Effects of drought stress on morphological, physiological, and biochemical characteristics of stock plant (*Matthiola incana* L.)

Sima Jafari, Seyyed Ebrahim Hashemi Garmdareh*, Behzad Azadegan

*Emam reza BLC., 3391653755, Tehran, pakdasht, Iran

1. Introduction

During the last few decades, world’s population growth and increasing water demand has revealed the importance of water resources management. Due to the low atmospheric rainfall and the inappropriateness of its distribution in time and place in Iran, this country is classified within arid and semi-arid countries of the world (Tabari and Talae, 2011). On the other hand, due to population growth, the development of health and the expansion of agricultural and industrial sectors, this country is constantly facing increasing water demand, which will increase the gap between supply and demand of this valuable substance in the future (Mombeni et al., 2013).

In arid and semi-arid areas, the allocation of new water resources to expand landscapes and growing ornamental plants is facing barriers because water resources in these areas are limited and water allocation to floral industry is in sharp competition with other water demands such as agriculture, urban management and even drinking water. Therefore, the water allocated to floral industry is of great value and should be used optimally and with high efficiency (Hashemi Garmdareh et al., 2007). The study of plants’ tolerance to drought in order to use more appropriate plants and determining their water needs is one of the most effective methods of water management.

Drought stress is the most common environmental stress which is considered as a serious threat to the successful production of agricultural crops (McWilliam, 1986). Drought occurs when a combination of physical and environmental factors leads to a shortage of water in the plant and thus, reduces the plant growth and yield (Anjum et al., 2011). This stress can occur as an abnormal metabolism in the plant and may occur as a growth failure, death of a part of the plant, or the death of the whole plant (Abdul Jaleel et al., 2009). Severity of drought stress has a great impact on the physiological and biochemical process of plants (Atkinson et al., 2000; Massonnet et al., 2007). Plant responses to drought stress are usually studied based on physiological parameters such as water potential, relative water content, stomatal reactions, photosynthesis, or osmotic adjustment (Reddy et al., 2004; Zlatev and Lidon, 2012). In recent studies, active oxygen species and antioxidative enzymatic responses have also been proposed as appropriate indicators of plant response to drought stress (Huang et al., 2016; Martinez et al., 2018).

The stock plant (*Matthiola incana* L.) is one of the most important cut flowers widely cultivated in Iran due to its aroma, beautiful shape and variety of color (Sanchez et al., 2005; Famil Irani and Arab, 2017). It is a species of the Brassicaceae family and is usually found in two forms of single-flowered and double-flowered which the latter is more popular
on the market (Eid et al., 2009). Seeds of the stock flower contain oil rich in linolenic acid (55–65%) which has medicinal importance (Yaniv et al., 1999). The height of this plant varies from 30 to 90 cm (Singh, 2005) and its flower quality is directly related to its environment conditions before flowering (Arab, 2005). A search in bibliographic databases reveals that drought stress is not studied on stock plant so far. However, several papers can be found in which the effects of saline water on stock plant is investigated. Heuer and Ravina (2004); Grieve et al. (2006), and Cassaniti et al. (2013) have reported that stock plants are tolerant towards low and moderate levels of salinity stress.

The objective of this study was to investigate the effects of moderate and severe drought stress on morphological, physiological and biochemical characteristics of two cultivars of stock flower in order to investigate the response of this flower to different levels of irrigation.

2. Materials and methods

2.1. Plant material and experimental conditions

In order to investigate the response of the stock flower to drought stress, a completely randomized block design with four replications was conducted on two cultivars of stock flower, namely PanAmerican and Cinderella. In the PanAmerican cultivar, the height of the plant is usually between 30 and 40 cm and it has a main stem where flower stalks grow on. Its leaves are spherical and oval, with a length of about 3–8 cm. However, in the Cinderella cultivar, the height of the plant is between 15 and 20 cm, and the leaves are slightly wider than the PanAmerican cultivar. The flowers grow at the ends of the stems and the number of petals is higher (Fig. 1).

This study was carried out during 2016–2017 in a research greenhouse at College of Abouraihan, University of Tehran, located in Pakdasht (35° 29′ N, 51° 40′ E). The height of the area is 1027 m above sea level. The climate is arid and rainfall is mainly in autumn and winter seasons. The average annual temperature is 16.8 °C and its annual evapotranspiration is 1390 mm per year.

Planting, maintenance and harvesting operations were similar for both cultivars. The planting began on October 2016, and the harvest was carried out 193 days later. The average day and night temperature of the greenhouse was 20 °C and 15 °C, respectively, during the cultivation period. Three seeds were planted in each pot consisting of coco peat (60%) and perlite (40%). After germination, the bushes were thinned in several steps and eventually, a plant was kept in each pot. After plant growth and reaching the second true leaf stage, the plants were transferred to plastic pots of size 8 with a height of 6.5 cm and a diameter of 7.5 cm. After the plants reached the eighth true leaf stage, they were finally transferred to pots of size 4 with a height of 18 cm and a diameter of 20 cm. Soil used to fill the pots contained a uniform mixture of loam (50%), decayed leaves (25%), rotten manure (12.5%), and river sand (12.5%). The soil texture used to fill the pots was sandy loam and its maximum water holding capacity was 83%. The bottom of the pots was filled with pebbles for proper drainage and plastic substrates were placed underneath the pots to collect possible water drainage and to void the intrusion of pathogenic agents.

2.2. Irrigation treatments

Complete and equally irrigation of all pots was continued until the eighth true leaf stage with 100% of field capacity. After reaching the eighth true leaf stage, water stress treatments were started and irrigation of each plant was performed using the soil moisture curve (Fig. 2). Field capacity (FC) and permanent wilting point (PWP) were 0.3 and 15 bar, respectively. For drought stress treatments, soil moisture was measured daily by a TDR device (PMS-714, LUTRON, Taiwan). For the control plants, tap water was supplied daily to maintain soil water potential close to the field capacity. Drought treated plants were stressed by withholding water until soil water potential became different with field capacity to study moderate and severe drought stresses (Beltrano and Ronco, 2008). In this study, control (W0) with 100% of field capacity along with four water treatments, i.e., 90% (W1), 80% (W2), 70% (W3) and 60% (W4) of field capacity were studied with four replications. Irrigation of the treatment samples was conducted until harvesting the flowers. Table 1 shows the chemical characteristics of the soil used in each treatment.

2.3. Morphological measurements

Morphological parameters including plant height, number of flowers, root fresh weight (RFW), root dry weight (RDW), shoot fresh weight (SFW) and shoot dry weight (SDW) were measured. The roots of the plants were removed instantaneously after harvesting of the plants, and then, the fresh weights of the root and shoot, including stem and inflorescence, were measured using a scale with precision of 0.001 g. In order to determine the dry weight of the plants, roots and shoots of the samples were placed in an oven at 80 °C for 48 h, and weighed using a scale with precision of 0.001 g (Sheikh et al., 2005). The plant height was measured by a line with a precision of 0.01 cm.
physiological and biochemical measurements

Leaf relative water content (RWC) and chlorophyll index (CI) were measured from the upper fully expanded young leaves according to Yamasaki and Dillenburg (1999). Leaf RWC was calculated according to Eq. (1).

\[
RWC(\%) = \left( \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Saturated weight} - \text{Dry weight}} \right) \times 100
\]

CI was evaluated in the upper fully expanded young leaves with a Field Scout CM1000 chlorophyll meter. This chlorophyll meter estimates chlorophyll content based on ratios of the amount of ambient and reflected light at 700 and 840 nm.

Young fully expanded leaf samples were also collected for biochemical measurements. One-gram sample of leaves was weighed and ground with an ice-cold pestle and mortar with 10 mL 50 mmol L\(^{-1}\) phosphate buffer (pH 7.0). The homogenates were then centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatant filtered through two layers of cheesecloth was used for the determination of enzymatic activities as well as protein determination.

Catalase (EC. 1.11.1.6) activity (CA) was measured by following the decomposition of H\(_2\)O\(_2\) at 240 nm with a UV spectrophotometer (UV-1700, Shimadzu, Japan) as described by Havir and McHale (1987). The reaction mixture consisted of 0.1 mL enzyme extract, 2.8 mL phosphate buffer (pH 7.4, 0.1 mol L\(^{-1}\)) containing 4 mM Na\(_2\)EDTA. The biochemical reaction was started by adding 0.1 mL 0.01 mol L\(^{-1}\) H\(_2\)O\(_2\) in the reaction system. Samples without H\(_2\)O\(_2\) were used as blank. CA was calculated by the differences obtained at OD240 values at a 30 s interval for 2 min after the initial biochemical reaction. A change of 0.01 units per minute in absorbance was considered to be equal to one-unit CA.

Anthocyanin contents (AC) of leaves were determined according to the method explained by Mancinelli (1990). One-gram leaf sample was firstly extracted in 3 mL methanol – HCl (1% HCl, v/v); the leaf samples were then kept at 4 °C for 48 h. Finally, the extract was filtered through two layers of cheesecloth and the anthocyanin content was measured using a spectrophotometry device (UV-1700, Shimadzu, Japan) at 530 and 657 nm wavelengths.

Phenol content (PhC) of leaves was determined according to Singleton and Rossi (1965) using Folin-Ciocalteu solution with slight modifications. Fresh leaf tissues of 0.5 g were homogenized in liquid nitrogen, then extracted with 5 mL 80% methanol, and centrifuged at 10,000 rpm for 10 min. 100 µL of the supernatant was mixed with 1.8 mL Folin-Ciocalteu solution and then kept 5 min at room temperature. Then, 1.2 mL 20% Na\(_2\)CO\(_3\) was added and the volume made up to 6 mL with distilled water. The mixture was incubated for 1 h at 80 °C and the absorbance was measured at 765 nm against methanol.

Proline content (PC) of the leaves was measured according to Bates et al. (1973). Proline was extracted from 0.5 g of leaf sample by grinding in 10 mL 3% sulpho-salicylic acid and the mixture was then centrifuged at 10,000 rpm for 10 min. Two mL of the supernatant was then added into test tubes to which 2 mL of freshly prepared acid-ninhydrin solution and 2 mL of glacial acetic acid were mixed. The tubes were placed in a water bath for 1 h at 90 °C and the reaction was terminated in ice-bath. The mixture was then extracted with 5 mL toluene and vortexed for 15 s. After allowing standing at least for 20 min in darkness at room temperature to separate the toluene and aqueous phase, the toluene phase was then carefully collected into test tubes and the absorbance of the fraction was read at 520 nm with a spectrophotometer (UV-1700, Shimadzu, Japan).

2.4. Physiological and biochemical measurements

2.5. Statistical analysis

The data of the experiment were subjected to analysis of variance (ANOVA) using LSD test at the significance level of 0.01 in SAS 9.0 programming environment.

3. Results and discussion

In this study, moderate and severe drought stress treatments were applied on two cultivars of stock flower and their morphological, physiological and biochemical effects were examined. The results of the mean comparison of the studied traits for both cultivars indicate that there is a significant effect (P < 0.01) in most traits due to drought stress conditions (Fig. 3).

The results of the mean comparison of the data shows that plant height was decreased by applying drought stress in PanAmerican cultivar (Fig. 3a), so that the highest plant height belonged to control (37.32 cm) and the lowest belonged to W\(_4\) (27.45 cm). According to this figure, there was no significant difference in plant height in W\(_1\) treatment with plant height in control, but with a decrease in irrigation rate less than 90% of irrigation requirements, this difference was significant. Therefore, it can be stated that the threshold of plant tolerance to...
change in plant height during drought stress is 90% of field capacity. The results of the mean comparison of data for Cinderella cultivar indicated that the plant height decreased with increasing water shortage. However, this height decrease, except for W3 and W4 treatments, was not significant (P < 0.01) in the other treatments compared to the control (Fig. 3a). Therefore, the threshold of plant tolerance to change in plant height during drought stress in Cinderella cultivar is about 70% of field capacity. Studies of Davodnia et al. (2016) on poppy cultivars showed that applying drought stress to 50% of field capacity resulted in a significant decrease in the plant height compared to control. In another study on barley, it was found that the drought stress at the end of the planting season caused a 5% reduction in plant height (Paknejad et al., 2017). Furthermore, Sabzi et al. (2016) reported a 26% reduction in bean plant height under drought stress from 70% of field capacity to 30%.

The results of the mean comparison of the effect of drought stress on the SFW indicate a decrease in this trait against drought stress in both cultivars (Fig. 3b), so that the highest and lowest SFW of the PanAmerican and Cinderella cultivars were observed in control (W0) and W4 treatments, respectively. The results showed that there is a significant difference in W1 with control in term of SFW which does not allow replacement of this treatment with 100% of field capacity irrigation. The study conducted by Davodnia et al. (2016) shows similar results for poppy plants. They showed that with increasing drought stress by 50%, the SFW decreased significantly about 38.5% compared with the control. Also, in a study on rosemary, a 47% reduction in SFW was observed under drought stress conditions of 25% field capacity (Ziaei et al., 2014).

The results of SDW of both PanAmerican and Cinderella cultivars were similar to the SFW, so that the SDW decreased with increasing water stress in both cultivars (Fig. 3c). For PanAmerican cultivar, the highest SDW was obtained from control (16.97 g) and lowest for W4.
(6.82 g). The highest and lowest SDW was obtained in control (12.19 g) and W4 treatment (5.5 g), respectively, in Cinderella cultivar. In accordance with the results of this study, Paknejad et al. (2017) showed that drought stress on the barley plant reduced its dry matter from 19750.7 g to 15397.2 kg g−1. Also, studies showed that increasing drought stress levels from 75% of field capacity to 25% of field capacity caused a significant reduction in the SDW of lentil plant (Ahmadpour and Hossain Zade, 2017). In a research conducted by Ebrahimi et al. (2017) on Calendula officinalis, it was found that the SFW of the plant during the variation of drought stress from 75% of field capacity to 25% of field capacity (severe stress) significantly reduced by 46%.

Mean comparison of RFW of PanAmerican cultivar showed that there was a significant difference due to drought stress and with increasing drought stress, RFW decreased (Fig. 3d). The highest RFW was observed for the control (12.48 g) and the lowest in the most severe drought stress, i.e., W4 (6.75 g). In Cinderella cultivar, by applying the drought stress, the RFW belonging to W1 had no significant difference (P < 0.01) compared to control (Fig. 3d). Also, with increasing drought stress, the RFW decreased and reached its lowest value (3.22 g) at the highest stress level (W4), which is 47% lower than the RFW in the control (6.08 g). The findings of this study were consistent with the results of Davodnia et al. (2016), which showed that increasing drought stress by 50% of field capacity resulted in a reduction of 39.1% of RFW in poppy plant. Ziaei et al. (2014) showed that in rosemary plant, RFW was 14.49 g and 11.16 g at 100% and 25% (severe stress) of field capacity, respectively.

Data analysis of RDW for PanAmerican cultivar showed that W4 to W4 treatments had a significant difference (P < 0.01) with control (Fig. 3e). The highest RDW was observed for control (3.3 g) and lowest for W4 treatment (1.28 g). It can be said that the critical plant tolerance to changes in RDW is 90%. Contrary to the results obtained for PanAmerican cultivar, the results of the mean comparison of RDW in Cinderella cultivar showed that the RDW of this cultivar has no tolerance towards drought stress even at 90% of field capacity (Fig. 3e). Davodnia et al. (2016) showed that drought stress equal to 50% of field capacity caused a significant difference (P < 0.01) on RDW of poppy plant and reduced its RDW by about 35%.

According to the results of the mean comparison, the effects of drought stress on the number of flowers in PanAmerican cultivar showed that the number of flowers significantly changed (P < 0.01) as a result of drought stress (Fig. 3f). In contrast with the results of PanAmerican cultivar, although the number of flowers of Cinderella cultivar decreased from 28.88 in control to 18.17 in W4 treatment, there was no significant difference between control and W4 treatment (P < 0.01). This indicates that all the morphological characteristics of the stock flowers, except for the number of flowers, are affected by moderate and severe drought stress.

Physiological and biochemical parameters of stock leaves grown in various drought stress levels are brought in Table 2. RWC is considered as a measure of plant water status and used as a most meaningful index for dehydration tolerance (Pirzad et al., 2011; Anjum et al., 2011). In this study, leaf RWC was found equal to 94.3, 93.6, 93.4, 92.7, and 92.5% in PanAmerican cultivar and 89.2, 84.6, 77.1, 72.0, and 67.8% in Cinderella cultivar for control and W1 to W4 treatments, respectively (Table 2). As shown in this Table, although RWC significantly decreased with the increasing levels of water stress in Cinderella cultivar, its reduction was not significant in PanAmerican cultivar. Similarly, it is reported that the negative effects of water stress on leaf RWC are reduced depending on the plant cultivar (Sharma and Sharma, 2008; Wang et al., 2012). Significant decrease in leaf RWC in Cinderella cultivar could have been due to unavailability of water in the soil, or root systems, which are not able to compensate for water (Gadallah, 2000). RWC of leaves is higher in the initial stages of leaf development and declines as the dry matter accumulates and leaf matures (Anjum et al., 2011).

Water stress treatments also resulted in a significant decrease in CI levels in both cultivars during the period of stress (Table 2). Leaf CI in the most severe water stress case was reduced by 55% in PanAmerican and 56% in Cinderella cultivar compared to the control. Similar findings were also reported by several researchers (Gholami et al., 2012; Bolat et al., 2014). Chlorophyll is one of the major chloroplast components for photosynthesis and an indicator of agricultural product quality (Asfurem Vakilian and Massah, 2017). Relative chlorophyll content has a positive relationship with photosynthetic rate. The decrease in total chlorophyll content under drought stress has been considered a typical symptom of oxidative stress (Anjum et al., 2011). Although drought stress has caused a large decrease in the chlorophyll content of different plants’ leaves (e.g., sunflower (Manivannan et al., 2007), olive (Guerfel et al., 2009), and apple shootstock (Bolat et al., 2014)), decreased or unchanged chlorophyll level during drought stress has been also reported in many species, depending on the duration and severity of drought (Zhang and Kirkham, 1996).

When biochemical parameters were evaluated, CA in both cultivars was significantly higher compared with plants under control conditions (Table 2). A similar phenomenon was also observed by Gholami et al. (2012) and Bolat et al. (2014) who found that enzyme levels were significantly higher in drought affected plants than in control plants. Plants have an internal protective enzyme-catalyzed clean up system which ensures normal cellular function (Horváth et al., 2007). The balance between reactive oxygen species production and activities of antioxidative enzyme determines whether oxidative signaling and/or damage will occur (Møller et al., 2007). To reduce the affections of oxidative stress, plants have complex enzymatic antioxidant system, such as catalase scavenging systems (Apel and Hirt, 2004). Similar to the results of this study, Yang et al. (2009) reported that as compared with 100% of field capacity, at 25% of field capacity, the CA increased 4.3% and 8.1% in P. cathayana, and P. kangdingensis, respectively. The capability of antioxidative enzymes to scavenge reactive oxygen species and reduce the damaging effects may correlate with the drought resistance of plants.

According to Table 2, similarly, anthocyanin and phenol contents in water-stressed plants of both cultivars were higher than the control. A similar trend was also shown for proline contents. Although much attention was given to biochemical parameters in drought stressed plants such as vegetables and trees (Bolat et al., 2014), the results obtained from ornamental plants is of great importance to evaluate stress mechanisms. Biochemical parameters in this study showed that PanAmerican and Cinderella cultivars performed relatively similar towards drought stress. According to these studies, to achieve high quality stock plants, plant materials should not be water-stressed during growing.

### Table 2

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Water treatment</th>
<th>RWC (%)</th>
<th>CI (%/%)</th>
<th>CA (units mg−1 g−1 Fwt)</th>
<th>AC (AS300 mg−1 g−1 Fwt)</th>
<th>Phc (mg GAE g−1 Fwt)</th>
<th>PC (g µg−1 Fwt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PanAmerican</td>
<td>W0</td>
<td>94.3 a</td>
<td>294.1 a</td>
<td>0.22 a</td>
<td>0.92 e</td>
<td>0.22 c</td>
<td>3.52 e</td>
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<tr>
<td></td>
<td>W1</td>
<td>93.6 b</td>
<td>248.6 b</td>
<td>0.24 b</td>
<td>0.97 d</td>
<td>0.20 e</td>
<td>3.88 b</td>
</tr>
<tr>
<td></td>
<td>W2</td>
<td>93.4 c</td>
<td>221.9 c</td>
<td>0.24 c</td>
<td>1.04 b</td>
<td>0.21 e</td>
<td>4.13 c</td>
</tr>
<tr>
<td></td>
<td>W3</td>
<td>92.7 d</td>
<td>175.6 d</td>
<td>0.24 d</td>
<td>1.11 b</td>
<td>0.35 b</td>
<td>4.17 b</td>
</tr>
<tr>
<td></td>
<td>W4</td>
<td>92.5 e</td>
<td>132.5 e</td>
<td>0.25 e</td>
<td>1.31 a</td>
<td>0.43 a</td>
<td>4.22 a</td>
</tr>
<tr>
<td>Cinderella</td>
<td>W0</td>
<td>89.2 a</td>
<td>271.4 a</td>
<td>0.21 a</td>
<td>0.90 b</td>
<td>0.27 c</td>
<td>3.18 b</td>
</tr>
<tr>
<td></td>
<td>W1</td>
<td>84.6 b</td>
<td>230.1 b</td>
<td>0.23 b</td>
<td>1.02 d</td>
<td>0.28 c</td>
<td>3.76 c</td>
</tr>
<tr>
<td></td>
<td>W2</td>
<td>77.1 c</td>
<td>219.2 c</td>
<td>0.24 c</td>
<td>1.15 b</td>
<td>0.30 b</td>
<td>3.99 b</td>
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<tr>
<td></td>
<td>W3</td>
<td>72.0 d</td>
<td>167.4 d</td>
<td>0.29 d</td>
<td>1.23 b</td>
<td>0.31 b</td>
<td>4.10 b</td>
</tr>
<tr>
<td></td>
<td>W4</td>
<td>67.8 e</td>
<td>119.7 e</td>
<td>0.27 e</td>
<td>1.44 a</td>
<td>0.38 a</td>
<td>4.11 a</td>
</tr>
</tbody>
</table>

RWC: Relative Water Content, CI: Chlorophyll Index, CA: Catalase Activity, AC: Anthocyanin Content, Phc: Phenolic compounds, PC: Proline Content. The means followed by the same letter in each column are not significantly different according to LSD test (P < 0.01).
4. Conclusion

The results of this study can be summarized as follows:

1) Morphological characteristics of PanAmerican and Cinderella cultivars were adversely affected by drought stress. Among the studied traits, only the number of flowers in the Cinderella cultivar was not significantly affected by drought stress ($P < 0.01$).

2) Physiological characteristics including RWC and CI were studied to investigate the effects of drought stress on cultivars of stock flower. Results showed that a decrease in RWC for PanAmerican cultivar was not significant due to drought stress up to 60% of field capacity.

3) Biochemical characteristics of both cultivars were significantly affected by drought stress, which shows weak tolerance of stock flower to drought stress at the intracellular level.

4) The irrigation program of stock flower should be carefully monitored since this plant cannot tolerate the drought stress even in moderate levels.

Conflict of interests

The authors declare that they have no conflicts of interest.

References


