

## The RESPONSE of Eight Pure Tomato Lines (*Lycopersicon esculentum*) for Drought Stress Induced by Polyethylene Glycol *in vitro*

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### Abstract

The study was carried out at Sweida research center/ the General Commission for Scientific Agriculture Research (GCSAR)/ Syria during 2018-2019 in order to investigate the effect of *in vitro* drought stress induced by Polyethylene Glycol (PEG6000) on eight pure tomato lines from the 5<sup>th</sup> generation at the first seedling growth development. Four different concentration of PEG6000, 2%, 4%, 6% and 8% were used in addition to control treatment. Results showed that increasing drought stress treatment has led to a decrease in the studied growth parameters involved mean number of roots and root diameter, seedling length and diameter, number of leaves and shoots, root and plant dry weight and leaf area in all tomato lines. Concerning root length, drought stress resulted in reducing root elongation except for Jerdy line which developed a longer roots under drought stress treatments, hence it is announced as a superior line for drought stress tolerance. Similar trend was recorded for root-to-shoot length ratio which was increased with increasing drought stress in all tomato lines. Using cluster analysis, based on the sum of relative values of drought stress responses, four groups were distinguished: (1) consisting of Jerdy line. (2) consisting of 2 lines; Daraa, and Daher-Aljabal. (3): consisting on line Brie and (4) consisting of 4 lines; Baskanta, Abosfair, Kafer-Selwan and Wardyat.

**Keywords:** Tomato, pure line, screening, drought stress, cluster analysis

### Introduction:

Tomato (*Lycopersicon esculentum*) is the second most consumed vegetable crop in the world (Iqbal *et al.*, 2019) after potato (Rashid *et al.*, 2012). It is grown in practically every country in the world, in outdoor fields, greenhouses and net houses (Bredy *et al.*, 2015). Being a tropical plant, tomato is well adapted to almost all climatic regions of the world; however, environmental stress factors are the primary constraints of this crop's yield potential (Gerszberg and Hnatuszko-Konka, 2017) and production area is limited by irrigation water scarcity (Aazami *et al.*, 2010) taking into account that most commercial tomato cultivars are drought sensitive at all stages of plant development (Foolad *et al.*, 2003) with seed germination and seedling establishment are potentially the most critical stages for water stress (Queiroz *et al.*, 2019). Drought stress reduces plant growth and crop production (Sallam *et al.*, 2019). So that, selecting of water stress tolerant cultivars and identifying tomato genotypes that can tolerate water stress are an important strategy to overcome this problem and vital

for increasing crop production (Basha *et al.*, 2015) by manipulating the genetic variability within a species which is a valuable tool for screening and breeding for drought tolerance. (Wahb-Allah *et al.*, 2011). However, conventional screening methods are time, cost and labor intensive. Tissue culture based *in vitro* selection has emerged as a feasible and cost-effective tool for developing stress-tolerant plants (Rao and Jabeen, 2013). *In vitro* techniques make it possible to screen the required number of genotypes rapidly (Aazami *et al.*, 2010). Additionally, it is considered to be very suitable because it reduces the time required for selection of abiotic stress tolerance (Patade *et al.*, 2012) by handling a large population in a controlled and disease free environment (Patade *et al.*, 2008). Such screening methods must be incorporated into plant breeding programs to facilitate meaningful genetic improvement (Longenberger *et al.*, 2006). High molecular weight PEG (6000 or above) is metabolically inactive compound (Magar *et al.*, 2019), virtually impermeable to cell membranes and can induce water stress uniformly without causing direct physiological damage (Ghebremariam *et al.*, 2013), and providing conditions closely matching the effect of the reduced matric potentials (Álvarez-Iglesias *et al.*, 2017). Thus, PEG solutions have been the most feasible option for simulating drought conditions in short-term experiments. (Liu *et al.*, 2019) So that, *in vitro* selection is the most trustworthy method for screening desirable genotypes and to study further the effects of water scarcity on plant germination indices (Esan *et al.*, 2018).

Tomato is one the most important vegetable crop in Syria. The total annual production reaches about one million tons of fresh fruits, and the invested area occupies 11% of the total area invested in growing vegetables, and its production constitutes about 10% of the total production of various vegetables. The cultivated area for year 2022 reached (10082 hectares), of which 9345 hectares are irrigated agriculture and 738 hectares are rain-fed agriculture. The total production reached 522328 tons, with a productivity of 51807 kg/ha (Annual Agricultural Statistical Group, 2023).

In recent years, Syria has experienced a steady reduction in its water resources, with diminishing rainfall (Rainfall in Syria is unevenly distributed in space and time, which negatively affects agriculture), a decline in the groundwater level due to poor consumption, depleted aquifers, and reduced surface water flow. The decrease in water availability and drought conditions affect tomato production. The present study was undertaken to investigate the effect of drought stress induced *in vitro* by PEG6000 on eight pure tomato lines. These lines were morphologically characterized by Alsafadi *et al.*, (2009) and shown to be of great importance especially for their adaptability to local environments and the desired customer characteristics, and it is very important to screen their tolerance toward drought stress taking into account the increasing drought severity during the last decade.

## Materials & Methods

### Research site and plant material:

The experiments were carried out at Sweida Agricultural Research Center/ General Commission for Scientific Agricultural Research (GCSAR)/Syria, during 2018-2019. Eight pure tomato lines (5<sup>th</sup> generation) were used from the gene bank of GCSAR (Daher-Aljabal, Brieh, Baskanta, Kafer-Selwan, Daraa, Wardyat, Jerdi and Abosfair).

### Drought screening treatments:

Different concentrations of PEG6000 (polyethylene glycol): 2%, 4%, 6%, 8% (w/v) in addition to control (0%) were used to induce *in vitro* drought stress in order to investigate the effect of drought stress at early seedling growth. Seeds were surface-sterilized by washing under running tap water, then immersed in 0.6g/l topsin M (fungicide) for 15 min. and rinsed three times with distilled water. Seeds then were sterilized with 1% sodium hypochlorite (NaOCl) for 20 min., rinsed three times with autoclaved distilled water under aseptic conditions. The seeds were dried on autoclaved filter papers

for 15min. then cultured in tubes (one seed/tube) containing 10mm MS basal medium (Murashige and skoog, 1962) supplemented with 30 g/l sucrose and 7g/l agar in addition to the selection agent PEG 6000 at studied concentration, in addition to the control (MS free of stress agent).

Each treatment was replicated three times, 10 seeds in each replication. Cultures in all experiments were kept in 16 hrs light and 8 hrs dark photoperiod and  $22\pm 2^{\circ}\text{C}$  temperature, provided by fluorescent tubes with light intensity of  $2.5 \mu\text{mol.m}^{-2}\text{S}^{-1}$ .

In order to screen the tomato pure line for drought stress tolerance, the plantlets were subjected to stress for a sufficient period to stimulate long-term effects (45 days) for measuring the morphological parameters.

#### **Studied parameters:**

After 45 days of stress treatment, application *in vitro* plantlets were removed carefully from tubes and agar was removed gently. Ten plantlets for each replication with total of 30plants/treatment were used. Length and diameter of main root, seedling length, and stem diameter were measured. Root to stem length ratio, number of roots, leaves and shoots were recorded. Leaf area ( $\text{mm}^2$ ) were measured with a Li-Cor 3100 area meter (Li-Cor, Lincoln, NE), plant and root dry weight were determined (oven-dried at  $70^{\circ}\text{C}$  for at least 72h) (Schafleitner *et al.*, 2007). The reduction rate of the studied parameters was calculated as the following:

$$\text{Reduction rate} = \frac{\text{the value of the control} - \text{the value of the treatment}}{\text{the value of the control}}$$

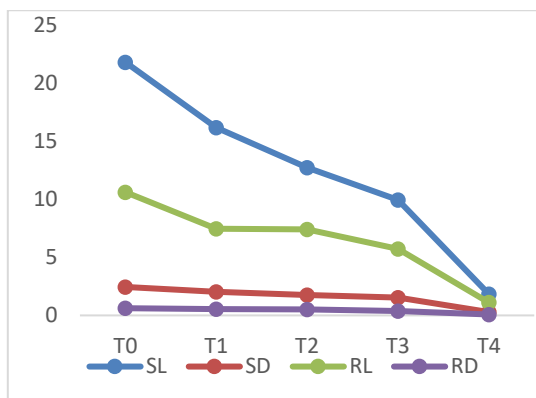
#### **Statistical analysis:**

The experiment was performed as a completely randomized design (CRD) in a factorial system. Each local line was replicated three times for each treatment. Data were subjected to ANOVA analysis, mean values were compared according to at least significant difference test (LSD) with p value  $\leq 0.01$ . The obtained results were statistically analyzed using GenStat12 program. Cluster analysis was performed based on the sum of relative values of the differences between the control and stressed plants for morphological parameters.

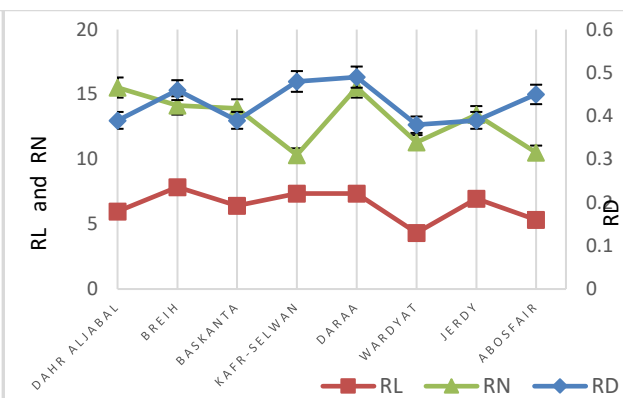
#### **Results**

##### ***Roots and seedling growth parameters:***

Root length and diameter in addition to seedling length and diameter decreased significantly with increasing PEG6000 concentration as illustrated in Figure 1. The mean root length decreased by 29.6%, 30.25%, 46.8% and 89.54% in T1, T2, T3 and T4 respectively as compared to T0 (10.61 cm). Regardless of PEG concentration, pronounced differences were recorded between pure-tomato lines. Breih and Daraa (Figure 2) recorded maximum mean root diameter (0.46, 0.49 cm) and maximum mean root length (7.86, 7.49 cm) with a reduction in root length by 41.3% and 32.07 %, respectively as compared to Wardyat ana Abosfair lines (Figure2) with no significant differences as compared to other lines. While the maximum reduction in root length was recorded for line Abosfair and Wardyat as presented in Table1. However, it is worth mentioning that the root length of Jerdy line increased with increasing drought stress from 6.15 cm in T0 to 9.47, 8.65 and 7.57 cm in T1, T2 and T3, respectively by 53.98%, 40.65% and 23.09 % respectively as compared to T0, then it decreased by 50.73 % in T4.



**Figure(1): Effect of drought stress on root and shoot growth of tomato lines**



**Figure(2): Effect of tomato lines on root growth parameters under drought stress**

SL (seedling length) SD (stem diameter) RL (mean root length) RD (mean root diameter) RN (mean number of roots per seedling)

Number of roots decreased significantly when PEG increased. On the other hand, no significant differences were found between tomato lines even though Daraa and Daher Aljabal had the maximum mean number of roots (8.13, 7.93 roots/seedling), respectively as presented in Table 1.

**Table(1): The effect of different drought stress treatments on root length and number of roots for eight pure tomato lines.**

| Tomato line        | Root Length (RL) |                |                |                |               | Mean for the tomato line | Number of roots (RN) |        |        |            |           | Mean for tomato line |
|--------------------|------------------|----------------|----------------|----------------|---------------|--------------------------|----------------------|--------|--------|------------|-----------|----------------------|
|                    | Treatment        |                |                |                |               |                          | Treatment            |        |        |            |           |                      |
|                    | 0%               | 2%             | 4%             | 6%             | 8%            |                          | 0%                   | 2%     | 4%     | 6%         | 8%        |                      |
| Dahr aljabal       | 9.75<br>bed      | 6.87<br>efghij | 7.73<br>defgh  | 5.50<br>hijklm | 0             | 5.97 CD                  | 19a                  | 11.67a | 6.33ab | 2.67abc    | 0         | 7.93 A               |
| Breih              | 11.75<br>b       | 9.15<br>bcdef  | 11.45a         | 6.97<br>efghij | 0             | 7.86<br>A                | 18.67a               | 2.78a  | 2.67b  | 3.67ab     | 0         | 5.56 A               |
| Baskanta           | 10.98<br>bc      | 6.95<br>efghij | 7.33<br>defghi | 6.87<br>efghij | 0             | 6.42 AB                  | 15.33a               | 7.5a   | 5.17ab | 2.67ab     | 0         | 6.13 A               |
| Kafr-selwan        | 17.60<br>a       | 4.97a          | 7.50<br>defghi | 6.67<br>fghijk | 0             | 7.35 AB                  | 10a                  | 7.33a  | 3.33b  | 1bc        | 0         | 4.33 A               |
| Daraa              | 9.90<br>bcd      | 7.63<br>efghij | 7.63<br>defgh  | 7.27<br>defghi | 5.00<br>ijklm | 7.49 AB                  | 16a                  | 10.67a | 8a     | 4a         | 2a        | 8.13 A               |
| Wardyat            | 7.30<br>defghi   | 6.00<br>ghijkl | 4.07<br>klmn   | 3.40<br>lmno   | 0.87<br>op    | 4.33<br>E                | 13.33a               | 7.17a  | 2.33b  | 1.5abc     | 0.67b     | 5 A                  |
| Jerdy              | 6.15<br>ghijk    | 9.47<br>bcde   | 8.65<br>cdefg  | 7.57<br>defghi | 3.03<br>mno   | 6.97 ABC                 | 18.5a                | 16a    | 1.67b  | 1bc        | 0.3b      | 7.49 A               |
| Abosfair           | 11.50<br>b       | 8.70<br>cdefg  | 4.87<br>jklm   | 1.63<br>nop    | 0             | 5.34 DE                  | 11a                  | 7.67a  | 3b     | 1.6c       | 0         | 4.65 A               |
| Mean for treatment | 10.61A           | 7.47B          | 7.40B          | 5.73C          | 1.11D         |                          | 15.23<br>A           | 8.85 B | 4.06 C | 2.26<br>CD | 0.37<br>D |                      |
| LSD Line           | 1.219            |                |                |                |               |                          | 2.489                |        |        |            |           |                      |
| LSD Treat.         | 0.964            |                |                |                |               |                          | 2.759                |        |        |            |           |                      |
| LSD Line*Treat.    | 2.726            |                |                |                |               |                          | 6.803                |        |        |            |           |                      |

In each column, different small letters indicate significant differences between treatments and different capital letters in each column indicate significant differences between tomato lines ( $P \leq 0.01$ )

Concerning stem diameter, no significant differences was recorded between tomato lines concerning stem diameter (Table 2). Moreover, seedling length varies due to PEG depending on the tomato line. The reduction in seedling length due to drought stress was significant between tomato lines as summarized in Table 2. Jerdy line had the least reduction percentage (32.45%) of seedling length in all treatments as compared to T0, followed by Daraa (42.05%) with significant difference between them.

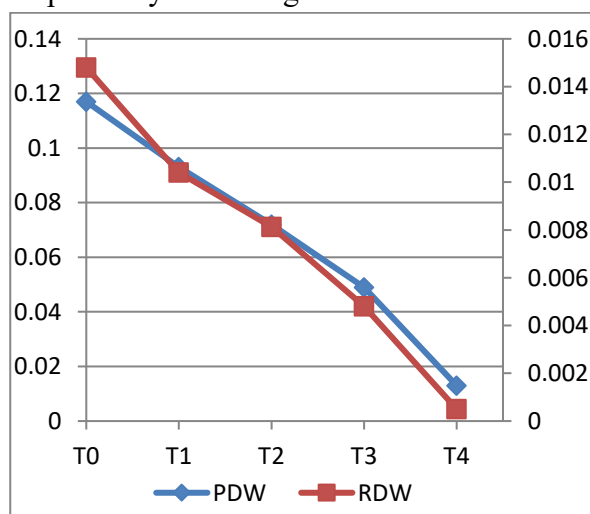
Table(2): The effect of different drought stress treatments on seedling length and stem diameter for eight pure tomato lines.

| Tomato line        | Seedling length (SL) |            |             |            |        | Mean for tomato line | Stem diameter (SD) |            |             |            |         | Mean for tomato line |
|--------------------|----------------------|------------|-------------|------------|--------|----------------------|--------------------|------------|-------------|------------|---------|----------------------|
|                    | Treatment            |            |             |            |        |                      | Treatment          |            |             |            |         |                      |
|                    | 0%                   | 2%         | 4%          | 6%         | 8%     |                      | 0%                 | 2%         | 4%          | 6%         | 8%      |                      |
| Dahr aljabal       | 13.93 A              | 12.73 Ab   | 5.4 hijkl   | 3.33 mnopq | %8     | 7.08 A               | 2.99a              | 2.40 bcdef | 2.36 bcdefg | 2.04 efgh  | 0       | 1.96 A               |
| Breih              | 12.46 abc            | 7.28ab     | 5.57 hijkl  | 5.57 hijkl | 0      | 6.18 B               | 2.49 bcd           | 2.06 defgh | 2.02 efgh   | 2.21 cdefg | 0       | 1.76A                |
| Baskanta           | 10.07 def            | 9.77 Ef    | 6.35 hijk   | 4.17 lmno  | 0      | 6.07 B               | 2.59 abc           | 2.46 bcde  | 2.14 defg   | 2.21 cdefg | 0       | 1.88A                |
| Kafr-selwan        | 10.67 Cde            | 8.6 Fg     | 4.67 klmn   | 5.63 hijkl | 0      | 5.91 B               | 1.64 Hij           | 1.63 hij   | 1.36 jkl    | 0.95 lm    | 0       | 1.11A                |
| Daraa              | 11.87 Bcd            | 9.95 def   | 6.73 ghij   | 4.62 klmn  | 0      | 7.08 A               | 2.44 bcde          | 2.19 cdefg | 1.62 hij    | 1.26 Jkl   | 0.97 lm | 1.69A                |
| Wardyat            | 9.60 Ef              | 5.23 Ijklm | 4.17 lmno   | 2.87 opq   | 2.25pq | 4.81 C               | 2.30 bcderg        | 1.98 fghi  | 1.44 jk     | 1.133 klm  | 0.69 m  | 1.51A                |
| Jerdy              | 10.85 Bcde           | 6.75 Ghi   | 4.78 ijklmn | 4.27 lmn   | 2.17qr | 5.62 BC              | 2.73ab             | 1.55 ijk   | 1.43 jk     | 1.25 jkl   | 0.69 m  | 1.53A                |
| Abosfair           | 10.03 def            | 9.93 Def   | 4.10 lmnop  | 3.20 nopq  | 1.43r  | 5.45 BC              | 2.42 bcdef         | 1.94 ghi   | 1.66 hig    | 1.26 jkl   | 0       | 1.46A                |
| Mean for treatment | 11.19 A              | 8.78 B     | 5.22 C      | 4.21 D     | 0      |                      | 2.82 A             | 2.02 B     | 1.75 C      | 1.54 D     | 0.2 E   |                      |
| LSD Line           | 0.882                |            |             |            |        |                      | 0.202              |            |             |            |         |                      |
| LSD Treat.         | 0.698                |            |             |            |        |                      | 0.159              |            |             |            |         |                      |
| LSD Line*Treat.    | 1.973                |            |             |            |        |                      | 0.451              |            |             |            |         |                      |

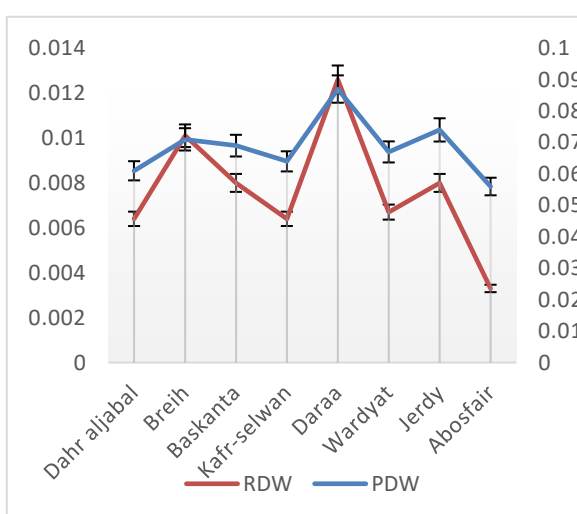
In each column, different small letters indicate significant differences between treatments and different capital letters in each column indicate significant differences between tomato lines ( $P \leq 0.01$ )

### Plant and root dry weight:

Overall, plant and root dry weight decreased as the PEG concentration increased and it was significant at high concentration of PEG when compared with control and low PEG concentration (Figure 3). Tomato lines were affected significantly by drought stress with significant differences between them. Daraa had the maximum mean plant dry weight (0.087g) followed by Jerdy (0.074g). Concerning the root dry weight, Daraa and Breih had the maximum mean root dry weight with 0.013 and 0.010g, respectively but no significant differences were noticed between the other tomato lines.



Figure(3): Effect of drought stress on plant and root dry weight (g) of tomato lines



Figure(4): Effect of tomato lines on plant and root dry weight(g) under drought stress

PDW (plant dry weight) RDY (roots dry weight)

**Root/ shoot length ratio**

The root/shoot length ratio increased with increasing PEG concentration in Jerdy, Daraa, Breih, Daher-Aljabal lines (Table 3). The ratio were 1.60, 1.44, 0.93 and 0.89 respectively. On the other hand, root/shoot ratio decreased with increasing drought stress severity induced by PEG for the other tomato lines and the lowest ratio was recorded for line Abosfair (0.74) by a mean reduction rate of 55.65% (PEG 6%) as compared to control treatment (PEG 0%).

**Table (3): The effect of different drought stress treatments on root/shoot length ratio**

| Tomato line               | PEG concentration |        |         |        |        | Mean for tomato Line |
|---------------------------|-------------------|--------|---------|--------|--------|----------------------|
|                           | %0                | %2     | %4      | %6     | %8     |                      |
| <b>Dahr aljabal</b>       | 0.73bc            | 0.54b  | 1.41a   | 1.75a  | 0      | 0.89ab               |
| <b>Breih</b>              | 0.95bc            | 1.35ab | 2.05a   | 1.73a  | 0      | 1.22ab               |
| <b>Baskanta</b>           | 1.10b             | 0.73ab | 1.16a   | 1.65a  | 0      | 0.93ab               |
| <b>Kafr-selwan</b>        | 1.65a             | 0.56b  | 1.58a   | 1.20a  | 0      | 0.99ab               |
| <b>Daraa</b>              | 0.83bc            | 0.70ab | 1.21a   | 2.16a  | 2.29a  | 1.44ab               |
| <b>Wardyat</b>            | 0.76bc            | 1.15ab | 0.98a   | 1.26a  | 0.39b  | 0.91ab               |
| <b>Jerdy</b>              | 0.59c             | 1.45a  | 1.98a   | 1.78a  | 2.22a  | 1.60a                |
| <b>Abosfair</b>           | 1.15b             | 0.88ab | 1.19a   | 0.51a  | 0      | 0.74b                |
| <b>Mean for treatment</b> | 0.97 B            | 0.92 B | 1.44 AB | 1.50 A | 0.61 B |                      |
| LSD Line                  | 0.384             |        |         |        |        |                      |
| LSD Treat.                | 0.304             |        |         |        |        |                      |
| LSD Line*Treat.           | 0.859             |        |         |        |        |                      |

In each column, different small letters indicate significant differences between treatments and different capital letters in each column indicate significant differences between tomato lines ( $P \leq 0.01$ )

**Leaf area (mm<sup>2</sup>)**

It was determined from the results illustrated in Table 4 that drought stress treatments had a very significant effect on leaf area so that the leaf area decreased significantly with increasing the PEG concentration in all tomato lines. Among the tomato lines, Daraa was affected the least by drought stress because it gave the lowest reduction rate for leaf area (52.41%) but the highest value for leaf area reduction rate was recorded for Abosfair (87.61%) at 6% PEG concentration as compared to 0% PEG.

**Table (4): The effect of drought stress treatments on leaf area (mm<sup>2</sup>) for 8 pure tomato lines.**

| Tomato line               | PEG concentration |           |          |          |          | Mean for Line |
|---------------------------|-------------------|-----------|----------|----------|----------|---------------|
|                           | %0                | %2        | %4       | %6       | %8       |               |
| <b>Dahr aljabal</b>       | 1309.63a          | 1163.45ab | 471.93ab | 273.37bc | 0        | 634.7a        |
| <b>Breih</b>              | 1404.47a          | 705.18abc | 296.22ab | 220.65ba | 0        | 525.3a        |
| <b>Baskanta</b>           | 1383.83a          | 1245.18a  | 484.82ab | 231.97ab | 0        | 669.2a        |
| <b>Kafr-selwan</b>        | 916.92a           | 527.43c   | 381.25ab | 256.13b  | 0        | 436.3a        |
| <b>Daraa</b>              | 1255.78a          | 858.75ab  | 631.83a  | 597.68a  | 153.33a  | 656.4a        |
| <b>Wardyat</b>            | 1105.58a          | 586.72bc  | 381.25ab | 165.87c  | 127.35ab | 473.4a        |
| <b>Jerdy</b>              | 1130.70a          | 584.55bc  | 416.60ab | 139.35c  | 92.37b   | 472.7a        |
| <b>Abosfair</b>           | 1195.20a          | 580.53bc  | 236.45b  | 148.03c  | 0        | 432.0a        |
| <b>Mean for treatment</b> | 1212.76A          | 781.47B   | 412.54C  | 254.13D  | 46.63E   |               |
| LSD Line                  | 129.9             |           |          |          |          |               |
| LSD Treat.                | 102.7             |           |          |          |          |               |
| LSD Line*Treat.           | 290.4             |           |          |          |          |               |

In each column, different small letters indicate significant differences between treatments and different capital letters in each column indicate significant differences between tomato lines ( $P \leq 0.01$ ).



### Number of leaves and shoots per seedling

As presented in Figure 5, total number of leaves and shoots per seedling decreased as the PEG concentration increased whereas the mean number of leaves decreased from 5.79 leaves/plant in T0 to 0.29 leaves/ plant in T4 by a reduction rate of 94.99%. Furthermore, mean number of shoots was 8.89 shoots/plant in T0 then decreased to 0.75 shoots/plant in T4 by a reduction rate of 91.56%.

It was determined from Figure 6 that tomato lines varied significantly in respect to the total number of leaves and shoots/plant. The maximum number of leaves (4.32) and shoots (7.04) was recorded in Daraa line which exceeded significantly the other lines, in contrast, the lowest number of leaves (1.5) and shoots (1.73) was recorded in Abosfair and Daher-Aljabal, respectively.

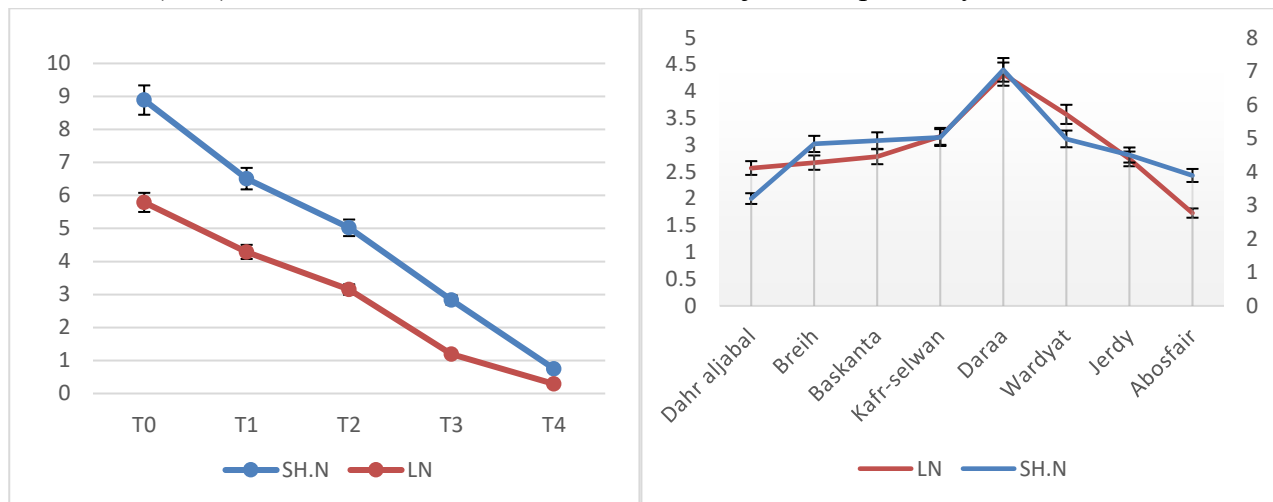


Figure (5): Effect of drought stress on total number of leaves and shoots/ tomato plant

Figure (6): Effect of drought stress on total of leaves and shoots / tomato plant

SH.N (mean number of shoots per seedling) LN (mean number of leaves per seedling)

### Cluster analysis

The cluster analysis based on the sum of relative values of the differences between the control and stressed plants for morphological parameters resulted in four districted groups: (1) drought tolerant group consisting of Jerdy line. (2) a moderately drought tolerant group consisting of lines: Daraa and Daher-Aljabal, (3): a moderately drought susceptible group consisting of line Breih and (4): a susceptible group consisting of Baskanta, Abosfair and Kafer-Selwan and Wardyat as shown in Figure 7

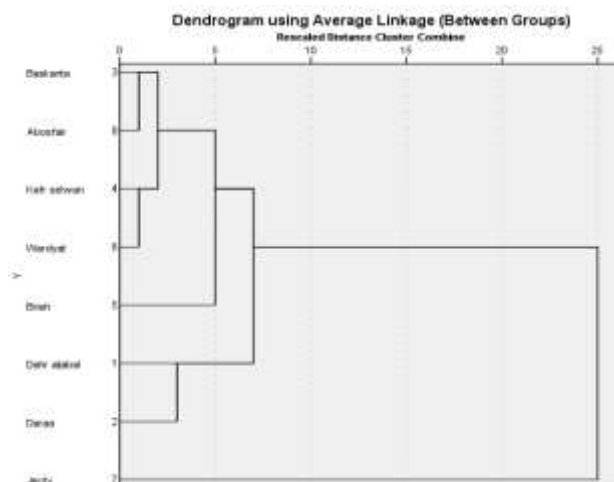


Figure (7): Dendrogram of the hierarchical clustering analysis.

## Discussion

*In vitro* development of drought stress tolerant plants has been reported in many plant species including tomato, potato, maize, corn, rice, banana (Kripkyy *et al.*, 2001; Naveed *et al.*, 2019; George *et al.*, 2013; Andreea *et al.*, 2014; Bidabadi *et al.*, 2012; He *et al.*, 2009; Khodarahmpour, 2011; Magar *et al.*, 2019; Partheeban *et al.*, 2017). Results of this study proved the efficiency of *in vitro* methods for screening tomato lines at early growth stage and indicated that drought stress induced by PEG6000 had a significant effect on the morphological characteristics of the studied tomato pure lines at seedling establishment stage which is potentially the most critical stages for water stress (Queiroz *et al.*, 2019).

On the other hand an increase in root-to-shoot length ratio was recorded as a result of the effect of drought stress on aerial parts growth recorded in this research. Our results are in accordance to Bredy *et al.*, (2015) who found a decrease in growth parameters with PEG concentrations increasing and agree with those obtained by Aazami *et al.*, (2010); Kulkarni and Deshpande, (2007) who recorded a limitation of tomato growth under drought condition induced by PEG.

Reduction of plant growth is a common response to water deficit. This is mainly due to the loss of turgor pressure which reduces cell elongation (Syversten, 1985; Karimi *et al.*, 2013). Additionally, water deficit inhibits cell division, expansion of leaf surface, growth of stem, and proliferation of root cells (Osmolovskaya *et al.*, 2018). Roots are the part that firstly affected by drought stress. In general, when plants are exposed to water stress, a significant inhabitation of root growth is noticed as recorded in many previous reports carried out on tomato (Jokanović and Zdravković, 2015, Bredy *et al.*, 2015). This fact was proved by our result except for Jerdy tomato line whereas a significant increase in root elongation was recorded in water stress condition induced by PEG6000 at concentrations of 2, 4, 6% in media. Since root length is an important trait against drought stress in plant varieties; so that variety with longer root growth has resistant ability for drought as mentioned by Kaydan and Yagmur, 2008 and earlier by (Leishman and Westoby, 1994) who reported that longer root growth has resistant ability for drought. This fact gave the Jerdy line the superiority over other evaluated tomato lines as a drought-tolerant line and this was clearly insulated by cluster analysis performed in this study.

The root length reduction was recorded in all other evaluated tomato lines under PEG6000 stress and that is in agreement with George *et al.*, (2013) and this may be associated to a reduced cellular division and elongation during germination (Muscolo *et al.*, 2014). However, seedling length was more affected than root length by water stress which is in line to Abdel-Raheem *et al.*, (2007) but disagreed with George *et al.*, 2013 who reported that drought stress was non-significant in case of shoot length. Concerning the root-to-shoot length ratio, the results of this study showed that root-to-shoot length ratio increased with increasing drought stress. These results are consistent with those reported by Queiroz *et al.*, 2019; Khodarahmpour 2011) High root to shoot ratio has been reported as a component trait for drought avoidance by Xu *et al.*, (2015).

In the present study, a reduction in root and plant dry weight was recorded in stressed conditions in all the genotypes. Present investigation is in confirmation with Kumar *et al.*, (2017); Khan *et al.*, (2015) and Nahar & Gretzmacher (2002). Root dry weight depends on the germination percent and root length, low water uptake and restricted metabolic activities were given to decrease in the root dry weight Wani *et al.*, (2010). Nevertheless, more pronounced effect on shoot dry weight than on the roots which is in line to Brdar-Jokanovic *et al.*, (2014).

Drought also decreased leaf area owing to loss of turgor and reduced leaf numbers (Farooq *et al.*, 2010). Plant showed reduced number of leaves and shoots in all PEG treatments. These results are also confirmed by Kiani *et al.* (2007) who observed that plant reduced number of leaves is mainly



due the loss of turgor. Moreover Karimi *et al.*, 2013 reported that reduced water absorption probably is the main reason for leaves growth reduction.

Cluster analysis was performed in order to classify tomato lines in similar groups as a final evaluation of lines to be recommended for breeding programs for drought tolerance. In our results, eight tomato lines were grouped into 3 clusters based on various drought related traits. Cluster analysis was used by many researchers (Murshed *et al.*, 2013; Bredy *et al.*, 2015; Zdravkovic *et al.*, 2013; Brdar-jokanovic *et al.*, 2014

### Conclusion

Data of this study suggested the efficiency of *in vitro* selection method for screening drought tolerant tomato lines. The inbred tomato lines Jerdy, Daraa, Brieh and Daher-Aljabal exhibited a good level of drought tolerance related to the measured parameters at the early seedling development stage. Therefore, they can be used as parents in breeding programs. Moreover, the evaluated tomato lines could be successfully used as positive tolerant or susceptible controls to be compared to any tomato genotype in future studies. So that, tomato lines with higher root length and dry weight besides higher root-to-shoot ratio and leaf area should be selected while screening for drought tolerance as positive tolerant controls in future researches. Nevertheless, physiological assessment is required for advanced studies.

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## استجابة ثمان خطوط نقية من البندورة للإجهاد الجفافي المحدث مخبرياً باستخدام بولي إيثيلين غليكول

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### المخلص

تم تنفيذ البحث في مركز بحوث السويداء / الهيئة العامة للبحوث العلمية الزراعية خلال العام 2018-2019 بهدف دراسة تأثير الإجهاد الجفافي المحدث مخبرياً باستخدام بولي إيثيلين غليكول PEG 6000 في ثمان سلالات نقية من البندورة من رتبة الجيل الخامس وذلك في المرحلة الأولى من تطور البادرات. تم استخدام أربعة تراكيز من PEG 6000 وهي 2% و 4% و 6% و 8% إضافة إلى الشاهد. أظهرت النتائج أن زيادة تركيز معاملة الإجهاد الجفافي أدت إلى انخفاض مؤشرات النمو المدروسة وهي قطر وعدد الجذور، طول وقطر البادرة، عدد الأوراق والأفرع، الوزن الجاف للجذور وللبادرة إضافة إلى المساحة الورقية. أما فيما يتعلق بطول الجذور فقد أدت معاملات الإجهاد الجفافي إلى خفض معدل استطالة الجذور في جميع السلالات المدروسة عدا السلالة جردى. التي أظهرت زيادة في طول الجذور بزيادة معاملة الإجهاد الجفافي الأمر الذي جعلها متفوقة على باقي السلالات وظهرت كسلالة متحملة للإجهاد الجفافي. اعتماداً على نتائج التحليل العنقودي المعتمدة على مجموع القيم النسبية لمؤشرات النمو المدروسة؛ تم تمييز أربع مجموعات: (1) تتضمن السلالة جردى. (2) تتضمن السلالتين درعا وضهر الجبل. (3) تتضمن السلالة بريج والمجموعة (4) التي تتضمن السلالات بسكننا وكفر سلوان وبصفير وورديات.

**الكلمات المفتاحية:** البندورة سلالة نقية، غرلة، إجهاد جفافي، تحليل عنقودي.