Somaclonal Variation Selection for Tolerance to PEG-Induced Drought Stress in Four Local Tomato (Solanum lycopersicum L.) Varieties

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ABOUT THE ARTICLE

Growth and development of tomato is restricted by numerous abiotic stress factors especially drought. Crop genetic improvement through traditional methods is time-consuming and conducted under uncontrolled conditions; thus, in vitro selection offers an efficient alternative. This research was carried out at the National Commission for Biotechnology (NCBT) and the General Commission for Scientific Agricultural Research (GCSAR), Syria during 2018 and 2019. The main objective was to select callus tolerant to drought stress and regenerate tolerant somaclonal variant plants in four local tomato varieties. Callus (formed from root explants on MS medium supplemented with 2 mg/L BAP and 0.2 mg/L NAA) was subjected to four different levels of drought stress induced by PEG 6000 (2%, 4%, 6%, 8% in addition to the non-treated control). After 8 weeks, cell viability, relative growth rate (RGR), proline content, and tolerance index (TI) were determined. Callus was then transferred to regeneration medium (MS supplemented with 2 mg/L zeatin, 0.2 mg/L GA3 and the sub-lethal concentration of PEG (4% for Daher-Aljabal, 6% for Brieh and 6%, 8% for Daraa variety)). The regenerated somaclonal variant plants were evaluated, based on morphological characteristics, for drought tolerance compared to mother plant. Results showed that RGR, TI and cell viability decreased while free proline content increased in response to increasing PEG concentration. Based on the studied parameters, Daraa was the most tolerant variety, followed by Daher-Aljabal, Brieh and Baskanta. A single regenerated plantlet was obtained from Daher-Aljabal variety named DH1 and another plantlet from Brieh variety named BR1, while two regenerated plantlets were obtained from Daraa variety named DA1 and DA2. All of them showed better tolerance to drought stress than mother plants at 8% PEG concentration in terms of root length, plant height, root-shoot ratio and dry weight. In conclusion, a successful callus formation and plant regeneration protocol has been developed under drought stress treatments induced by PEG 6000. Four somaclonal variant plants were successfully developed and they were different from mother plants based on the studied parameters.

KEYWORDS:
Callus culture, Drought stress, Proline, Induced Drought Stress, PEG, Somaclonal Variation, Selection for Tolerance, Callus, Drought stress, Proline, Somaclonal Variation Selection for Tolerance to PEG, Four Local Tomato (Solanum Lycopersicum L.) Varieties.

ALNADAF, O., OBAID, H. et MOHSEN, W. 2020. Selection of somaclonal variants tolerant to drought stress induced by PEG in four local Syrian tomato varieties. In The Scientific Journal of King Faisal University, 2020, Volume (OnlineFirst), Issue (OnlineFirst).
1. Introduction

Tomato (Solanum lycopersicum L.) is the second most consumed vegetable in the world (Idbali et al., 2019) after potato. Recently, this crop has gained huge popularity due to its antioxidant and anti-cancer characteristics (Khuong et al., 2013). Being a tropical plant, tomato is well adapted to almost all climatic regions of the world; however, environmental stress factors are the primary yield determinants of this crop’s yield potential (Gerszberg and Hnatuszko-Konka, 2017). Drought is one of the most limiting factors for agricultural productivity worldwide (Hamdi et al., 2020). Drought stress can be simply defined as a shortage of water that causes significant changes in plant morphology, physiology, and biochemistry (Liang et al., 2020). All of these changes reduce tomato growth rate and production (Krishna et al., 2019). With the increasing human population and depleting water resources, the development of drought-resistant crops is of prime importance to preventing crop yield losses (Kim et al., 2020). However, traditional methods for tomato breeding can be expensive, time-consuming (Khuong et al., 2013) and very hard due to the different soil properties (Nemesci and Helyes, 2019). Therefore, plant biotechnology could help plant breeder by creating and detecting the genetic differences then enhance the yield and quality of tomatoes by increasing the plant tolerance for different stress factors (Abdel-Raheem et al., 2007). In vitro selection to obtain drought tolerant genotypes can use selection agents in the form of osmotic compounds that can simulate dry conditions in the field (Yunita et al., 2020). Polyethylene glycol (PEG) is widely used to induce drought stress under controlled laboratory conditions. High molecular weight PEG (6000 or above) is metabolically inactive compound (Magar et al., 2019). Thus, PEG solutions have been the most feasible option for simulating drought conditions in short-term experiments (Liu et al., 2019). So that, it can be used as a selection agent in vitro for screening desirable genotypes to drought stress (Esan et al., 2018). It was used in many crop species such as tomato (Basha et al., 2015; Kumar et al., 2017), sweet potato (Sunaryo et al., 2019), soybean (Saepudin et al., 2017) and wheat (Rana et al., 2017). It is being assumed that in vitro propagation passing through callus phase is prone to epigenetic changes among the regenerated plants (Bednarek and Orłowska, 2020). Somaclonal variation through callus culture has been used for generating useful genetic variation for desired traits (Krishna et al., 2019). Various somaclonal variant plants were obtained by in vitro selection in tomato and pepper (Sargsyan et al., 2014), Brassica (Jan et al., 2018), tomato (Mohamed et al., 2004; Ali et al., 2017) and potato (Bordallo et al., 2004). Furthermore, significant improvement in drought tolerance was made in crops like sugarcane (Rao and Jabeen, 2013), wheat (Mahmood et al., 2012), tomato (Abdel-Raheem et al., 2007), chili pepper (Hossain et al., 2003), and date palm (Al-Khayri and Al-Bahrany, 2004). Many tissue culture studies concerning calllogenesis and regeneration have been conducted on tomato (Ishag et al., 2009; Chaudhry et al., 2010; Gerszberg et al., 2016; Durrani et al., 2017). On the other hand, callus formation and regenerate tomato plants from calli grown under drought stress were studied by many researchers who suggested that callus cells tolerate drought stress could produce tolerant plants as well due to somaclonal variation phenomenon (Abdel-Raheem et al., 2007; Aazami et al., 2010). Research efforts have been undertaken in order to improve tomato tolerant to drought stress. Aazami et al. (2010) studied in vitro response of some tomato genotypes for tolerance to osmotic stress and found that the presence of PEG in the medium decreased relative growth rate and increased dry matter content in all treatments compared with the control. In all genotypes, proline levels increased in response to water stress. In addition, there was decreased shoot induction in all cultivars with increased PEG concentration. Abdel-Raheem et al. (2007) described the effect of PEG-induced drought stress on different tomato genotypes. Significant differences were found between genotypes under investigation for their calllogenesis and regeneration ability under drought stress condition. However, in their investigations no attempt is made to select PEG tolerant callus lines based on RGR and cell viability as selective criteria for regeneration stage and no analysis were made to compare somaclonal variants to their mother plants. However, there are few reports on in vitro selection for drought tolerance in tomato based on callus viability, relative growth rate and proline accumulation. It is worth to mention that somehow all previous studies found that callus formation and plant regeneration are highly dependent on tomato genotype. In Syria, this is the first report on somaclonal variation selection in tomato taking into account that there are many valuable local varieties of tomato that are adapted to the environment. The present investigation aimed to select tolerant callus to drought stress induced by PEG and characterize callus lines in relation to growth rate, cells viability, proline accumulation and assess the regeneration responses of selected drought tolerant callus lines then screening of somaclonal variant plants compared to the mother plants in four tomato varieties based on morphological characters.

2. Materials and Methods

The experiments were carried out at the tissue culture
laboratory of the General Commission for Scientific Agricultural Research (GCSAR) and at the National Commission for Biotechnology (NCBT)-Syria, during years 2018 and 2019. Four tomato varieties were used from the gene bank of GCSAR (Daher-Aljabal, Brieh, Baskanta and Darra). Seeds were surface-sterilized by washing under running tap water, then immersed in 0.6 g/L topsin M (fungicide) for 15 min. and rinsed three times with distilled water. Seeds were then sterilized with 1% sodium hypochlorite (NaOCl) for 7 min., and rinsed three times with autoclaved distilled water under aseptic conditions. The seeds were dried on autoclaved filter papers for 15 min. then cultured in sterilized petri dishes containing MS basal medium (Murashige and skoog, 1962) supplemented with 30 g/L sucrose and 7 g/L agar. For callus formation, roots of 15-day-old seedlings were used as explants for callus formation. Explants were cut into small pieces of about 3-5 mm segments and cultured in aseptically conditioned callus formation medium: MS medium supplemented with 30 g/L sucrose, 7 g/L agar, 2 mg/L BAP (6-Benzylaminopurine) and 0.2 mg/L NAA (1-Naphthaleneacetic acid). The best explants for callus formation and the best induction medium plus the hormonal supplements were selected in this study based on our previous experiment (unpublished data). The pH of the medium was adjusted to 5.8 then autoclaved at 120 °C and 1.04 kg/cm² pressure for 20 min. Cultures were maintained at 24±2 °C under 16 h photoperiod provided by cool fluorescent tubes with a light intensity of 40 μmol m⁻² s⁻¹. Callus was sub-cultured twice at 4-week intervals on the same induction medium then, 2 month old homogenous callus (nodular and light green or creamy color with friable texture) was re-cultured on callus proliferation medium supplemented with different concentrations of PEG (2%, 4%, 6% and 8%). Eight weeks after incubation, the following parameters were determined:

**Callus viability:** According to TTC method described by (Lutts et al., 2004). Cell viability was measured by reduction of 2,3,5-triphenyltetrazolium chloride (TTC) in viable cells to a red colored water-insoluble formazan by the action of dehydrogenases. Samples of 25 mg callus were incubated in 2 ml TTC solution (dissolved in a mixture of 0.05 M sodium phosphate buffer, pH 7.1) for 15 h at room temperature in darkness. The produced formazan was extracted from the callus with 5 ml of ethanol 94% (v/v) for 60 min. Absorption was read at 455 nm with the spectrophotometer. The formazan content was expressed as a percentage of the control, calculated as (absorption of stressed tissue/absorption of control) x 100. Thus, Formazan content calculated will be termed viability. Black and non-colored cells are considered dead (Silva and Menéndez-Yuffá, 2006).

**Relative growth rate (RGR):** RGR of callus was determined on a fresh weight basis using the formula: 

\[ \text{RGR} = \frac{\text{GR}_{\text{PEG}} - \text{GR}_{\text{control}}}{\text{GR}_{\text{control}}} \times 100 \]  

-GR=Wi-Wi/GP (where: Wi, W0 are the Final and initial weight of callus, respectively, GP is the growth period under stress condition) (Birsin and Ozgen, 2004).

**Free proline content:** Free proline was estimated according to method described by (Bates et al., 1973). 0.5 g of fresh callus was homogenized in 3% (w/v) aqueous sulphosalicylic acid and centrifuged at 10000 g for 10 min. In a test tube, 2 ml of the filtrate was mixed with 2 ml acid ninhydrin and 2 ml glacial acetic acid and incubated in 100 °C water bath for 1 h. The reaction mixture was terminated on ice bath then it was extracted with 4 ml toluene, and the chromophore phase was separated from the aqueous phase. The absorbance of proline-ninhydrine product was read at 520 nm using spectrophotometer. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

\[ \text{μmoles proline/g of fresh weight material} = \left( \frac{[\text{μg proline/ml} \times \text{ml toluene}]}{115.5 \text{ μg/μmole}} \right) / [(g \text{ sample})/5]. \]

**Tolerance index (TI):** to compare the varieties-related responses to drought stress, a tolerance index (TI) based on RGR was determined according to the following formula:

\[ \text{TI} = \frac{\text{RGR treatment}}{\text{RGR control}} \]

**PEG 6000 sub-lethal determination:** The concentration of PEG 6000 that resulted in 50% decrease in cell viability and RGR was selected as sub-lethal concentration for tomato varieties to evaluate against drought stress (El-Yacoubi et al., 2010). Callus that survived at this concentration was considered tolerant and selected for the plant regeneration stage.

**Plant regeneration stage of stressed callus:** Callus were inoculated in the proliferation medium (2 mg/L BAP and 0.2 mg/L NAA) supplemented with the sub-lethal concentrations of PEG 6000 for two months (4% for Daher-Aljabal, 6% for Brieh, 6% and 8% for Darra variety). After that, callus were transferred to the regeneration medium (MS medium supplemented with 2 mg/L zeatin and 0.2 mg/L GA3 (gibberellic Acid-3) supplemented with the sub-lethal concentration of PEG 6000 determined for each variety. After two months, regeneration percentage (%), days for regeneration (day) and number of regenerated shoots (shoots/callus) of each callus were assessed as compared to control (regeneration medium free of PEG 6000).
Somaclonal variant plants evaluation: Regenerated shoots were multiplied on MS medium supplemented with 1 mg/L BAP to obtain enough number of shoots. Then regenerated plantlets were cultured on MS supplemented with PEG 6000 at 4%, 6%, 8% concentration for each variety. The somaclonal variant plants were compared to the mother plants based on the following parameters: plant height (cm), root length (cm), shoot/root ratio and plant dry weight (g).

Acclimatization stage: Somaclonal variant plantlets were cut into small explants and transferred to a rooting medium (MS supplemented with 1 mg/L IBA) for 60 days in order to develop a good root growth. *In vitro* rooted plantlets were washed with sterile distilled water to remove the remains of the medium. They were cultured in pots containing a sterilized mixture of perlite: peat moss (1:1) and subjected to acclimatization. The pots were maintained under growth condition of 16:8 photoperiod and 24±2 ºC. The pots were covered with polythene bags to maintain the relative humidity (70 to 80%). Bags were opened gradually every week until total removal after 4 weeks depending on the growth. The acclimatized plantlets were later shifted to greenhouse.

Experimental design and statistical analysis: The experiments were performed as completely randomized design (CRD) in a factorial system with the variety being the first factor and the PEG treatment being the second factor. In the callus selection experiment, four varieties x five PEG concentrations (control, 2%, 4%, 6% and 8%) were examined. At the plant regeneration stage, five callus lines x two PEG treatments (control and sub-lethal concentration) were examined. For somaclonal variation assessment, somaclones were compared to their mother plants at four PEG concentrations.

In the callus selection experiment, somaclones were compared to the mother plants at four PEG concentrations (2%, 4%, 6% and 8%) were examined. For somaclonal variation assessment, somaclones were compared to the mother plants at four PEG concentrations (2%, 4%, 6% and 8%) were examined. For somaclonal variation assessment, somaclones were compared to their mother plants at four PEG concentrations (2%, 4%, 6% and 8%) were examined. For somaclonal variation assessment, somaclones were compared to the mother plants at four PEG concentrations (2%, 4%, 6% and 8%) were examined. For somaclonal variation assessment, somaclones were compared to their mother plants at four PEG concentrations.

### Table 1: Mean square of studied parameters according to analysis of variance in CRD

<table>
<thead>
<tr>
<th>Source of variance</th>
<th>d.f.</th>
<th>Mean square</th>
<th>Prob.</th>
<th>RM</th>
<th><strong>p</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Variety</td>
<td>3</td>
<td>1349.5</td>
<td>6090.0**</td>
<td>0.00146</td>
<td></td>
</tr>
<tr>
<td>Treatment (PEG concentration)</td>
<td>4</td>
<td>848.5**</td>
<td>2190.3**</td>
<td>0.000444**</td>
<td></td>
</tr>
<tr>
<td>Variation</td>
<td>12</td>
<td>18.9</td>
<td>88.5</td>
<td>0.00001</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>40</td>
<td>777.9</td>
<td>550.3</td>
<td>0.000326</td>
<td></td>
</tr>
</tbody>
</table>

**The differences are significant at p value ≤ 0.01**

3. Results and Discussion

Drought stress induced by PEG 6000 had a significant effect on callus of the examined tomato varieties as shown in Fig. 1.

**Relative growth rate (RGR):** Increasing PEG 6000 concentration has led to decreasing relative growth rate RGR of callus. The maximum value of RGR (0.064 g/day) was determined in control treatment while the lowest (0.018 g/day) value was determined in 8% PEG treatment regardless of tomato variety and the differences were significant between PEG concentrations.
Free proline content: Accumulation of proline constitutes a general response to abiotic stress (Hazzouri et al., 2020). It is a way that plant survives through stress conditions by reducing the osmotic potential of plant tissue at cellular level thus maintaining plant growth under stressful condition. Our findings illustrated in Fig. 4 confirmed this fact where cells accumulated higher content of proline in response to increasing PEG concentration in all tomato varieties. The differences between PEG concentrations as well as varieties were significant as presented in Table 1. The highest mean content of accumulated proline was (103.51 µmol/g FW) in Daher-Aljabal followed by Baskanta (99.87 µmol/g FW), Daraa (63.57 µmol/g FW) then Brieh (57.09 µmol/g FW). These results are in line of many researchers who found that proline content increased with increased stress treatment in tomato (Khan et al., 2015; Kareem and Karrar, 2018), sugarcane (Rao and Jabeen, 2013), cucumber (Abu-Romman, 2010) and rice (Tripathy, 2015). Our results suggested that sensitive tomato varieties may accumulate more proline content compared to tolerant ones and this agree with those obtained in tomato crop by Aazami et al. (2010) who reported that in some cases drought sensitive cultivars accumulated more proline than tolerant ones. However, our results disagree with Hassan et al. (2004) who reported that the proline level showed a positive correlation with the degree of tolerance for water stress in sunflower. These different results may due to the genetic differences among the studied varieties. It is worth to mention that the role of proline is very important for plant growth under stressful conditions that protect the cells and their membranes during osmotic stress (Cardoso et al., 2019). Furthermore it contributes to increase plant tolerance by inhibition of reactive oxygen species (ROS) production (Hayat et al., 2012) as it is a physiological and biochemical indicator for stress effects on plant tissue which was first suggested by (Bates et al., 1973) as a screening indicator against drought stress.

Tolerance index (TI): TI was used to estimate callus growth in order to exclude variations associated to (RGR) of tomato varieties. Significant differences (p<0.01) were recorded among examined tomato varieties related to TI at different PEG concentrations (Table 1). Daraa variety exhibited the highest tolerance to PEG treatment with a TI value of (0.815) while Baskanta gave the lowest TI value (0.317) as illustrated in Fig. 5. This is due to the reduction of water availability and loss of turgor pressure (TP) in callus cells (Haque et al., 2017). Our results were in accordance to those obtained by (Al-Khayri and Al-Bahrainy, 2004) in date palm callus as response to PEG-induced drought stress.
PEG 6000 sub-lethal determination: The concentration of PEG 6000 that resulted in 50% decrease in cell viability and RGR (4% of PEG) was selected as sub-lethal concentration for Daher-Aljabal and Baskanta varieties while it was (6%) for Brieh variety and two sub-lethal concentrations (6% and 8%) of PEG were selected for Daraa variety.

Plant regeneration stage: Tolerant cells induced by the selective agent PEG 6000 were selected and plant regeneration for the surviving tolerant cells was assessed. The concentration of PEG 6000 that resulted in 50% decrease in cell viability and RGR (4% of PEG) was selected as sub-lethal concentration (LD50) for Daher-Aljabal and Baskanta varieties while it was (6%) for Brieh variety and two sub-lethal concentrations (6% and 8% of PEG) were selected for Daraa variety. Callus was inoculated in the proliferation medium supplemented with the sub-lethal concentrations of PEG 6000 for two months. After that, callus was transferred to the regeneration medium (MS medium supplemented with 2 mg/L zeatin, 0.2 mg/L GA3 and the sub-lethal concentration of PEG 6000).

Results presented in Table 2. showed that regeneration was observed after 16 days of callus inoculation in control treatment. The days for regeneration increased to 25 days in stressed treatment. On the other hand, the mean regeneration percentage was 75.29% in control treatment and was decreased to 34.13% in stressed treatment by 45.33%. The mean number of regenerated shoots per callus was lower in stressed callus (2.58 shoots) compared to control treatment (4.2 shoots).

Table 2: Regeneration responses of tomato varieties for drought stress

<table>
<thead>
<tr>
<th>Tomato Variety</th>
<th>Regeneration percentage</th>
<th>Days for regeneration</th>
<th>Number of shoots/callus</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>PEG concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control: 0% PEG</td>
<td>26.2 ± 1.4</td>
<td>4.2</td>
</tr>
<tr>
<td>Daraa</td>
<td>45.89 ± 2.1</td>
<td>23.5 ± 1.7</td>
<td>4.08 ± 1.4</td>
</tr>
<tr>
<td>Brieh</td>
<td>85.89 ± 2.1</td>
<td>21.24 ± 1.7</td>
<td>4.59 ± 1.7</td>
</tr>
<tr>
<td>Baskanta</td>
<td>10.10 ± 2.1</td>
<td>34.13 ± 1.7</td>
<td>3.42 ± 1.7</td>
</tr>
<tr>
<td>Daraa</td>
<td>41.50 ± 2.1</td>
<td>33.33 ± 1.7</td>
<td>2.05 ± 1.7</td>
</tr>
<tr>
<td>Brieh</td>
<td>60.88 ± 2.1</td>
<td>26.33 ± 1.7</td>
<td>2.85 ± 1.7</td>
</tr>
<tr>
<td>Daraa</td>
<td>30.24 ± 2.1</td>
<td>25.59 ± 1.7</td>
<td>2.7 ± 1.7</td>
</tr>
<tr>
<td>Contr</td>
<td>0.522</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Different letters in each column indicate significant difference at p value ≤0.01

Each value is a mean of three replications, ten plants in a replication
1Control: 0% PEG, 2 Sub-lethal concentration of PEG determined for each variety (4% for Daher-Aljabal, 6% for Brieh, 6%, 8% for Daraa). Daraa was examined at *90%, 2*8% sub-lethal concentrations.

Callus induction, regeneration, plantlet rooting and acclimatization are illustrated in Fig. 6, which shows the process of obtaining somaclonal variant from callus to a complete plant. The somaclonal variant plants were successfully acclimatized and transferred to the greenhouse for adaptation before transferred to field for further in vitro screening.

Fig. 6: a, b, c, d Callus formation and proliferation from roots explants. e+f shoot formation, g, regenerated shoots were cut aseptically to remove callus. h, then cultured on multiplication medium. i, growth of regenerated shoots. j, rooting of regenerated shoot. k, rooted plantlet was removed from medium and washed to remove agar. l, acclimatization of rooted plantlet.

Somaclonal variant plants evaluation: Physiological changes of callus may lead to changes in shoot and plant morphology then produce somaclonal variant plants (Sahara et al., 2019). This fact was proved by our results of morphological traits of regenerated plantlets. Root length, plant height, root-to-shoot ratio and dry weight were measured after 2 months in order to compare each somaclonal variant plant with their perspective mother plant. The regenerated plantlets from stressed callus were dead, and only one plant was obtained from Daher-Aljabal and Brieh varieties and 2

Fig. 5: Effect of polyethylene glycol concentration on tolerance index TI of callus cultures in four tomato varieties

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plantlets from Daraa variety.

Regenerated plantlets from tolerant callus were named as following DH1, from PEG 4% tolerant callus of Daher-Aljabal tomato variety, BR1 from PEG 6% tolerant callus of Brieh tomato variety, DA1 from PEG 6% tolerant callus of Daraa tomato variety and DA2 from PEG 8% tolerant callus of Daraa tomato variety. These four somaclones were screening based on morphological parameters at PEG concentrations of 4%, 6% and 8% in addition to control. Results illustrated in Fig. 7 showed a decrease in root length in all somaclonal variant plants as compared to their controls (mother plant) in all PEG treatment. The lowest decrease in root length was determined in DA1 (41.63%) followed by BR1 (49.85%), DH1 (51.49%) then DA2 (63.06%) as compared to their controls. It is important to mention that somaclonal variant plants DH1 and BR1 could withstand higher PEG concentration 8% while their mother plants dead at this concentration. Furthermore, plant height followed the same trend as for root length. The decrease in plant height was 28.38%, 30.19%, 38.52%, 51.08% in DH1, DA2, DA1 and BR1, respectively as shown in Fig. 8. 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 (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 determined in many previous reports carried out on tomato (Jokanović and Zdravković, 2015; Bredy et al., 2015). On the other hand, root-to-shoot ratio was decreased with increased PEG concentration in DH1 and DA1 by (61.93% and 73.77%) while it is increased in BR1 and DA2 by (32.48% and 3.57%) as compared to their controls (Fig. 9). Xu et al. (2015) have reported a high root-to-shoot ratio as a component trait for drought avoidance. This increase was determined because of the effect of drought stress on aerial parts growth determined in this research. Moreover, root-to-shoot ratio indicates that the drought tolerance mechanism adapted in the genotype as the increased ratio helps plant to cope with transpiration effect during drought and against lodging at maturity (Claeys et al., 2014).

In the present study, a decrease in plant dry weight (Fig. 10) was determined only for DH1 and BR1 by 38.23%, 17.31%, respectively compared to their controls. While in DA1 and DA2, plant dry weight increased as compared to mother plant by an increase rate of 25.45%, 52.57%, respectively. It is important to mention that somaclonal variant plants DH1 and BR1 could withstand higher PEG concentration (8%) while their mother plants died at this concentration. Present investigation is in confirmation with (Kumar et al., 2017; Khan et al., 2015).

4. Conclusions

A successful callus formation and plant regeneration protocol has been developed under drought stress treatments induced by PEG 6000. Four somaclonal variants were successfully developed form PEG tolerant callus. DH1 from PEG 4% tolerant callus of Daher-Aljabal tomato variety, BR1 from PEG 6% tolerant callus of Brieh tomato variety, DA1 from PEG 6% tolerant callus of Daraa tomato variety and DA2 from PEG 8% tolerant callus of Daraa tomato variety. Based on the studied morphological
parameters, those somaclones were different as compared to their mother plants. Whereas, regeneration from stressed callus of Baskanta variety was not successful. However, genetic variation could be somatically or genetically stable so it is important to undergo molecular detection at DNA level to detect the genetic variation among somaclonal variant plants at the molecular level and carry out a field screening as well as physiological and biochemical assessments. It is worth mentioning that these experiments are under investigation as a part of continuous research program.

Acknowledgments
The authors wish to thank Dr. Fahed Albisiki and Mr. Majdi Gharzeddin for the scientific help both practically and theoretically to achieve this work.

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References


