Effect of water stress on morphological traits, mucilage percentage and yield of *Alyssum homolocarpum*

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Abstract

A pot experiment was carried out to study the impacts of water stress upon physiological and biochemical properties as well as seed yield and yield components of *Alyssum homolocarpum*. The factors were water stress (80%), the capacity of field (60%) as well as, field capacity (40%) within three stages of growth, stem elongation, flowering and seed setting, as well. Result showed that water stress within three stages of growth was reduced plant height, component yield and seed yield and increased superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activity. Water stress increased content of proline, soluble sugar and malondialdehyde (MDA). The content of calcium (Ca) and magnesium (Mg) decreased in 40% and 60% field capacity at stem elongation stage compared to control. As water stress increased, mucilage percentage increased. In 40% field capacity at elongation stage *Alyssum* did not have seed yield. In this stress level, at flowering stage and seed setting, seed yield decreased by 85% and 68% compared to control, respectively. Result indicated that most sensitive growth stage to the water stress in *Alyssum homalocarpum* was stem elongation, Thus, It was suggested that this plant must be irrigated at stem elongation to produce acceptable yield.

Keywords: Alyssum, Field capacity, Antioxidant enzyme, Proline.

Introduction

*Alyssum homalocarpum* is of flowering plants in the family of Brassicaceae with main distribution in Asia and Eastern Europe (Marcotte et al., 2001). The utility of mucilage and poly saccharide exudates for medical purposes has also been proven. Lately, the power of its seed mucilage as a novel source of hydrocolloid has been investigated (Koocheki et al., 2009), using the methodology of response surface, researchers optimized of *Alyssum homalocarpum* seed mucilage extraction (Marvdashti et al., 2017). *Alyssum homolocarpum* gum, like many commercial gums, acts as a weak gel in 1.5-3% concentration. Properties of this gum include thickening, gelling, stabilizing, and fat replacing represent its potential as an alternative for commercial gums in food and pharmaceutical formulations (Koocheki et al., 2009).

One of the critical problems today in the agricultural world, and food production is the limited resource of water (Pandey and Shukla, 2015). Water stress can cause certain plants to experience drought stress due to insufficient supply going through the root system (Pandey and Shukla, 2015). Depending on the medicinal plant species, drought stress may inhibit or affect growth, developmental stages, tissues, and numerous physiological adaptations (Chiante et al., 2006).

Hence, understanding the physiological and biochemical mechanisms of resistant varieties in response to water stress is important. Genetic variation for water stress tolerance has been

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observed in many plant species (Pandey and Shukla, 2015). Depending upon the degree of stress, diverse concomitant stress variables, their species as well as stages of development, plants’ susceptibility to drought could vary (Feng et al., 2017). Water stress during various developmental stages can change the amount of yield (Navarro et al., 2011). Water stress reduced seed yield of medicinal plant through the negative impact on yield components. Drought stress during the flowering phase can reduce the spacing from seed formation to pollen shed and reduce the seed setting stage. Also, there is a large amount of research on the effect of water stress on various medicinal plants, and aromatic plant yields components (Barnabas et al., 2008). Water deficiency in different stages of plant growth can have different effects on physiological and morphological traits (Tanaka et al., 2017).

Drought stress has culminated in a significant decrease in the content of chlorophyll as well as in the amount of leaf water (Chiane et al., 2006). Therefore, decrease of this can be considered as a non-stomata limiting factor in the water stress conditions. There are some reports about decrease of chlorophyll in the water stress conditions (Kuroda et al., 1990).

Under water stress condition, proline accumulates in plant as the main osmoprotectant (Chakhchar et al., 2015; Azarmi et al., 2015). The concentration of proline in the cells increases in response to water stress and results in the transfer of water to the plant (Sofo et al., 2004). Proline can contribute to scavenging of the ROS, thereby protecting the cell membrane from oxidative damages of drought stress. Proline accumulation under drought stress is due to the activation of proline biosynthesis enzymes and decreased use of proline in protein synthesis (Tsugane et al., 1999). Particularly, due to prolin efficiency for monitoring peroxidative damage because of drought stress conditions, thiobarbituric acid might react with different oxidized products of amino acids, for example proline and carbohydrates for the purpose of cosmetic regulation (Ji et al., 2014).

Biosynthesis of specifically effective antioxidants like phonelic compounds can be regarded as one of the most important defensive mechanism (Georgive et al., 2014). Phenolic compounds’ ant oxidative capabilities could be attributed to their capacity of high reaction as hydrogen or electron donors, the specificity of derived radical for the stabilization and delocalization of the unpaired electron as well as their ability to chelate transition metal ions (Skrovankova et al., 2015). In response to stress, it was proposed to activate the synthesis of phenolic compounds (especially flavonoids), carotenoids and ascorbic acid (Subbarao et al., 2000). Thus, phenolic compounds provide important physiological and ecological duties, being mainly involved in protection against different types of stress. Besides numerous enzymes (superoxide dismutase, peroxidase etc.), phenolic compounds are strong antioxidants that help plants to survive stress conditions (Sánchez-Rodríguez et al., 2010).

The alteration of antioxidant metabolisms is one of the essential metabolic processes that may influence the drought stress tolerance of medicinal Plant (DaCosta and Huang, 2007). Drought stress, though boosting the production of reactive oxygen species (ROS), like superoxide (O₂⁻), singlet oxygen (O₂•), hydroxyl (OH), and hydrogen peroxide (H₂O₂), can be harmful for proteins, lipids, carbohydrates, and nucleic acids (Smirnoff, 2000). Plants have developed enzymatic as well as non-enzymatic defense systems to scavenge and detoxify ROS. It is believed that superoxide dismutase (SOD) scavenges O₂ to H₂O₂ in enzymatic systems (Smirnoff, 2000). The decomposition of H₂O₂ to H₂O at diverse cellular sites by peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and dehydroascorbate reductase (DHAR) has also been observed (Mittler, 2002). The equilibrium between the production of ROS on one hand and antioxidative enzyme’s activities on the other hand could clarify the status of oxidative signaling and, or damage will happen (Moller et al., 2007).

More studies have shown that Alyssum homolocarpum seed gum can have many pharmaceutical and industrial uses (Anvari et al., 2016). Our aim in this research was the
determination of water stress impacts upon physiological, biochemical, and morphological characteristics, components yield and seed yield of *Alyssum homolocarpum*.

**Materials and Methods**

*Growth conditions and treatments*

The pot experiment was carried out in Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran. The factors were water stress (80% field capacity (control), 60% field capacity and 40% field capacity) within three growth stages including the stem elongation, flowering and seed setting. *Alyssum homolocarpum*’s seeds were accessed from the institute of seed and plant breeding improvement located in the city of Karaj, Iran. The soil used in this research was accessed from, autoclaved, and passed through a 3-mm sieve. Soil samples were collected from 0 to 30 cm depth (Table 1).

<table>
<thead>
<tr>
<th>Textural classification</th>
<th>Organic matter (%)</th>
<th>N (%)</th>
<th>K</th>
<th>P</th>
<th>Fe</th>
<th>Cu</th>
<th>Zn</th>
<th>Mn</th>
<th>Mg</th>
<th>Ca</th>
<th>pH</th>
<th>EC (mS/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sand</td>
<td>0.870</td>
<td>0.130</td>
<td>391.000</td>
<td>9.000</td>
<td>1.238</td>
<td>0.730</td>
<td>0.470</td>
<td>7.870</td>
<td>0.410</td>
<td>0.810</td>
<td>8.120</td>
<td>2.630</td>
</tr>
<tr>
<td>Clay</td>
<td>0.870</td>
<td>0.130</td>
<td>391.000</td>
<td>9.000</td>
<td>1.238</td>
<td>0.730</td>
<td>0.470</td>
<td>7.870</td>
<td>0.410</td>
<td>0.810</td>
<td>8.120</td>
<td>2.630</td>
</tr>
</tbody>
</table>

Soils (5 kg) were placed in plastic pots with a height of 25cm and a diameter of 3cm. In a thoroughly randomized design with four replications, the pots’ arrangement was conducted. Plants were grown in a greenhouse between October and May 2019 at a temperature of 15–30°C, with 60–75% relative humidity and 1300 mol.m⁻².s⁻¹ photo synthetically active photon density. Experiments were conducted in natural light. Our greenhouse was composed of steel structure of permanent type which was covered by glass roof and walls, a total area of which was nearly 50 m². Equipped with heating as well as cooling systems, it is composed of an evaporative cooling unit as well as two unit heaters. Instantly after sowing, watering the pots was done. Coming out 3 to 4 days after sowing, the seedlings were thinned to 5 plants each pot (Fig. 1).

![Figure 1. View of Alyssum seedlings during the growth period in greenhouse](image-url)
The capacity of the field was recognized via Souza et al. (2000) methodology distinguishing between the wet soil after saturation and free drainage as well as the weight of the dry soil. For the purpose of water treatment maintenance, the pots were weighed on a daily basis substituting the lost water by transpiration utilizing a precision scale. Irrigation was done regularly until stem elongation stage, flowering, and seed setting. At this stages, the pots will be weighed and until the pots reached the appropriate weight of 40%, 60% and 80% of the field capacity. Over time and measuring the weight of the pots, the amount of water scarcity is added to the pots to reach the field capacity (40%, 60% and 80% field capacity). This was done until the end of the experiment. Plants which had already been treated were harvested at the end of the stress. Leaves samples were stored at −80°C.

Proline content

The homogenization of frozen leaves (0.2 g) 3% sulfosalicylic acid was performed. Then, the homogenate was centrifuged at 10 000 rpm. The analysis of the content of proline was conducted in reactions composed of 2 ml of acid ninhydrin, 2 ml of glacial acetic acid and 2 ml of the extract. Then, it was heated at 100°C for 1 h. The extraction of the reaction mixture was performed with 4 ml of toluene; Afterward, absorbance reading was carried out at 520 nm. For the purpose of the determination of the proline content of corn cultivars, a standard curve was formed utilizing pure proline (Bates et al., 1973).

Lipid peroxidation

Following the technique proposed by Health and Packer (1968), MDA concentration was calculated using an extinction coefficient of 155 mM−1 cm−1. 0.2 g frozen leaves were homogenized in an aqueous solution of trichloroacetic acid (5% w: v). Afterward, the homogenate centrifugation was conducted at 14 000 rpm for 20 min and the supernatant were heated in 0.25% thiobarbituric acid. At the next stage, the absorbance of supernatant was recorded at 532 and 600 nm by spectrophotometer.

Soluble sugar content

The homogenization of 0.2 g of frozen leaves with 3 ml of 95% ethanol was performed. Afterward, homogenate configuration took place at 3 000 rpm for a duration of 10 min. The components of the reaction mixture included 2.5 mL of concentrated sulfuric acid (96%), 0.5 mL of phenol (5%), and 50 μL from the extract. Having utilized a spectrophotometer at 490 nm, the total soluble sugar content was determined, expressed in milligrams of glucose per gram of fresh matter (Dubois et al., 1956).

Total Phenolic Content

Adapting Sharma and Guja’s (2010) framework, phenolic content was measured by the Folin-Ciocalteu spectrophotometric method. Total contents of polyphenol as a gallic acid equivalent from the calibration curve of gallic acid standard solutions, and expressed as gallic acid equivalents (GAE) in milligrams per gram of dry plant material.

Antioxidant enzyme activities assays

Utilizing mortar and pestle at 4°C, we mashed 0.2 g of frozen leaves; and was homogenized with 4 ml of 50 mM potassium phosphate buffer (pH 7.0). The centrifugation of homogenate
at 20,000 rpm for 20 min at 4°C. Afterward, crude extraction enzyme supernatant for enzyme assay was employed.

We assessed catalase (CAT) and peroxidase (POD) activities by method Cakmak and Marschner (1988) and Pandolfini et al. (1992). The activity of CAT was assessed in reactions composed of 50 mM potassium and phosphate buffer (pH 6.8), 10 mM H₂O₂, and enzyme extract. Absorbance reduction was recorded at 240 nm for 1 min. The analysis of POD activity was performed in reactions composed of 50 mM phosphate buffer (pH 6.8), 28 mM guaiacol, 5 mM H₂O₂ and an enzyme extract. Also, enzyme extract was measured at 470 nm for 1 min. we also assessed the activity of superoxide dismutase (SOD) Giannopolitis and Ries (1977). The reaction solution was composed of 50 mM potassium phosphate buffer (pH=7.8), 12 mM L-methionine, 1 mM riboflavin, 50 mM calcium carbonate, 75 mM nitro blue tetrazolium (NBT), and 200 μl of the enzyme extract with non-enzyme solution as control. Irradiation of test tubes composed of the reaction mixture was done under white fluorescent lamps (120 W) at 40 cm distance for a time span of 15 min. One unit of SOD was described as the enzyme amount needed to cause 50% hindrance of NBT decrease as assessed at 560 nm.

Ca and Mg concentration

The collection of the samples was carried out at the final stage of the experiment. The Ca and Mg concentration was recognized in the roots as well as the leaves of the plant. Oven dried plant materials were smashed into a tiny powder. 0.5 g samples were incinerated at 500°C heat for 8 h. Then, the obtained ash was dissolved in 5 mL 2N hydrochloric acid (HCl). When digestion was finished, Utilizing distilled deionized water, we made up the volume of the sample up to 100 mL (Tandon, 1998). Moreover, employing an atomic absorption spectrometer, we analyzed Ca an analyses of Ca and Mg were carried out with an atomic absorption spectrometer Mg (Sepaskhah and Maftoun, 1981).

Photosynthetic pigment estimation

The determination of the content of chlorophyll was carried out in an extract of 80% acetone. As centrifugation (20,000 g, 20 min) was terminated, the spectrophotometric reading of the absorbance was conducted at 663 and 645 nm. The concentrations of chlorophyll a and b as well as the total chlorophyll were estimated (Arnon, 1949). Furthermore, we carried out a spectrometric estimation of carotenoids (Arnon, 1949).

Mucilage extraction

*Alyssum homolocapum* seed gum was dispersed in preheated deionized water (Milli-Q, Millipore, and Bedford, USA) (water to seed ratio of 60:1) at pH 7. The pH was adjusted with 0.1 M HCl or NaOH at a constant temperature of 55 ± 1.0 °C. The seed-water slurry (a semiliquid mixture) was stirred continuously with a mechanical mixing paddle throughout the entire extraction period (1 h). The seeds were discarded, and the rest of the supernatant was subjected to ethanol precipitation (97% ethanol/mixture ratio of 3:1). The precipitate was then kept in the solvent for approximately 10 min with occasional gentle stirring. Then, the precipitate was recovered using a sieve to allow the drainage of excess solvent and was dispersed in deionized water. The dispersion stored overnight at 4°C with continuous stirring. Ultimately, the dispersion was dried in a conventional oven (overnight at 45°C), milled and sieved using a mesh 18 sifter (Koocheki et al., 2009).
Morphological traits, Yield, and Yield Components

To measure morphological traits, we collected 5 plants of pot at the final phase of the experiment. Then we recorded the traits that included: The height of the plant, number of branches, seed and biological yield, 100 seed weight and seed number per raceme.

Statistical analysis

Mean comparison was performed using the LSD test at the 5% level of significance (P<0.05). The statistical analysis was performed using SAS (SAS Institute Inc, 2011) software.

Results

Proline, Soluble Sugar, Lipid Peroxidation and Phenolic Contents

Under water stress, proline and soluble sugars increased (Table 2). The highest proline and soluble sugars content observed under 40% field capacity at stem elongation stage. At this growth stage, proline and soluble sugars content increased by 3 and 4.5 times compared to the control (80% field capacity), respectively. In 60% field capacity, the highest and lowest proline and soluble sugars content was observed at the stem elongation and seed setting stages.

MDA content increased with increasing water stress. MDA content at stem elongation stage under 40% and 60% field capacity increased 4 times more than control (Table 2). Under water stress, phenol content increased. The highest content phenol was in 60% field capacity at the stem elongation (Table 2). In 80% field capacity (control), free proline, soluble sugars, MDA and phenol content did not differ at three growth stages (Table 2). At the stem elongation, the content phenol increased 2.65 time compared to control at this growth stage (Table 2).

Table 2. Effects of water stress at different stages of growth on proline, total soluble sugars, MDA, and phenol content in Alyssum homolocarpum

<table>
<thead>
<tr>
<th>Water stress</th>
<th>Growth stage</th>
<th>Prolin (mg/g FW)</th>
<th>Soluble sugar (mg/g FW)</th>
<th>MDA (µM/g FW)</th>
<th>Phenol (mg/g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% field capacity</td>
<td>Stem elongation</td>
<td>1.854±0.022a</td>
<td>426.43±19.06a</td>
<td>0.565±0.069a</td>
<td>8.95±0.177cd</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>1.362±0.154b</td>
<td>351.04±16.01b</td>
<td>0.429±0.047b</td>
<td>10.3±0.749bc</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>0.771±0.028d</td>
<td>305.52±4.73c</td>
<td>0.175±0.086c</td>
<td>7.7±305d</td>
</tr>
<tr>
<td>60% field capacity</td>
<td>Stem elongation</td>
<td>1.098±0.053bc</td>
<td>275.35±7.33dc</td>
<td>0.555±0.095ab</td>
<td>13.3±0.272a</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>0.9239±0.031dc</td>
<td>252.7±8.65d</td>
<td>0.291±0.064c</td>
<td>11.1±0.641b</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>0.7532±0.042d</td>
<td>183.58±7.35e</td>
<td>0.121±0.063d</td>
<td>8.22±0.232d</td>
</tr>
<tr>
<td>80% field capacity</td>
<td>Stem elongation</td>
<td>0.4188±0.039e</td>
<td>137.13±9.72f</td>
<td>0.135±0.062de</td>
<td>5.025±0.335e</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>0.4755±0.043e</td>
<td>135.5±5.44f</td>
<td>0.133±0.022de</td>
<td>5.17±0.221e</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>0.369±0.032e</td>
<td>132.43±5.13f</td>
<td>0.122±0.01e</td>
<td>5.55±0.132e</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter are not significantly different at P<0.05 according to LSD test. Values are means±SE obtained from four replicates.
**Antioxidant enzyme activities**

With increasing water stress SOD, CAT and POD activities increased. The highest SOD and POD activities were in 40% field capacity at stem elongation stage (Table 3), that had no significant difference with flowering stage in this level of stress. The highest CAT activities was in 40% field capacity at stem elongation stage and its activity increased 3.7 times compared to the control (Table 3). In 80% field capacity (control), there was no significant difference in SOD, CAT and POD activities among the three growth stages.

**Photosynthetic pigment**

With increasing water stress total, chlorophyll content decreased, whereas carotenoid increased. In 40% and 60% field capacity total chlorophyll content decreased at stem elongation stage. However, in 80% field capacity (control), total chlorophyll content increased at stem elongation and flowering stages (Table 3). The highest carotenoid content was at the stem elongation stage in 40% field capacity and its content increased two times more than control (Table 3). In 80% field capacity (control), carotenoid content did not differ at three growth stages.

**Calcium and Magnesium concentration**

Calcium concentration in leaves was mostly dependent on the growth stage where water stress occurs. The lowest leaves calcium concentration was in the 40% field capacity at the stem elongation stage and the highest leaves calcium concentration was in 80% field capacity at the flowering stage (Table 4). In roots, the calcium in 40% field capacity and 60% field capacity did not differ with control at three growth stages (Table 4).

Magnesium concentration was affected by drought stress, so that, the magnesium concentration in leaves decreased with increasing drought stress. In this regard, the lowest of leaves magnesium concentration was in 40% field capacity at the stem elongation stage and seed setting. The highest leaves magnesium concentration was in 60% field capacity at flowering stage (Table 4). In 80% and 60% field capacity, magnesium concentration in roots did not differ at three growth stages but in 40% field capacity at stem elongation, magnesium concentration in roots decreased 35% compared to control condition in this growth stage (Table 4).

**Morphological traits**

Water stress decreased plant height and number of branches per plant. Under 40% and 60% field capacity at stem elongation stage, plant height decreased, 54% and 37% compared to control condition, respectively (Table 4). In 80% field capacity, plant height did not differ at three growth stages.

The highest number of branches per plant belonged to the flowering stage under 80% field capacity that had no significant difference with stem elongation stage in 80% field capacity. The lowest number of branches was at stem elongation stage in 40% field capacity that had a decrease of 55% compared to control at this growth stage (Table 4).
Table 3. Effects of water stress at different stages of growth on SOD, POD, CAT, total chlorophyll content and carotenoid in *Alyssum homolocarpum*

<table>
<thead>
<tr>
<th>Water stress</th>
<th>Growth stage</th>
<th>SOD (units /mg protein/min)</th>
<th>POD (units /mg protein/min)</th>
<th>CAT (units /mg protein/min)</th>
<th>Total chlorophyll (mg/g)</th>
<th>Carotenoid (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% field capacity</td>
<td>Stem elongation</td>
<td>94.07±2.57a</td>
<td>12.289±0.24a</td>
<td>14.732±0.188a</td>
<td>2.223±0.0505ed</td>
<td>1.49±0.02a</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>93.56±2.04ab</td>
<td>11.828±0.59ab</td>
<td>13.756±0.151b</td>
<td>2.6±0.0785d</td>
<td>1.408±0.04ab</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>92.56±9.55bc</td>
<td>10.64±0.101dc</td>
<td>13.84±0.79b</td>
<td>2.86±0.05c</td>
<td>1.285±0.01b</td>
</tr>
<tr>
<td>60% field capacity</td>
<td>Stem elongation</td>
<td>92.26±1.13c</td>
<td>10.97±0.281bc</td>
<td>11.57±0.133c</td>
<td>2.74±0.043f</td>
<td>1.335±0.03b</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>90.66±4.81d</td>
<td>9.7±0.209de</td>
<td>10.23±0.49d</td>
<td>3.17±0.0636e</td>
<td>1.307±1.04b</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>83.37±5.81e</td>
<td>8.95±0.309e</td>
<td>8.14±0.497e</td>
<td>2.91±0.0759d</td>
<td>0.992±0.03c</td>
</tr>
<tr>
<td>80% field capacity</td>
<td>Stem elongation</td>
<td>79.39±4.23f</td>
<td>4.45±0.1f</td>
<td>3.98±0.47f</td>
<td>4.21±0.044a</td>
<td>0.727±0.02d</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>80.27±4.11f</td>
<td>4.35±0.881f</td>
<td>3.6±0.179f</td>
<td>4.06±0.092a</td>
<td>0.717±0.04d</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>80.32±3.74f</td>
<td>4.51±0.205f</td>
<td>2.92±0.158</td>
<td>3.68±0.072b</td>
<td>0.722±0.02d</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter are not significantly different at P<0.05 according to LSD test. Values are means±SE obtained from four replicates.

Table 4. Effects of water stress at different stages of growth on shoot Ca, root Ca, shoot Mg, root Mg, plant height and number of branches in *Alyssum homolocarpum*

<table>
<thead>
<tr>
<th>Water stress</th>
<th>Growth stage</th>
<th>Shoot Ca (%)</th>
<th>Root (Ca) (%)</th>
<th>Shoot Mg (%)</th>
<th>Root Mg (%)</th>
<th>Plant height (cm)</th>
<th>Number of branches (per plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% field capacity</td>
<td>Stem elongation</td>
<td>1.43±0.721d</td>
<td>1.542±0.119ba</td>
<td>0.27±0.011d</td>
<td>0.389±0.023b</td>
<td>16.25±0.478d</td>
<td>2.5±0.288e</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>1.58±0.0696dc</td>
<td>1.359±0.109b</td>
<td>0.36±0.033c</td>
<td>0.50±0.022a</td>
<td>23±0.683c</td>
<td>4.33±0.333cd</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>1.9±0.02a</td>
<td>1.301±0.06b</td>
<td>0.277±0.035d</td>
<td>0.545±0.002a</td>
<td>26±1b</td>
<td>5±1bcd</td>
</tr>
<tr>
<td>60% field capacity</td>
<td>Stem elongation</td>
<td>1.66±0.063bc</td>
<td>1.89±0.047a</td>
<td>0.46±0.012b</td>
<td>0.528±0.013a</td>
<td>22.5±0.645c</td>
<td>4±0.408d</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>1.76±0.0751bac</td>
<td>1.405±0.08b</td>
<td>0.527±0.005</td>
<td>0.583±0.034a</td>
<td>28±0.408b</td>
<td>5.5±0.286bc</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>1.822±0.0561ba</td>
<td>1.967±0.046a</td>
<td>0.464±0.102b</td>
<td>0.537±0.035a</td>
<td>27.75±0.47b8</td>
<td>5.25±0.25bc</td>
</tr>
<tr>
<td>80% field capacity</td>
<td>Stem elongation</td>
<td>1.86±0.047ba</td>
<td>1.533±0.065ba</td>
<td>0.35±0.1113c</td>
<td>0.59±0.047a</td>
<td>35.25±1.43a</td>
<td>5.5±0.5a</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>1.96±0.054a</td>
<td>1.54±0.259ba</td>
<td>0.36±0.112b</td>
<td>0.527±0.035a</td>
<td>36.75±0.478a</td>
<td>6.75±0.25ab</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>1.935±0.032a</td>
<td>1.72±0.175ba</td>
<td>0.277±0.008c</td>
<td>0.584±0.019a</td>
<td>36.5±0.645a</td>
<td>6±0.408bc</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter are not significantly different at P<0.05 according to LSD test. Values are means±SE obtained from four replicates.
Mucilage percentage

Data from three field capacity (40, 60 and 80%) showed the mucilage percentage was different in three growth stages (stem elongation, flowering and seed setting). Water stress increased mucilage percentage in the seed. With an increase in water stress, mucilage percentage increased. The highest mucilage percentage was at flowering stage in 40% field capacity. In 60% field capacity, the highest mucilage percentage was in seed setting stage, but in stem elongation with flowering mucilage percentage did not differ. In 80% field capacity, mucilage percentage did not differ at three growth stages (Table 5).

Yield and Yield Components

Water stress during three growth stages, decreased seed number per raceme, 100 seed weight, biological yield and seed yield (Table 5).

No seeds per raceme was produced in 40% field capacity at stem elongation stage. In 40% field capacity at flowering stage and seed setting stage, seed number per raceme decreased by 53% and 43% respectively compared to control. In 60 and 80% field capacity, there was no significant difference in seed number per raceme among the three growth stages (Table 5).

In 60% field capacity, 100 seed weight decreased at stem elongation stage. However, in 80% field capacity, 100 seed weight did not differ at three growth stages (Table 5). The highest biological yield was in 80% field capacity at flowering stage. The lowest biological yield was at stem elongation in 40% field capacity that decreased 36% compared to control (Table 5).

The highest seed yield obtained in 80% field capacity at three growth stages. In 40% field capacity, no seed was produced at stem elongation stage. However, at the flowering and seed setting stages, seed yield decreased by 85% and 27% compared to control respectively (Table 5).

Table 5. Effects of water stress on mucilage percentage, seed number per raceme, 100 seed weight, biological yield and seed yield in Alyssum homolocarpum

<table>
<thead>
<tr>
<th>Water stress</th>
<th>Growth stage</th>
<th>Percentage mucilage (%)</th>
<th>Seed number per raceme</th>
<th>100 seed weight (g)</th>
<th>Biological yield per pot (g)</th>
<th>Seed yield per pot (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40% field capacity</td>
<td>Stem elongation</td>
<td>17.75±2.33a</td>
<td>49±1.52c</td>
<td>0.0725±0.0058c</td>
<td>68±0.43d</td>
<td>0.259±0.0326cd</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>17.65±1.99ab</td>
<td>55±0.899c</td>
<td>0.085±0.0015ba</td>
<td>66.78±1.05d4</td>
<td>0.523±0.0765d</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>16.6±2.66c</td>
<td>81±1.0587b</td>
<td>0.0805±0.0011bc</td>
<td>78.075±0.82c2</td>
<td>1.02±0.0517b</td>
</tr>
<tr>
<td>60% field capacity</td>
<td>Stem elongation</td>
<td>16.8±3.24c</td>
<td>92.25±1.528ab</td>
<td>0.907±0.0016a</td>
<td>90.75±0.842b</td>
<td>1.251±0.0307b</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>17.22±1.8ab</td>
<td>78.75±1.045b</td>
<td>0.092±0.001a</td>
<td>88.073±1.093b</td>
<td>1.036±0.0435b</td>
</tr>
<tr>
<td></td>
<td>Seed setting</td>
<td>17.65±1.96ab</td>
<td>105.75±1.63a</td>
<td>0.0927±0.006ac</td>
<td>91.2±0.93b</td>
<td>1.656±0.008a</td>
</tr>
<tr>
<td>80% field capacity</td>
<td>Stem elongation</td>
<td>17.53±2.33ab</td>
<td>103.5±1.245a</td>
<td>0.094±0.0012a</td>
<td>101.62±0.76a</td>
<td>1.703±0.0247a</td>
</tr>
<tr>
<td></td>
<td>Flowering</td>
<td>17.45±2.01ab</td>
<td>95.25±1.178ab</td>
<td>0.0917±0.0008a</td>
<td>93.45±1.178b</td>
<td>1.608±0.0178a</td>
</tr>
</tbody>
</table>

In each column, means followed by the same letter are not significantly different at P<0.05 according to LSD test. Values are means±SE obtained from four replicates.
Discussion

There is a consensus on the negative impact of water deficits upon plant growth as well as its development. Along with the gradual water deficit developments, physiological as well as biochemical events take place in a sequential manner. In this respect, as an osmoprotectant, proline can be associated with the drought tolerance under drought stress (Richards et al., 2011). The key role of soluble sugars in cell’s osmotic adjustment during the conditions of stress has been corroborated (Nayyar and Gpta, 2006). Usually evaluated as the end-product of lipid peroxidation, MDA enhanced during drought stress (Ayala et al., 2014). The results of ours research demonstrated that water stress could have a significant impact upon proline, soluble sugar, and MDA accumulation rates was achieved under stress conditions (Table 2). The highest total proline, soluble sugars and MDA contents observed in 40% field capacity at stem elongation (Table 2). Increase in proline and soluble sugar can be useful in maintaining the cell density, which is the protective reaction of Alyssum homolocarpum, by improving water storage capacity and increasing water absorption over the period of water stress. It appears that there is a relationship between proline accumulation and damages caused by water stress (Mazzaferra and Teixeira, 2006). Baher et al. (2002) demonstrated that the rate of proline accumulation rose under the condition of drought stress. As a result, an increase in the rate of proline accumulation under the condition of drought stress is predictable (Bayat and Moghadam, 2019). Moreover, the results of some research highlighted an increase in the rate of soluble sugar and proline as well as oil palm under the condition of water stress (Ji et al., 2014). There have also been some reports on MDA’s accumulation under the condition of water stress in wheat (Mickky and Aldesuquy, 2016), pitanga (Toscano et al., 2016), melon (Sarabi et al., 2017), desi chickpea (Farooq et al., 2018). Also, we observed that phenol contents increased by water stress. In different plants, phenol contents increased in diverse tissues under water stress (Agastian et al., 2000; Muthukumarasamy et al., 2000). Phenolic and flavonoid compounds protect crops against environmental stress through non-antioxidant systems. These compounds act as detergents for the ROS and thus stabilize the membrane of the cell and prevent the peroxidation of lipids (Di Ferdinandou et al. 2014).

Drought tolerance or susceptibility in the plant is well correlated with antioxidant responses. Drought-tolerant medicinal plant species generally have a better capacity to protect themselves from drought stress and induced oxidative stress via the increase of antioxidant enzyme activity (Cao et al., 2011; Turkan et al., 2005). The results of our research demonstrated an increase in POD and CAT activities under water stress condition at three stages of growth. Water stress during stem elongation had a great effect on antioxidant enzymes activity (Table 3). The results of many studies have proven the excessive accumulation of O$_2$ in plant cells under the condition of stress, and superoxide dismutase, peroxidase and catalase were among three outstanding supportive enzymes scavenging reactive oxygen (Kurutas, 2015). The results conducted on many plant species have shown that some plants exposure to abiotic stresses even at low levels water stress, increased antioxidant enzymes such as rice (Lee et al., 2001), mulberry (Harinasut et al., 2003) and quinoa (Fghire et al., 2013, Panuccio et al., 2014).

In this study, chlorophyll content decreased under water stress whereas, carotenoid content increased (Table, 3). Reactive oxygen species through damaging chloroplasts are the primary culprits of Chlorophyll depletion under the condition of drought stress (Smirnoff, 2000). The leaf chlorophyll content is considered as an appropriate criterion for evaluating the physiological status of the plant. The ROS compounds are produced in the cell during the drought stress, the destruction of the photosynthetic system and ultimately the decomposition of chlorophyll (Farooq et al. 2009). Nayyar and Gpta (2006) reported a reduction in the content of chlorophyll in maize and wheat when confronted with drought stress.
Carotenoids have a protective role, and protect chlorophyll form oxidation. Protection against oxidative damage caused by quenching of triplet chlorophyll inhibiting singlet oxygen generation. Radiation energy absorbed by chlorophyll and carotenoids, which is used for photosynthesis (Abid et al., 2018). Among the non-enzymatic antioxidants, carotenoids (Car) are particularly important because they decrease ROS contents and thereby protect the photosynthetic machinery (Cazzonelli, 2011). Also, via decreasing thermal impacts of drought stress condition, carotenoids might exhibit their defensive response (Farooq et al., 2009). There have also been some reports of an increase in the content of carotenoid under the condition of water stress in cotton (Mssacci, 2008) as well as Catharanthus roseus (Jaleel et al., 2009).

It has been shown that calcium concentration in the leaves relies on the stage of growth as well as water stress intensity, but in roots, the calcium concentration did not differ with control at three growth stages with increasing water stress. Integrating the structure as well as the function of plant membrane and other structures, Calcium plays a crucial role (Shao et al., 2008). Sardans et al., (2008) reported that drought reduced concentrations of Ca in the aboveground biomass of Mediterranean evergreen Quercus ilex forest due to the decrease in transpiration flux. Pomper and Grusak, (2008) concluded that transpiration and growth-induced water uptake by developing plant organs appear to drive the transport of Ca in the xylem pathway to vegetal tissues of the snap bean plant. Azzeme et al., (2016) concluded that absorption calcium by sorghum was negatively affected by irrigation. Drought stress reduced the concentration of calcium in different tomato varieties (Nahar and Gretzmacher, 2002).

The magnesium concentration in leaves decreased with increasing water stress, but in root the magnesium concentration in 40% field capacity magnesium concentration decreased at stem elongation stage (Table 4). The major function of magnesium is its role as the central atom of chlorophyll molecules, but magnesium is also involved in energy conservation and conversion (Amtmann and Blatt, 2009). Grabaová and Martinková, (2001) found that drought induced a decrease in nitrogen and magnesium content more than phosphorus and potassium in growth periods of the Norway spruce. According to them, in accordance with the degree of stress, the time needed for the sufficient restoration of mineral nutrition would be one year or more. Brown et al., (2006) observed concentrations of Mg in roots and shoots of Spartina alterniflora (coastal smooth cordgrass) decreased under drought condition.

In our experiment, was mucilage percentage in the seed affected by water stress (Table 5). Mucilage percentage increased with increasing in intensity of water stress. Pollak, (1990) reported that water-soluble mucilage content extracted from Boraginaceae increased with increasing in intensity of water stress. Also, Jeremias, (1996) showed that mucilaginous substances increased during drought stress in Brunella grandifolia. Yield depends on morphological, physiological and biological factors. These factors are affected by increasing water stress. In the current study, yield components and seed yield had the highest decrease at the stem elongation stage in 40% field capacity (Table 5). It seems that this stage was the most sensitive to water stress in Alyssum homolocarpum. In 40% field capacity and stem elongation stage, Alyssum was subjected to a long water stress period. At this stage, although plant increase of antioxidant enzymes activities, but growth and yield decreased. In 60% field capacity at stem elongation stage, yield components and seed yield of Alyssum were acceptable. Our findings demonstrated that flowering stage has a priority over other stages under the condition of mild water shortage because it allows stress acclimatization to take place over time. Growth response’ analyses to drought over time demonstrated flowering plants’ recovery of their growth in the second half of their vegetative development (Baerenfaller et al., 2012). While Farooq et al., (2009) reported that water stress at the stage of grain filling decreased maize yield about 79–81%. In durum wheat, this decrease was about 40% (Daryanto et al., 2017). Rebey et al., (2012) showed an increase in seed yield of
cumin under moderate condition, though in severe drought stresses, a decrease was observed. It was reported that inadequate photosynthesis because of stomata closure resulting in a decrease in CO$_2$ uptake is one of the main culprits of a decrease in seed yield (Seghatoleslami and Forutani, 2015).

**Conclusions**

Present results showed that plants under water stress condition make changes in some of their physiological biochemical and morphological characteristics. Water stress reduced plant height, as well as seed yield and yield component, whereas SOD, POD, CAT activities and mucilage percentage increased. Also, water stress caused an increase in the content of proline, soluble sugars MDA. Most of these changes were at the stem elongation stage in 40% and 60% field capacity. The most sensitive growth stage to water stress is stem elongation stage in alyssum. Growth response analyses demonstrated that water stress in flowering stage is capable of recuperating yield. On the contrary, severe water stress at the stem elongation stage is associated with the risk of yield loss. The result revealed that *Alyssum homolocarpum* should be irrigated at stem elongation for high and good quality yields.

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