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Prolonged Water Stress on Growth and Constituency of Iranian of Oregano (*Origanum vulgare* L.)

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ABSTRACT

Oregano (*Origanum vulgare*), a popular flavoring herb, is widely used throughout the world for flavoring foods and beverages. Water stresses in the arid and semiarid regions of the Middle Eastern countries, severely limiting growth, production, and survival of oregano. To determine the growth and ecophysiological responses of native Iranian oregano to prolong water stress, six populations sourced from different bioclimatic zones in Iran were studied. The plants, grown in pots, were subjected to three water stress conditions (no water stress, mild water stress, and moderate water stress) that were continuously maintained throughout the entire plant development and growth period. Relative water content, cellular injury, leaf pigmentation, proline content, leaf area, biomass production, and antioxidant enzymes (peroxidase, ascorbate peroxidase, catalase, and superoxide dismutase) were monitored. The relative plant yield, water content, pigmentation, and leaf area were reduced under water stress, although the ratio of carotenoids to total chlorophyll in the studied populations increased. Under water stress conditions, three of the oregano populations exhibited increased plant yield, superoxide dismutase, peroxidase, and catalase activity along with proline accumulation as compared with other populations. The positive relationship was observed in the activity of superoxide dismutase (SOD) and the proline content, however, a negative correlation between ion leakage and activity of SOD was indicated. Our results demonstrated that populations of Banch, Ardabil, and Naim oregano had a higher tolerance to water deficit conditions than the other investigated populations of oregano.

INTRODUCTION

The genus *Origanum*, a member of the Lamiaceae family, is comprised of 39 species distributed throughout the Mediterranean, Euro-Siberian, and Irano-Siberian regions (Aligiannis et al., 2001; Skoula et al., 2002; Sozmen et al., 2012). In Iran, the genus consists of one species (*Origanum vulgare* L.) and three subspecies (ssp. *vulgare*, ssp. *viride*, and ssp. *gracile*) (Rechinger, 1982). Commonly known as oregano, *O. vulgare* is widely used as a flavoring herb and a composition constituent of essential oil mixtures and extracts in the world. The plant is commonly used as a spice in food production (Olivier, 1997) and a medicinal plant in ethnopharmacological preparations of Iranian traditional medicines (Mozafarian, 2012). Previous studies (Lagouri et al., 1993; Kokkini, 1996; Kulisic et al., 2004; Bakkali et al., 2008; Kursat et al., 2011; Gulluce et al., 2012; Sarikurkcu et al., 2015) have demonstrated that *O. vulgare* has important chemical, biological, and health effects, including antimicrobial, antifungal, antigenotoxic, insecticidal, anti-oxidant, and anti-inflammatory activity (Ocana-Fuentes et al., 2010).

In Iran and other areas of the Middle East, the detrimental effects of drought periods and lack of alternative water sources for oregano production are of great concerns to farmers as crop yields of oregano are reduced due to the lack of water. In Iran, more than 75% of the arid and semiarid regions have been classified as water deficient regions (Tabari and Talaee, 2011). The issue of water deficient limits the distribution and survival of plants in arid and semi-arid regions (Lu and Zhang, 1999; Liu et al., 2011; Tabari et al., 2012). Under water stress, the growth and yield of plants along with changes in aromatic constituents in

oregano and other herb and spice crops can be significantly affected (Singh et al., 2000; Zehtab-Salmasi et al., 2001; Delfine, et al., 2005; Azizi et al., 2009; Yadav et al., 2014; Morshedloo et al., 2015), thus affecting farmers' income.

The lack of water leads to oxidative stress in plants due to stomatal closure and increased electron leakage that result in the formation of reactive oxygen species (ROS) in chloroplasts and mitochondria (Asada, 1999; Mittler, 2002; Ozkur et al., 2009; Liu et al., 2011). ROS damage lipids, proteins, nucleic acids, photosynthetic pigments, and enzymes, preventing the normal functions of cells (Fu and Huang, 2001; Mittler, 2002; Apel and Hirt, 2004; Farooq et al., 2009; Ozkur et al., 2009).

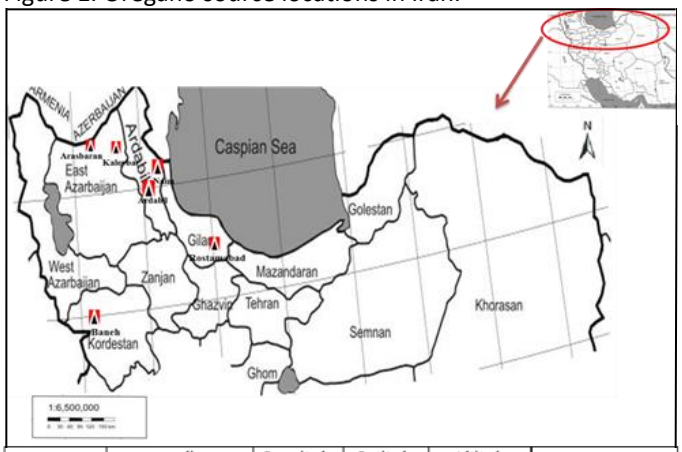
With oregano being an important medicinal crop in Iran and other areas, the development of oregano selections being able to grow and produce a viable crop yield under water stress is needed. Field observations in Iran suggest that some wild populations of oregano appear to have some drought resistance mechanisms. Understanding and elucidating these mechanisms through selection and breeding programs could improve drought tolerant oregano populations for domestication and cultivation.

MATERIALS AND METHODS

Plant material. In this study, six populations of oregano (*O. vulgare* L.) sourced from different bioclimatic zones of Iran (Figure 1) were seeded in a greenhouse at the College of Agriculture and Natural Resources, University of Tehran, Iran (longitude 50˚59'51" E, latitude 35˚48˚20" N, altitude 1343 m above mean sea level). The seeds (obtained from the seed gene bank of the Forest and Rangeland Research Institute, Tehran, Iran) were germinated in a coco peat: perlite mix (70:30, w: w) contained in a plastic germination tray.

Uniform sized seedlings with six leaves were randomly selected from each population 30 days after seeding for transplanting (one plant per pot) into a prepared media contained in round pots (volume = 7 L). To ensure all plants have the same root environment, the pots were filled with a uniform bulk media mixture containing two parts field soil (collected from 0-20 cm deep and screened to remove stones), sand, and leaf mold. Each pot was subsequently supplemented with 1.4 mg N, 6.9 mg P, and 12.8 mg K that were thoroughly mixed into the potted media before the seedlings were transplanted. The soil mixture was subsequently fertilized with 1 g per pot using Plant-Prod 20-20-20 water soluble fertilizer (Master Plant Prod, Inc., U.S.A.)

Figure 1. Oregano source locations in Iran.



Populations	sampling collection area	Longitude (E)	Latitude (N)	Altitude (m)	Habitat
Gilan	Gilan province Roudbar	49°29′	36°51′	177	Mountain-semi humid
Ardabil	Ardabil provinceArdabil	48°15′	38°20′	1320	Mountain-semiarid
Naim	Ardabil province-Aliabad	48°30′	38°24′	1370	Mountain-semiarid
Kaleybar	East Azerbaijan province-Kaleybar	47°08′	38°54′	1360	Mountain-semiarid
Arasbaran	East Azerbaijan province-Arasbaran	46°53′	38°57′	950	Woody-semiarid
Baneh	Kordistan province-Baneh	45°59′	36°06	1750	Mountain-semiarid

The pots containing the seeds were placed in a glass greenhouse at the University of Tehran for germination and seedling growth under natural daylight and a maximum day temperature of 32.5 ºC and night temperature of 17.5ºC. All seeded pots were labeled with voucher numbers for positive identification. Observations of plant characteristics and origins of the studied populations were recorded.

Water stress. Water stress conditions were initiated at field capacity (100% FC), mild water stress (60 ± 5% FC), and moderate water stress (40% ± 5% FC) two weeks after transplanting the oregano seedlings into pots. The soil moisture conditions were maintained by a daily weighing of pots and following the procedure of adding replacement water outlined by Azizi et al. (2009). Briefly, nutrient and water leachate were

collected in a dish under each pot and returned to the potted soil along with any additional water needed to bring the pots to their original weight.

The percentage soil water content, calculated according to the procedure of Talebi et al. (2013), was confirmed 24 h after watering. A 50g soil sample collected from a randomly chosen pot under each water stress condition was oven dried at 100ºC and reweighed. Any additional water determined as necessary to bring the soil to the designated stress moisture content was added to all pots within that stress level (Formula 1).

Formula 1. Soil water content.

$$\% \text{ Soil water} = \frac{\text{Fresh soil weight} - \text{Dry soil weight}}{\text{Fresh soil weight}} \times 100$$

Plant growth. The effect of water stress on plant growth and development was measured on six plants randomly chosen from each oregano population at each water stress level. The plants were harvested at full bloom by cutting the stem 6 cm above the growing media and weighed to determine fresh weight. Subsequently, 20 fully expanded middle leaves were removed from each harvested plant, and the leaf area was determined using a commercial leaf area meter (Delta-T, Cambridge, England). All vegetative plant tissue, including the remaining and removed leaves plus stem tissue, were subsequently dried in the shade for five days. The ratio of plant leaves to stem tissue was determined by comparing dried leaf weight to dried stem weight.

Relative water content (RWC) was determined on a second set of oregano plants following the procedure outlined by Turner (1986). From each oregano population in the water stress treatments, ten fully extended leaves were removed from the plant stem, weighed and then floated on double distilled water for 5 h at 4ºC to ensure turgidity of the leaf tissue. The turgid leaves were quickly weighed and then oven-dried at 70ºC for 72 h and reweighed for a dried leaf weight. The dried leaves were then soaked in distilled water for 24 h and weighed, followed by drying at 105ºC for 24 h and reweighed to determine the RWC of the leaves (Formula 2).

Formula 2. Relative water content of leaves.

$$(\%)\ RWC = \frac{\text{Fresh leaf weight} - \text{Dried leaf weight}}{\text{Water soaked leaf weight}} \times 100$$

Electrolyte leakage. Electrolyte leakage was measured according to the method of Nayyar (2003), using the youngest fully expanded leaves collected from water-stressed, flowering oregano plants. The collected leaves were thoroughly washed with double distilled water, and ten rectangular leaf disks (2 mm x 4 mm) were cut from the center of each leaf blade. The leaf disks were placed in clean glass vials filled with 20 mL of double distilled water as a bathing and ion collection media. The vials containing the leaf tissue were subsequently shaken for 3 h to uniformly distribute any leaked ions throughout the collection media. Initial electrolyte leakage (EC_1) from the leaf tissue samples was measured using a conductivity meter.

The leaf samples were subsequently heated at 121ºC for 15 min to degrade cellular membranes and cause full leakage of ions into the bathing solutions so that differences among plant populations and water stress levels could be determined (Nayyar, 2003). The electrical conductivity (EC_2) of the bathing solution was re-measured to determine total electrolyte leakage (Formula 3).

Formula 3. Determination of electrolyte leakage.

$$\% \text{ Electrolyte leakage} = \left(\frac{\text{Initial EC}}{\text{Final EC}}\right) \times 100$$

Chemical analyses. To determine the effects of water stress on plant chemistry, four replicate samples of each water-stressed oregano population were analyzed for chlorophyll, carotenoids, and proline content using the youngest, fully expanded leaves from flowering plants within each water-stressed population. The removed leaves were extracted with dimethyl sulfoxide (DMSO), and the concentrations of chlorophyll a, chlorophyll b, and total carotenoids in the extract were determined spectrophotometrically at 645 nm, 663 nm, and 430 nm (Bacelar et al., 2007, Hiscox and Israelstam, 1979).

Proline content of the leaf tissue was determined using a ninhydrin method according to Bates et al. (1973) with minor modifications. Briefly, 500 mg leaf samples from the leaves of flowering plants, were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid. The homogenate was then centrifuged at 12,000 g for 10 min at 4ºC, and a 2 mL sample of supernatant was boiled with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid in a test tube for 40 min. The reaction was terminated in an ice bath and the reaction mixture was extracted with 4 mL of

toluene. The absorbance of the extract was measured in a spectrophotometer at 520 nm for comparison with standard concentrations of proline as a measure of proline per g fresh weight (Zhang et al., 2010).

Lipid peroxidation. Lipid peroxidation was measured by analysis of malondialdehyde (MDA) according to the procedures of Estrada et al. (2013). Briefly, leaf tissues from three replicate samples collected from the youngest fully expanded leaves of each oregano population under each waster stress, were ground with a mortar and pestle in 4 mL of 50 mM phosphate buffer (pH 7.5). The ground tissue was then filtered through Whatman No. 1 filter paper and centrifuged at 14,000 g for 20 min to separate the supernatant from the leaf residue. After separation, 0.4 ml of the supernatant was added to 1.2 mL of reaction mixture containing 20% (v/v) trichloroacetic acid (TCA) and 0.5% 2-thiobarbituric acid (TBA). The mixture was incubated at 100 ºC for 30 min to form MDA and then quickly cooled to stop the reaction.

The cooled mixture was centrifuged at 10,000 g for 10 min to separate any precipitate from the supernatant. The supernatant was then measured spectrophotometrically at 532 nm. The amount of lipid peroxidation was expressed as nanomoles of MDA formed using an extinction coefficient of 155 nmol cm^{-1} (Heath and Packer, 1968).

Antioxidants and protein content. The antioxidant activity and protein content of the oregano populations under each water stress were measured on the youngest, fully-expanded leaf of flowering plants. The leaves were removed from the plants and immediately frozen in liquid nitrogen for storage at −80°C. For enzymatic assays, 200 mg of the frozen tissue was extracted in 3 mL of extraction buffer (50 mM potassium phosphate, pH 7.5; 2 mM EDTA; and 1% polyvinylpyrrolidone), according to the procedures outlined by Stancheva et al. (2010). The homogenate was centrifuged at 15,000g at 4°C for 30 min to separate supernatant from tissue residue. Total soluble protein content of the enzymatic extracts was determined according to the procedures outlined by Bradford (1976) using BSA as a standard.

Superoxide dismutase (SOD; EC 1.15.1.1) activity was based on the method of Beauchamp and Fridovich (1971). One unit of SOD activity was defined as the amount of enzyme required to produce a 50% reduction in nitro-blue tetrazolium (NBT) at 560 nm. For the assay, 100 µL of supernatant, separated from tissue residue as previously described, was added to 2.9 mL of reaction mixture that contained 50 mM potassium phosphate buffer (pH 7.8), 75 µM NBT, 12 mM L-methionine, 0.1 mM EDTA, and 2 µM riboflavin. The reactions were maintained at 25 ºC, for 10 min under cool white fluorescent light (100 $\mu mol^{-12}s^{-1}$), and the absorbance was measured at 560 nm. The specific enzyme activity was expressed as unit mg^{-1} protein for each plant selection and water stress treatment.

Catalase (CAT) and ascorbate peroxidase (APX) activities were determined according to the methods of Turkan et al. (2005) with minor modifications. Briefly, CAT activity was determined by monitoring the decomposition of H_2O_2 at 240 nm for 1 min in a reaction mixture containing 50 mM K-phosphate buffer (pH 7) with 1 mM EDTA and 3% H_2O_2. The decrease in the absorption due to the loss of H_2O_2 per min was defined as one unit of CAT. APX activity was assayed by monitoring the oxidation of ascorbic acid at 290 nm for 30 sec. The reaction mixture contained 0.5 mM ascorbate (0.4 mg ascorbate + 0.01 mM Na_2EDTA in 50 mL buffer) in 50 mM K-phosphate buffer (pH 7), and H_2O_2 (3%). One enzyme unit was defined as a µmol mL^{-1} oxidized ascorbate per min.

POD was assayed using four replicate samples of the youngest fully expanded leaf from each plant population, following the procedure outlined by Polle, et al. (1994). Guaiacol was used as a base to measure the oxidation through an increase in absorbance at 470 nm over 1 min. The reaction mixture, contained 200 mM guaiacol in 50 mM K-phosphate buffer (pH 7), and H_2O_2 (3%), with POD activity is defined as the production of µmol mL^{-1} H_2O_2 per min.

Statistical analysis. Analyses were done using a randomized complete block design. All data were subjected to analysis of variance (ANOVA) followed by an LSD test with $P < 0.05$ as the significant cut-off. Mean values are presented with the standard errors. Pearson Correlation was investigated with IBM SPSS Statistics 23.0 (SPSS, Chicago, IL, USA).

RESULTS

Water deficit and plant growth. Oregano populations growing under the mild and moderate water stresses had significantly reduced growth in all six populations (Figure 2 and Table 2). Fresh weight, dry weight, and leaf weight of the plant selections were significantly decreased under water stress in all

populations as compared with oregano not under water stress. The decreases in fresh (leaf and inflorescence) and dry weights of the populations Arasbaran, Gilan, and Kaleybar were more severely affected by water stress than the other three water-stressed populations (data not shown).

Figure 2. Fresh weight of oreganos under water stress.

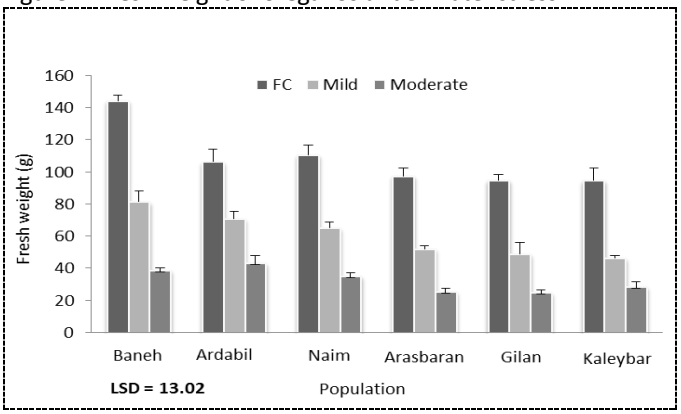


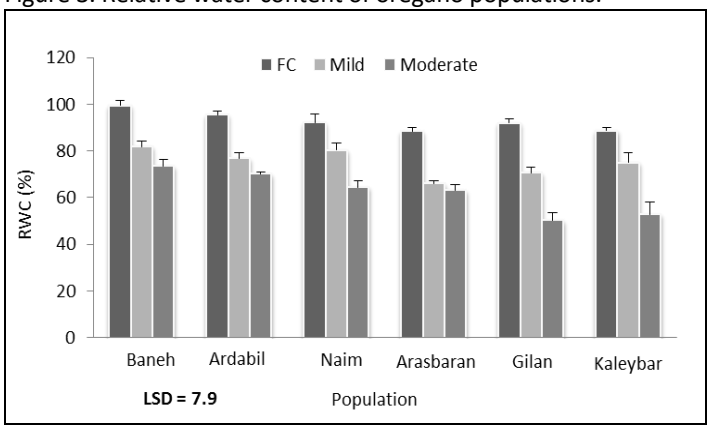
Table 2. Mean oregano plant growth response to water deficit.

Irrigation treatment	Dry weight (g)	Leaf & inflorescence (g)	Leaf area (mm^2)	Specific leaf area (mm^2/g)
Field capacity	27.40 ± 1.45	18.50 ± 0.65	315.50 ± 16.95	19871.83 ±668.50
Mild	20.00 ± 1.06	13.64 ± 0.56	227.57 ± 17.48	13136.43 ±733.80
Moderate	11.13 ± 0.80	7.37 ± 0.34	163.19 ± 14.50	12958.82 ±210.15
LSD**	1.53	0.99	18.60	980.11
CV*	14.39	16.11	16.94	13.38

Of the plant populations, Baneh oregano had the highest fresh weight under the well-watered and mild-water stress conditions. Leaf area and specific leaf area were decreased in the oregano populations under mild and moderate water stress, although the population of Naim oregano under water stress had a smaller loss of leaf area as compared with the other studied oregano populations.

Water stress and leaf water content. The relative water content in leaves was significantly reduced by water stress conditions in all six populations (Figure 3). In the mild water stress, leaves on the Arasbaran oregano population had the largest decrease in RWC. Under moderate water stress, the leaves of Gilan and Kaleybar oregano populations exhibited a more rapid decrease in RWC as compared with the other studied oregano populations.

Figure 3. Relative water content of oregano populations.



Water stress and plant pigments. While the mild water stress did not appear to affect chlorophyll a, chlorophyll b was reduced by 37% under the same water stress condition. The decrease in chlorophyll b at the mild water stress level caused a significant decrease in total chlorophyll. The moderate water stress significantly decreased all chlorophyll levels in all the tested oregano plants. The decrease in chlorophyll led to a relative increase in carotenoid content.

Water stress, proline, and cell damage. Proline content was significantly increased in all six oregano populations under mild and/or moderate water stress Figure 4). The accumulation of proline in the plant tissue brought different responses to the drought intensities with the Ardabil population exhibiting high proline content under both mild and moderate water stress, while the Baneh population exhibited a high proline accumulation under a moderate water stress. MDA content and electrolyte leakage occurred in all six oregano populations, increasing as the drought stress intensified (Figures 5 and 6).

Table 4. Proline production in water stressed oregano.

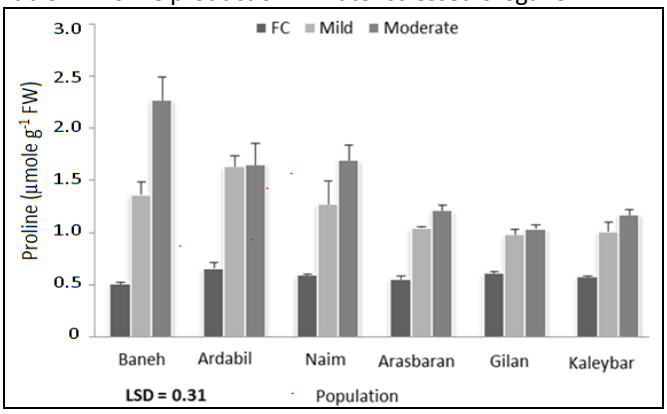


Figure 5. MDA accumulation in water stressed oregano.

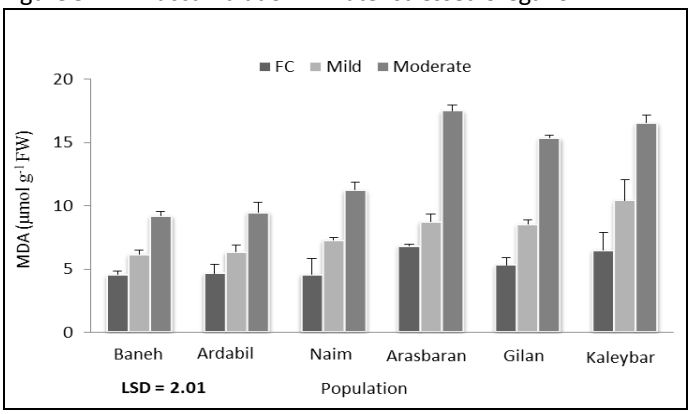
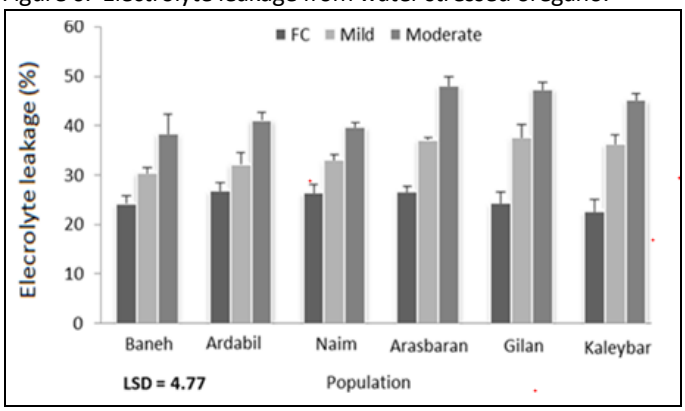


Figure 6. Electrolyte leakage from water stressed oregano.



Populations of Arasbaran, Gilan, and Kaleybar exhibited a larger increase in both MDA content and electrolyte leakage under moderate water stress, but the populations of Baneh and Ardabil exhibited smaller increases in MDA content as compared with the other four populations. Populations of Baneh, Ardabil, along with Naim, had smaller increases in electrolyte leakage as compared with the other three populations under mild and moderate water stress.

Water stress and antioxidant enzymes. Except for the Arasbaran population under the mild stress, the prolonged water stress increased SOD activity in all six populations of oregano as a function of water stress intensity. The populations of Baneh and Ardabil exhibited large increases in SOD activity under moderate water stress (Figure 7). In contrast, SOD activity did not increase in the oregano population Gilan under a moderate water stress.

CAT activity increased in all six populations under moderate water stress, and the population Naim had the highest activity of CAT under prolonged water stress. No significant differences in APX activity was observed under water stress conditions. Water stress, however, increased the activity of POD in all six oregano increased the activity of POD in all six oregano populations (Figure 8). POD activity was highest in the oregano populations of Baneh and Arasbaran under a mild water stress and the populations of Baneh, Ardabil, and Naim under a moderate water stress.

Figure 7. SOD activity in water stressed oregano.

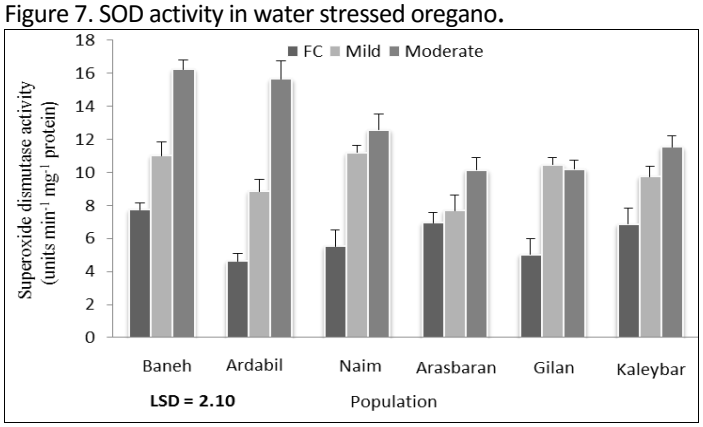
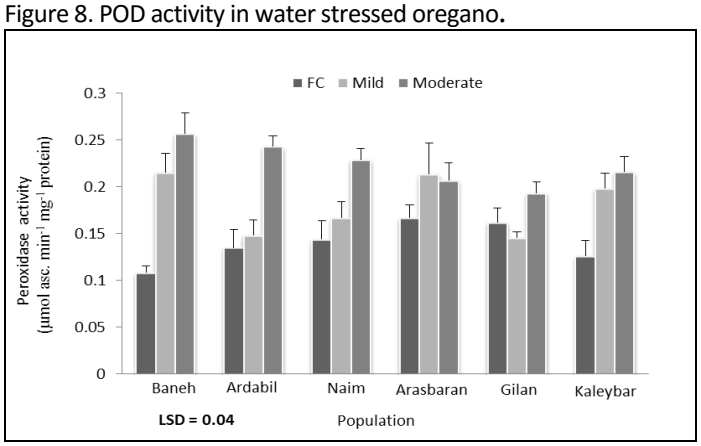


Figure 8. POD activity in water stressed oregano.



Correlations among treatments. Significant correlation coefficients were observed among indications of water stress, including a positive correlation between proline content and SOD activity (r = 0.91), proline content and dry weight, (r = 0.953), and proline content and RWC (r = 0.955). In addition, SOD was positively correlated with POD (r = 0.890), RWC (r = 0.823), and fresh weight (r = 0.922) of the oregano populations. MDA and ion leakage were negatively correlated with SOD activity, with fresh and dry weights, and with RWC in the oregano populations. In addition, total chlorophyll was positively correlated with total carotenoids and APX activity in the oregano populations, while the RWC was negatively correlated with MDA and electrolyte leakage.

DISCUSSION

The importance of oregano as a popular medicinal and flavoring crop is recognized throughout the world. The oregano plant is frequently used as a carminative, diaphoretic, expectorant, stimulant, tonic, antiviral, and antifungal agent among other medical applications (Charles, 2012; Mozafarian, 2012). In addition, the food industry uses oregano to flavor number foods, including processed meats, salads, soups, omelets, fish, pizza, processed meats, salads, soups, omelets, fish, pizza, and other eatables (Charles, 2012).

Due to limited rainfall in Middle Eastern countries, such as Iran, oregano production is frequently limited by dry conditions. Selection and development of oregano being able to withstand and produce a crop under water stress conditions would enhance production, making oregano a profitable crop for a number of Iranian and other Middle Eastern farmers. In current study, oregano populations from various environmental areas of Iran differed in the ability to withstand water stress, suggesting genetic variability among oregano populations enable some collections to survive and grow with only limited amounts of water. The current study demonstrated that oregano populations from dryer environmental areas were better able to withstand water stress as compared with plants originating in more humid areas.

Stress resistant oregano populations exhibited less leachate and MDA, but higher levels of proline and antioxidant enzyme activity as compared with populations susceptible to water stress. The metabolic differences support the concept that plant selection and breeding could be used to enhance oregano production in dry areas of Iran and other water stressed environments.

Oregano plants that tolerate osmotic stresses have apparently evolved mechanisms similar to other plants that survive and grow under water stress, including early flowering and structural traits, such as reduced leaf area and plant height, physiological adaptations such as stomatal closure and salt exclusion, and metabolic responses, such as photosynthetic alterations and proline accumulations (Delauney and Verma, 1993). In the current oregano study, a water deficit slowed vegetative growth, reducing plant canopy and biomass as an adaptive strategy to limit water loss to the atmosphere via transpiration, an adaptation similar to that observed in water stressed *Artemisia annua* (Talebi et al., 2013), barley (*Hordeum vulgare*) (Samarah, 2005), oregano (*Origanum vulgare*) (Azizi et al., 2009) and other plants (Farooq, et al., 2009).

While the growth in all the tested oregano populations were reduced under the mild and moderate water stress as compared with plants growing in media with water at field capacity, physiological differences apparently enabled the oregano populations Baneh, Ardabill, and Naim to maintain more growth under water stress than the populations Arabaran, Gilan, and Kaleybar.

Water stress reduces the relative water content (RWC) of leaves and is generally accepted as a reliable method to measure the state of osmotic stress and frequently used to evaluate the level of water balance in plants (Uziday et al., 2012). In addition, RWC is commonly used for the measurement of plant water status in terms of the physiological and biochemical consequences of water deficit in plant cells (Barrs and Weatherley, 1862). Plants with a higher tolerance to water stress often have a higher level of RWC (Arjenaki et al., 2012). In the current study, populations of Baneh and Ardabil had a relatively higher RWC under mild and moderate water stress compared to other oregano populations.

As an oxidative plant stress, the lack of water frequently causes a reduction in plant chlorophyll and carotenoid content (Farooq et al., 2009) due to a decreased rate of synthesis or an increased rate of constituent breakdown (Smirnoff, 1993). According to Misra and Srivastava (2000), water stress results in a significant reduction in fresh and dry weight, chlorophyll, and carotenoids in Japanese mint (*Mentha arvensis* L.). In contrast, our results showed that the amount of chlorophyll a significantly increased in oregano under mild water stress, but decreased under moderate water stress. Similarly, Pirzad et al. (2011) reported that the greatest amounts of chlorophyll a were obtained under mild water stress in *Matricaria chamomilla* L. Our investigations with oregano populations noted that chlorophyll b and total chlorophyll significantly decreased under mild and moderate water stress. In a majority of plant species, water stress decreases the level of chlorophyll a, chlorophyll b, and total chlorophyll (Talebi et al., 2013; Pirzad et al., 2011; Liu et al., 2011). Under stress conditions, the loss of chlorophyll content cold is expected to reduce photosynthesis (Talebi et al., 2009).

As water stress was intensified in the oregano populations. The ratio of chlorophyll a to chlorophyll b and the ratio of carotenoid to total chlorophyll significantly increased. The effect has also been noted in some woody plant species (Liu et al., 2011). An increased ratio of chlorophyll a to chlorophyll b under water stress conditions could be due to a decrease in peripheral light-harvesting complexes and light collection in relation to the rate of PSII photochemistry (Demmig-Adams and Adams, 1996). Carotenoids, which are known to play an important role in photo-protection of the photosynthetic apparatus from photo-oxidative damage to tissues (Young and Britton 1990; Moran et al. 1994; Demmig-Adams and Adams, 1996), could also promote photosynthesis in stressed oregano by transferring light energy to chlorophyll (Taiz and Zeiger, 2006).

Proline, as a potent antioxidant and potential inhibitor of programmed cell death, plays a key role in plant tolerance to water stress (Gill and Tuteja, 2010). Accumulation of proline in oregano populations under water stress, correlated with reduced water stress. The response was similar to observations on other plants, such as *Achillea tinctoria* (Shanjani et al., 2014) and wheat (*Triticum aestivum* L.) growing under water stress conditions (Khoshro et al., 2013), suggesting the presence of proline reduces water stress in various plant species. In the current research with water-stressed oregano, the populations Baneh, Ardabil, and Naim accumulated more proline than other populations and had more water stress tolerance.

Lipid peroxidation, a widely used marker of oxidative damage (Xu et al., 2008; Uzilday et al., 2012), leads to ion leakage and increasing MDA content, important indicators of cell membrane breakdown in plants under stress conditions (Uzilday, et al. 2012; Gill and Tuteja, 2010; Xu, et al. 2008). In the current study, ion leakage and MDA content were relatively low in normal, non-stressed oregano plants, but increased as water stress intensified from a mild to moderate level, indicating loss of cell stability and viability (Masia et al. 2003; Xu et al., 2008).

As water stress increased, electrolyte leakage and MDA content increased in all six populations of oregano tested. The increase, however, was smaller in the populations of Baneh, Ardabil, and Naim compared with other oregano populations subjected to a moderate water stress, demonstrating that these three oregano populations were more tolerant to water stress than the other studied populations. Ying et al. (2015) have reported large increases in MDA content in two populations of *Camptotheca acuminate* under water stress. Stress sensitive species exhibit a sharper increase in lipid peroxidation than regular species under water deficit stress (Liu et al., 2011; Khoshro et al., 2013; Shanjani et al., 2014).

Our results demonstrated that the ROS scavenging in Iranian oregano plants may contribute to the enhancement of SOD, POD, and CAT activity in response to oxidative stress. The most tolerant water stress oregano populations, Baneh and Ardabil, had significant increases in the antioxidant enzyme activity under both the mild and moderate water stress conditions. As compared with regular cultivars of *Phaseolus acutifolius*, higher levels of SOD, CAT, POD and APX activity have been noted in drought-tolerant cultivars of *Phaseolus acutifolius*, reducing lipid peroxidation as compared with drought-sensitive cultivars (Turkan et al., 2005). Among plant defense systems, SOD is recognized as one of the most protective antioxidant enzymes used against oxidative conditions, such as water stress (Uzilday et al., 2012).

The current study demonstrated differences in water stress tolerance among the tested populations of oregano. Those oregano plants with significant SOD and POD activity (Baneh, Ardabil and Naim) had protection against oxidative stress caused by lack of water. For example, the population of Naim oregano exhibited smaller increases in MDA and electrolyte leakage and higher fresh weight and dry weight under moderate water stress as compared with the other investigated oregano populations. The positive correlation between proline content and antioxidant activities, similar to those observed in oregano, has been reported in earlier studies (Turkan et al., 2005; Ahmed et al. 2009; Liu et al. 2011), suggesting that proline accumulation could activate the antioxidant defiance systems in various plants. Ozden et al. (2009) have proposed that proline may have a direct positive effect on antioxidant enzyme systems.

The positive correlations of proline with SOD and POD activity in the investigated oregano populations suggest that plant selection and breeding programs for higher levels of antioxidants in oregano could produce plants tolerant to water stress environments.

CONCLUSION

Cultivation of oregano, a traditional, Iranian culinary and medicinal crop, is severely limited due to a lack of water needed to produce satisfactory yields. To test if Iranian oregano sourced in different bioclimates could maintain or increase yields when transferred to water stressed areas, six populations of wild oregano were grown under three water regimes, field moisture capacity, mild water stress, and moderate water stress. Oregano sourced from dry areas proved more resistant to water stress than oregano sourced in other areas. Those populations of wild oregano tolerant to water stress exhibited more antioxidant enzyme activity and proline content than populations susceptible to water stress. Oregano tolerant to water stress had higher ratio of chlorophyll *a* to chlorophyll *b* and ratio of carotenoids to total chlorophylls that enabled the plants to maintain photosynthesis and limit photo-oxidation. A positive correlation was also observed between proline content and the activity of SOD, POD, RWC, fresh weight, and dry weight, while a negative correlation was observed between cellular damage and SOD activity and proline. Oregano collections of Baneh, Ardabil, and Naim tolerant to water stress exhibited higher SOD and POD activity, as well as higher proline content under water deficit stress along with smaller increases in ion leakage and MDA content. The presence of plants with higher antioxidant capacity and more water stress tolerance in oregano populations offers the possibility of incorporating desirable traits into domesticated oregano through the selection and breeding programs.

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