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Effect of Drought Stress on Total Protein, Essential Oil Content, and Physiological Traits of

Levisticum Officinale Koch

Davoud Akhzari¹ and Mohammad Pessarakli^{2,*}

¹Assistant professor, Department of Watershed and Rangeland Management, Malayer

University, Iran

²School of Plant Sciences, The University of Arizona, Tucson, Arizona 85721, USA

*Address Correspondence to Mohammad Pessarakli: pessarak@email.arizona.edu or pessarak@ag.arizona.edu

ABSTRACT

Levisticum officinale Koch is one of the most important plants producing essential oil. An experiment was conducted to determine the effect of drought stress on growth, total protein and essential oil content of *Levisticum officinale*. The experiment was conducted in a greenhouse, in a completely randomized design using 5 replications. Aridity levels of field capacity irrigation (as control), -0.6 and -1.0 MPa were applied. There was significant effect of aridity on leaf area in *Levisticum officinale*. The highest leaf area values were found in the lowest aridity (FC) level. The root weight and root length in the -0.6 MPa aridity level were more than that of the control aridity level, but, shoot height and shoot weight were highest in the control treatment. Total soluble protein contents under -0.6 MPa drought stress was numerically lower than that in the control treatment, but statistically there was no significant difference between protein contents in the -0.6 MPa and the control treatment. Compared to the control treatment, total soluble protein contents of *Levisticum officinale* were significantly decreased in the -1.0 MPa drought treatment. Essential oil content of *Levisticum officinale* was significantly increased in the -0.6 MPa drought

treatment compared to the control. Compared to control treatment, there was a significant reduction found in essential oil content of *Levisticum officinale* in the -1.0 MPa aridity treatment. The results suggest that in the drought levels between -0.6 to -1.0 MPa, *Levisticum officinale* could be used for arid and semi-arid lands economical use.

Keywords

Drought, total protein, essential oil, physiological traits

INTRODUCTION

Drought is known as a major abiotic factor limiting plants growth and production (Bhatnagar-Mathur et al., 2009). Numerous physiological and biochemical changes occur in response to drought stress in various plant species (Chen and Tabaeizadeh, 1992; Cheng et al., 2002). Drought stress is one of the most important factors influencing arid lands vegetation survival. Only the native and the well-adapted species are present in these areas (Lloret et al., 2005). The climate of the arid to semiarid region is defined by generally low and highly variable precipitation (Westoby et al., 1989; Behnke et al., 1993; Scoones, 1994; Heady, 1999; Rothauge, 2007; Hein and Weikard, 2008). Plants that can naturally be established in the drought soil conditions are called xerophytes (Richards, 1954). Drought has considerable adverse impacts on plant growth and development and productivity (Lauchli and Epstein, 1990). Drought stress causes reduced in nutrient uptake by roots and eventually plant death (Jafari, 1994). Thus, soil aridity stress can reduce rangeland production potential (Kafi and Mahdavi, 2012).

In aromatic plants, growth and essential oil production are influenced by various environmental factors, such as water stress (Burbott and Loomis, 1969). Changes in essential oils extracted from aromatic plants and their composition were observed under water stress condition (Sabih et al., 1999). Also, changes in protein expression, accumulation, and synthesis have been observed in many plant species as a result of plant exposure to drought stress during growth (Chen and Tabaeizadeh, 1992; Cheng et al., 2002). Both quantitative and qualitative changes in proteins were detected during the drought stress period (Riccardi et al., 1998).

Variation in protein contents is an essential part of plant response to environmental stress as well as for plants adaptation to changes in environmental conditions (Vierstra, 1993; Hieng et

al., 2004). Under water deficit (dehydration) conditions, numerous physiological processes in plants are modified or impaired (Bradford and Hsiao, 1982). Water stress affects the protein levels in plants, but the results of different investigators are contradictory. Some authors have reported decreased protein levels in plants under water stress (Pierre and Savoure, 1990; Roy-Macauley et al., 1992). Others have found an absence of deleterious effects of drought on protein levels in plants (Todd and Basler, 1965). Increases in plant protein levels have also been reported (Singh and Rai, 1982). One way of plants to tolerate abiotic stresses to some degree is by biosynthesis of so called “stress proteins”, i.e. heat shock protein. Among them are dehydrins that accumulate in plants in response to environmental stresses (Close, 1996, 1997; Svensson et al., 2002; Marian et al., 2003). Economical use of arid and semi-arid lands is a top priority and challenge for the Iranian natural resources experts. In this study, we investigated the effect of water stress on growth, essential oil and protein contents of *Levisticum officinale* Koch that is a suitable plant for arid and semi-arid lands economical use in Iran.

MATERIALS AND METHODS

The seeds of *Levisticum officinale* were taken from Research Institute of Forests and Rangelands of Iran. Plants were grown in a greenhouse at 15-28°C under a 14 h photoperiod. Scions were planted in pots of 14 cm diameter and 25 cm depth; each pot contained 3.5 kg soil. The soil characteristics were as follows: sandy loam in texture, sand 54.6%; silt 11.7%; clay 13.2%; pH 8.52 and organic matter 1.3%.

Three levels of aridity were implemented on pots with pressure plates. Aridity levels of field capacity irrigation (as control), -0.6, and -1.0 MPa were applied. Each pot was weighed every 3 days. The decreased weight of each pot in each round of observation showed the amount

of water evaporated or consumed by the plants. So, this weight of water was added to the pots by irrigation water (Alizadeh, 1999).

The plant height (cm), root system length (cm) and leaf area (cm²) were measured after 3 months. Plants were washed with distilled water and separated into shoots and roots. The dry weight (dw) was obtained after oven drying the plants at 60°C for 48 hours. The dry weights of the root and shoot systems were also determined.

Protein content of plants was estimated by Lowry et al. (1951) method. Reagent A: 1% sodium carbonate (Na₂CO₃) in 0.5 N sodium hydroxide (NaOH); Reagent B: 1% copper sulfate (CuSO₄). 5 water (H₂O); Reagent C: 2% sodium tartrate (Na₂C₄H₄O₆); Reagent D: Mixture of 0.5 ml Reagent C with 0.5 ml Reagent B and 10 ml Reagent A, and Reagent E: Folin 0.2 N. Soluble proteins were extracted from 2 g dry weight of each sample into 5 ml Tris-hydrochloric acid (HCl) buffer (pH = 8.0) containing 26.8 ml 0.2 N HCl 17.2% sucrose, 1% ascorbic acid and was then centrifuged. 1 ml of Reagent D was added into 0.05 ml of resultant solution and kept in room temperature. Then, 3 ml of Reagent E was added and the sample was kept in Bain-marie at 50°C. The absorbance was measured spectrophotometrically at 625 nm. Protein was calculated based on μMg^{-1} fresh weight (FW).

Plant samples were air dried and weighed for the essential oil extraction. Dry plant material (200 g) from each replicate of all treatments was subjected to steam distillation for 3 hours using a Clevenger type apparatus (Clevenger, 1928). The essential oil content was calculated as a percentage of the plant dry weight (Khalid, 2012).

The pot experiment was set up in completely randomized design using 5 replications. Analysis of variance (ANOVA) was employed for statistical analysis of data. Statistical significance was defined as $P < 0.05$.

RESULTS

Leaf area declined significantly along decreasing soil moisture. The highest leaf area values were found in the lowest aridity (FC) level. Compared to the FC treatment, leaf area was reduced by 72.41% and 11.43% in the -0.6 and -1.0 MPa aridity treatments, respectively. So, there was a significant effect of aridity on the leaf area in *Levisticum officinale* (Figure. 1).

Shoot dry weights at all stages of development reduced progressively, but reversibly, with increasing drought stress. Increasing drought stress in soil significantly retarded ($p < 0.05$) dry weight of stems (Figure 2). Dry weight significantly decreased ($p < 0.05$) for shoots and total biomass of plants in response to increasing aridity level (Figure 2). Root height and root dry weight were highest in the -0.6 MPa aridity level (Figure 2). The relative percentage of the shoot height, shoot dry weight, root length, and root dry weight of the plants under drought stress compared to those of the controls were computed as (drought stressed plants/control plants) x100 and illustrated in Figure 2.

The percentage values of the parameters under increasing soil aridity varied from 100, 32, and 17% for the shoots height, from 100, 28, and 21% for the shoot weight, from 92, 100, and 53% for the root height, and from 88, 100, and 48% for the root weight for the FC, -0.6 and, -1.0 MPa, respectively. Plant dry weight decreased dramatically with increasing aridity level the highest plant dry weight of *Levisticum officinale* was obtained under the first treatment in all

ranges of aridity treatments. The general tendency was that increasing aridity level induced a progressive decline in the length of shoots and in the weight of roots, stems and leaves.

Effect of Drought Stress on Essential Oil Content

Aridity stress significantly increased essential oil content of plants in the -0.6 MPa treatment. There were significant differences found between essential oil contents of plants at various aridity levels. Essential oil content reduced from 0.7 to 1.3 and 0.25%, for FC, -0.6 and -1.0MPa aridity levels, respectively (Figure 3).

Effect of Drought Stress on Total Protein Content

Increase in aridity level from FC and -0.6 MPa to -1.0 MPa resulted in a significant progressive decline in total protein content of the plants. Total protein content was reduced from 52 to 49 and 9 $\mu\text{M/g}$ FW, for FC, -0.6 and -1.0 MPa aridity levels, respectively (Figure 4).

DISCUSSION

Drought stress reduces the ability of plants to absorb water, and this causes reductions in growth rate along with a cascade of metabolic changes. Hence, the ability of a plant to grow under drought stress conditions is a key factor to improve rangeland vegetation in arid rangelands (Sharp and Davies, 1979, 1985; Netting, 2000; Bruce et al., 2002). So, aridity alters plant growth rate and nutrient uptake (Sharp and Davies, 1979, 1985; Netting, 2000; Bruce et al., 2002). Results of the present research showed leaf area declined significantly as level of aridity increased from FC to -0.6 and -1.0 MPa (Figure. 1). These variations are due to the different soil moistures treatments.

Results of the present research showed that different aridity levels had different effects on dry matter production in both shoots and roots. Several studies indicated that increasing in drought stress could decrease plant dry weights (Omidi, 2010; Jongrunklang et al., 2008; Abdalla and El-Khoshiban, 2007; Hamada, 1996). These were seen in shoot height and shoot weights that significantly reduced in *Levisticum officinale* under different aridity levels (Figure. 2). However, Kameli and Losel (1996) reported that drought stress may increase root weight and decrease shoot weight. The highest root height and root weight were seen under -0.6 MPa aridity level in *Levisticum officinale*. In other words, the plant could produce enough roots at -0.6 MPa drought stress. More stress caused a reduction in root growth, water uptake, mineral nutrient deficit and finally stopped growth. However, the root height and weight at the -0.6 MPa aridity stress were significantly higher compared with the control level (Figure 2).

The absorption of nutrients from the soil depends on the availability of water to roots. It has been reported that soil water deficit may reduce root growth and limit nutrient uptake by roots (Arndt et al., 2001). This significant reduction in nutrient uptake (compared with the control and -0.6 MPa aridity levels) has been seen at the -1.0 MPa aridity level (Figure. 2). Li and Wang (2003), Gazal and Kubiske (2004), Bargali and Tewari (2004) considered a high root to shoot ratio as a drought tolerant parameter. They have emphasized that the first and the most common feature of plant growth is increasing of root to shoot ratio in arid regions. The results of Baher et al. (2002) showed that greater soil water stress decreased plant height and total fresh and dry weight of plants. Colom and Vazzana (2002) also showed that the number of stems per plant and plant dry weights were negatively related to water stress in plants.

The maximum essential oil content of *Levisticum officinale* has been seen at the -0.6 MPa aridity level (Figure. 3). This result is in agreement with (Simon et al., 1992) report that stated essential oil contents increased in response to water stress, but plant growth decreased with increasing water stress (Figures. 2 and 3). Limited water supply is a major environmental constraint in productivity of plants. Moisture deficiency induces various physiological and metabolic responses like stomatal closure and decline in growth rate and photosynthesis (Flexas and Medrano, 2002). Minimum essential oil content of *Levisticum officinale* has been seen at the -1.0 MPa aridity level treatment. So, the results of the the present research is in contrast to Hendawy and Khalid (2005) report that stated severe water stress increased essential oil content more than moderate water stress.

Increase in aridity level from FC to -1.0 MPa resulted in a significant progressive decline in total protein content of plants (Figure. 4). So, this result is in agreement with Pierre and Savoure (1990) and Roy-Macauley et al. (1992). The results of the present study showed increasing aridity level caused decline in total protein content of the plants (Figure. 4). However, in some plant species, some proteins (i.e., dehydrins) are synthesized in response to drought stress (Close and Chandler, 1990). Dehydrin family of proteins are accumulated in a wide range of plant species under dehydration stress (Close, 1996). The results of this research shown in Figure 4 probably reflects the inability of *Levisticum officinale* in the production of dehydrins protein.

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FIGURE 1 Effect of aridity on the leaf area in *Levisticum officinale*.

FIGURE 2 Effect of aridity on root and shoot dry weights of *Levisticum officinale*. Different letters represent a significant difference ($P < 0.05$) between treatments.

FIGURE 3 Effects of various aridity levels on essential oil content. Different letters represent a significant difference ($P < 0.05$) between treatments.

FIGURE 4 Effect of drought stress on total protein content of *Levisticum officinale*. Different letters represent a significant difference ($P < 0.05$) between treatments.