Effects of two species of mycorrhiza fungi and drought stress on chlorophyll a, b and total of Ocimum basilicum

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ABSTRACT: Moisture deficits is a significant challenge to the future crop production. A plant responds to a lack of water by halting growth and reducing photosynthesis and other plant processes in order to reduce water use. The influences of arbuscular mycorrhizal (AM) fungus on chlorophyll concentration were studied in pot culture under well-watered and drought stress conditions. In order to investigate the effects of two species of mycorrhiza fungi and drought stress on chlorophyll content on Ocimum basilicum, a Pot experiment in factorial and completely randomized design with four replications was conducted on Ocimum basilicum L. Examined factors include four levels of drought (FC, 0.75 FC, 0.5 FC and 0.25 FC) and applying two species of mycorrhiza fungi (Glomus fasciculatum and Glomus mosseae) and control. Results showed that drought stress had a significant effect on traits. Also the results showed that the fungus Glomus fasciculatum and Glomus mosseae had not any significant differences on chlorophyll a, b and total of Ocimum basilicum. This is important to know that the knowledge about chlorophyll concentration of AM plants under drought stress are insufficient.

Keywords: mycorrhiza fungi, Ocimum basilicum, drought stress, Glomus fasciculatum and Glomus mosseae

INTRODUCTION

In nature or crop fields, water is often the most limiting factor for plant growth. If plants do not receive adequate rainfall or irrigation, the resulting drought stress can reduce growth more than all other environmental stresses combined. Drought can be defined as the absence of rainfall or irrigation for a period of time sufficient to deplete soil moisture and injure plants. Drought stress results when water loss from the plant exceeds the ability of the plant's roots to absorb water and when the plant's water content is reduced enough to interfere with normal plant processes. Osmolytic accumulation in plant cells can act as a mechanism of osmotic adjustment for decreasing the cellular osmotic potential and thus for maintaining water absorption and turgor. Osmolytic accumulation can also protect cellular components, such as cell membranes and proteins, and sustain the physiological activity of plants (Serraj and Sinclair 2002).

Plants are frequently subjected to different abiotic environmental stresses that determine geographic distribution and adversely affect growth, development, and agronomic yield. Drought is one of the major constraints on plant productivity worldwide and is expected to increase with climatic changes (IPCC 2007 and EEA 2011). The symbiotic relationship between arbuscular mycorrhizal (AM) fungi and the roots of higher plants is widespread in nature, and several ecophysiological studies have demonstrated that AM symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance, as demonstrated in a number of host plant and fungal species (Augé 2001; Ruiz-Lozano 2003; Smith and Read 2008; Ruiz-Lozano and Aroca 2010). Arbuscular mycorrhizal fungi (AMF) are integral functioning parts of plant root systems and are widely recognized for enhancing plant growth on...
severely disturbed sites. A pattern that has been repeatedly observed in interactions between arbuscular mycorrhizal (AM) fungi and plants is that plant competition is stronger with AM fungi than without (reviewed by Koide & Dickie 2002). However, plants and the mycorrhizal fungi that colonize their roots usually occur as diverse communities that vary in species composition at multiple spatial and temporal scales (Horton & Bruns 2001; Clapp et al. 2002). Because mycorrhizal fungus species often vary in their growth effects on particular plant species (e.g. Chu-Chou & Grace 1985; Hetrick et al. 1986; van der Heijden et al. 1998). The tolerance of plants to drought stress differed with the arbuscular-mycorrhizal fungal isolate which the plants were associated. The fungi has different traits that affected the drought resistance of host plants. This study was done for understanding effects of two species of arbuscular mycorrhizal fungi on plant under drought along fungi that can be effective on Chlorophyll content.

MATERIALS AND METHODS

This study was conducted in a greenhouse of Damghan Islamic Azad University. This factorial experiment was conducted in a completely randomized design with three replications. Drought treatments consisted of four levels including T0=FC, T1= 0.75 FC, T2=0.5 FC and T3=0.25 FC. Mycorriza treatments were including two species of mycorrhiza fungi (M1=Glomus fasciculatum and M2=Glomus mosseae) and control(M0). The experiment was 4 steps including: 1- preparation of soil samples 2- preparing pots and treatments 3- planting seed 4- adding treatments

The soil samples used for 1 h with a temperature of 121 °C for 5 min and then rinsed with tap water sterilized pots, were surface sterilized by alcohol. Physical and chemical properties of soil were determined. The research field had a sandy loam soil. Details of soil properties are shown in Table 1.

<table>
<thead>
<tr>
<th>FC</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Sand (%)</th>
<th>EC (dS/m)</th>
<th>CEC (meq/100g)</th>
<th>O.C (%)</th>
<th>Total N (%)</th>
<th>Available P (ppm)</th>
<th>Available K (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>24</td>
<td>38</td>
<td>38</td>
<td>2.94</td>
<td>11.26</td>
<td>0.465</td>
<td>0.03</td>
<td>7.78</td>
<td>78.68</td>
</tr>
</tbody>
</table>

According to Dan Kerr for surface disinfection, the seeds were soaked in 5% bleach for 7 min and were washed 8 to 10 times with distilled water. For each hole, a number of seed placements on the inoculum were covered with soil. Seedlings till Two leaf stage in pots were maintained at field capacity. Arriving the seedling to the next stage were thinned to 3 seedling in per pot. After thinning the seedlings in pots, desired level of stress was done. After about 2 months of planting seeds in every pots, roots and shoots of plants were harvested. Immediately after harvest, samples were placed in plastic bags and then transferred to the laboratory and were measured by a digital scale with an accuracy of one ten-thousandth. Other dishes were immediately washed in tap water. Finally distilled water was placed in a paper bag full of leaching and. They were set and dried in laboratory condition. Weighing the samples after 24 h and cooling in desiccator by a digital scale was measured with an accuracy of one ten-thousandth. Measurement of leaf chlorophyll: For determining chlorophyll a, b, total chlorophyll content of leaves Lichtenthaler and Wellburn, 1987 was used. For this purpose, 0.05 g leaves in 10 ml acetone and 80% pulverized was put. The obtained smooth was filtered with paper atman 1. Brought to a final volume of 20 ml and absorbance at wavelengths of 663/2 and 646/2 nm was measured using UV-Visible spectrophotometry. The concentration of chlorophyll a, b, and total chlorophyll content was determined by the following formulas. All data were statistically analyzed using SAS software.

RESULTS AND DISCUSSION

Results:
The Analysis of variance of mycorrhiza fungi and drought stress and their interaction on chlorophyll content Ocimum basilicum was shown in table 2. The results showed that effect of drought stress on chlorophyll content of Ocimum basilicum was significant but test indicated that mycorrhiza fungi and, their interaction on Ocimum basilicum was not significant.
Table 2. The ANOVA Table of mycorrhiza fungi and drought stress and the interaction on Ocimum basilicum

<table>
<thead>
<tr>
<th>ANOVA Table</th>
<th>Sources of Variation</th>
<th>MS</th>
<th>chlorophyll a</th>
<th>chlorophyll b</th>
<th>chlorophyll total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mycorrhiza fungi</td>
<td>0/0588 ns</td>
<td>0/033 ns</td>
<td>0/134 ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>drought stress</td>
<td>1/744 **</td>
<td>0/776 **</td>
<td>3/313 **</td>
<td></td>
</tr>
<tr>
<td></td>
<td>mycorrhiza fung*</td>
<td>0/0365 ns</td>
<td>0/013 ns</td>
<td>0/191 ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>drought stress</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FW: Fresh weight, DW: Dry weight, *: significant at %5, **: significant at %1, ns: not significant

Figure 1 shows effect of mycorrhizal fungi on mean of chlorophyll a, b and total in Ocimum basilicum. The fig shows that there were not any differences between groups.

![Figure 1](image1.png)

**Figure 1.** effect of mycorrhizal fungi on chlorophyll a, b and total

Figure 2 shows effect of drought stress on mean of chlorophyll a, b and total in Ocimum basilicum. The fig shows that there were differences between groups. The highest chlorophyll mean related to Fc and 0.75 Fc (A) and the lowest was to 0.25 Fc (C).

![Figure 2](image2.png)

**Figure 2.** effect of drought stress on mean of chlorophyll a, b and total in Ocimum basilicum

Figure 3 shows Interactions between mycorrhizal fungi and drought stress on chlorophyll a in Ocimum basilicum. The results showed that there were not any significant differences between treatments. The highest chlorophyll mean related to M0T0 and the lowest was to M0T3.
Figure 3 Interactions between mycorrhizal fungi and drought stress on chlorophyll a

Figure 4 shows interaction of drought stress and mycorrhizal fungi on chlorophyll b. The results showed that there were no significant differences between the various groups. The results showed that there were not any significant differences between treatments. The highest chlorophyll b mean related to M1T0 and the lowest was to M2T3.

Figure 4 Interactions between mycorrhizal fungi and drought stress on chlorophyll b

Figure 5 shows interaction of drought stress and mycorrhizal fungi on chlorophyll total. The results showed that there were no significant differences between the various groups. The results showed that there were not any significant differences between treatments. The highest chlorophyll total mean related to M1T0 and the lowest was to M0T3.

Figure 5 Interaction of drought stress and mycorrhizal fungi on chlorophyll total

**Discussion:**

Moisture deficits is a significant challenge to the future crop production. A plant responds to a lack of water by halting growth and reducing photosynthesis and other plant processes in order to reduce water use. As water loss progresses, leaves of some species may appear to change color—usually to blue-green. Foliage begins to wilt and, if the plant is not irrigated, leaves will fall off and the plant will eventually die. Drought lowers the water potential of a plant's root and upon extended exposure, abscisic acid is accumulated and eventually stomatal closure occurs.
plant with a large mass of leaves in relation to the root system is prone to drought stress because the leaves may lose water faster than the roots can supply it.

To summarize, mycorrhizal plants employ various protective mechanisms to counteract drought stress. Considerable progress has been made in understanding the role of AM symbiosis in conferring drought resistance to plants, but different aspects still require attention for unraveling novel metabolites and hidden metabolic pathways. The accumulated physiological, biochemical, and molecular data based on classical approaches will benefit from the various ‘omic’ techniques and their combinations. An in-depth investigation using the advanced methodologies could help to the mechanisms of drought avoidance and/or tolerance induced by AM symbiosis and to discriminate the drought-induced processes of the protective mechanisms regulated by AM symbiosis. Symbiosis has a variety of effects on the defensive responses of host plants, depending on the species of host plant and the AM fungus involved (Bezemeres and van Dam 2005). In this study Mycorrhizal fungi in plants treated, there was no change in chlorophyll content. The concentrations of chlorophyll a, chlorophyll b and chlorophyll a + b were similar in mycorrhizal and non-mycorrhizal plants under well-watered conditions. The results of the study did not show a significant difference by Mycorrhizal fungi but drought stress reduced chlorophyll content which is probably due to reduced water uptake. When water is limiting, decreased stomatal conductance and increased diffusive resistance to CO2 could lead to increased plant water potential. To maintain water uptake from the soil, though, the water potential must be reduced. To achieve such an effect, plants can rely on mechanisms of ‘osmotic adjustment’ or ‘osmoregulation’ that decrease the osmotic potential resulting from the accumulation of compatible solutes or osmolytes (Munns 1988; Serraj and Sinclair 2002). The contribution of mycorrhizal symbiosis to drought tolerance is due to a combination of physical, nutritional, physiological, and cellular effects (Aroca et al. 2008, Miransari 2010). Osmolytic accumulation in plant cells can act as a mechanism of osmotic adjustment for decreasing the cellular osmotic potential and thus for maintaining water absorption and turgor. Osmolytic accumulation can also protect cellular components, such as cell membranes and proteins, and sustain the physiological activity of plants (Serraj and Sinclair 2002). AM and non-AM plants often show different photosynthetic characters. Wu and Xia (2006) confirmed that AM citrus seedlings had higher photosynthetic rate (Pn), stomatal conductance (gs) and transpiration rate (E) than corresponding non-AM plants under drought stress. It is known that the concentration of chlorophyll is associated with Pn, and the characterization of chlorophyll fluorescence reflects the state of the photosynthetic apparatus. However, the knowledge about chlorophyll concentration and chlorophyll fluorescence of AM plants under drought stress is insufficient.

REFERENCES


