought treatments

Discussion

Water stress negatively affects water balance of the plant body and causes changes in water uptake patterns of plant [32]. Plant species and cultivars vary greatly in their response to drought [33].

Screening a large number of genotypes for drought tolerance in the field is very hard, due to difference of soil chemical and physical properties.

The effect of drought stress on *in-vitro* tomato growth has been reported to be similar to that observed under field conditions [34]. Many studies have been proposed the *in-vitro* screening of tomato genotypes for water stress tolerance as an alternative approach to costly, labor-intensive and sometimes problematic field-based screening [35]. Poly ethylene glycol (PEG) was used in this study since it is often used to impose low water potentials in solution culture [36].

The genotypes used in this study responded differentially to drought stress.

Drought stress effects on plant length and number of leaves [37]. Also it was recorded reduced growth rate in tomato cultivars at varying PEG simulated drought stress. Some studies describe the reduction of leaf area as the first morphological parameter affected by drought, which led to photosynthesis reduction and accumulation of dry matter [38].The reduction of leaf area is directly correlated with plant length and number of leaves.

Water stress causes biochemical and physiological changes responses in different plants. The synthesis and storage of osmolites differs in different plants[39].The increase of free proline occurs in decrease in water supply [40]. Thesynthesis of proline in plants extensively protects cell membrane and protein content in plant leaves[40&39].

The increase of proline content under drought stress were reported in different plants such as tomato[41], potato [42],and green gram [43]. The results of this study are in agreement with other investigations [18].

Chlorophyll content is a basic way to evaluate the effects of environmental stress [44].Photosynthesis is the main ROS-producing process in chloroplasts, and ROS can cause photoinhibitory and photooxidative damage [45].Oxidative stress generated in plant cells as a result of extended drought causes a reduction of carotenoid content in many species [46]. Nevertheless, carotenoids as a part of the plant antioxidant defense system also play additional roles in plants resisting to drought [47].

[48]demonstrated that drymatter partitioning and biomass disposition are strongly connected with plant productivity under drought stress conditions. Increasing accumulation of dry biomass is related to two processes: dehydration and new material synthesis required for maintenance of higher osmoticum to continue water absorption [49].

The drought stress affected the different plant growth parameters like stem height, foliage weight, root number and root dry weight [50].

A decrease of development of new leaves, leaf area, leaves' number, total fresh and dry matter, root volume, shoot and root growth were reported on various crops suffering from drought stresses[51].

This study showed that the PEG stress tolerance of tomato genotypes could be easily evaluated by the *in-vitro* screening, based on growth parameters, for the identification of suitable genotypes with improved PEG tolerance. A number of mechanisms relating to improved stress adaptation in crops have been suggested [52]. Therefore, a well-focused approach combining the molecular, physiological, and metabolic aspects of abiotic stress tolerance is required to establish a screening approach.

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