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Effects of drought stress and manure on relative water content and cell membrane stability in dragonhead (*Dracocephalum moldavica*)

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Abstract

In the current study, the effect of water deficit stress or drought on relative water content and cell membrane stability of dragonhead (*Dracocephalum moldavica*) was studied in a greenhouse experiment carried out at Islamic Azad University, Jiroft branch, in 2009. It was a split plot experiment based on Randomized Complete Block Design with three replications, in which vertical factor included three levels of drought stress (irrigation when soil moisture reached 75% of field capacity [mild stress], irrigation when soil moisture reached 50% of field capacity [moderate stress] and irrigation when soil moisture reached 25% of field capacity [severe stress]). The results showed that the effect of water deficit stress was significant on relative water content at 5% level and on cell membrane stability at 1% level. Water stress significantly decreased relative water content from 77.69% under mild stress under severe stress. Relative water content increased as manure level increased. As the stress was intensified, the electrolyte leakage increased, but it started to decrease under severe stress. The manure treatment of 40 t/ha had the greatest electrolyte leakage (328.89 $dS.m^{-1}$).

Keywords: Dragonhead; drought stress; manure; relative water content; electrolyte leakage.

Introduction

Medicinal herbs have been extensively studied in this century mainly because chemical medicines have proved to have side effects and humans tend to use natural products as much as possible (Azizi, 2000). Dragonhead or dragon's-head (*Dracocephalum moldavica*) is herb from mint family (Hussein *et al.*, 2006). The effective substances of its body are sedative and appetizing. Its essence is antibacterial and is used in curing stomachache and flatulence as well as in food industries, soda manufacturing and health and make-up industries (Omidbeigi, 1997). Although the effects of drought stress on crops have been extensively studied, the researches on the behavior of medicinal and aromatic herbs under water deficit have not been so extensive (Letchamo and Gosselin, 1996).

Nowadays water deficit is known as an important limiting factor of yield increase in arid and semiarid regions and growth decrease is much greater under water deficit than that under other environmental stresses (Rodrigues, 2006). It is more important in regions which experience the problem due to climate change but have not been paid attention (Chaves and Oliveira, 2004) because global environment change programs show the growth of water deficit in future and the recurrence of much more severe events in most parts of the world. Environmental stresses bring about a wide range of responses in plants from genetic changes to the changes in growth speed and yield (Reddy *et al.*, 2004). Therefore, in order to understand the conditions for the survival of medicinal herbs in arid regions, their responses

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to water deficit need to be evaluated and their appropriate growing conditions should be determined (Letchamo and Gosselin, 1996).

Levitt (1980) suggests measuring the accumulation of soluble sugars, relative water content (RWC) or leaf water potential (LWP), cell membrane stability (CMS), accumulation of minerals and saturated and unsaturated fatty acid content in various plants under environmental stresses.

Blum *et al*., (1999) reported that plant RWC was the best criterion for measuring plant water status among parameters like plant RWC, plant water potential (PWP) and turgor potential. Several studies have been carried out about measuring RWC and drought resistance in different plants, all of which have shown the decrease in RWC with the increase of water deficit stress [e.g. in wheat (Rezaie and Borzooei, 2006), barley (Dadashi, 2006), rice (Lakshmi *et al*., 2005) and faba bean (Khan *et al*., 2007)].

In a study on the effects of dew and drought stress on medicinal herb *Melissa officenalis* L., Munne and Alegre (1999) found that drought stress decreased PWP by 3 MPa and RWC by 34%. Also, Munne *et al*. (1999) reported that drought stress decreased RWC of *Rosmarinus officinalis* L. by 40% and that of *Melissa officenalis* L. by 30% but increased essence percentage and soluble sugars content.

Abbaszadeh *et al*. (2007) reported the decrease in RWC of *Melissa officenalis* L. with the increase in the severity of drought stress so that no-stress treatment had the highest RWC (93.369% on average) and most-severe-stress treatment had the lowest one (3[illegible].75%) which was 25% of field capacity.

In a study on the metabolic changes due to drought stress in medicinal herb *Saturej hortensis* L., Bahernik *et al*. (2004) reported that the decrease in irrigation and consequently, the increase in potential water capacity decreased leaf RWC so that RWC in no-stress treatment to light-stress treatment (2/3 of field capacity) in vegetative growth period and flowering stage and severe-stress treatment in flowering stage was 65.30, 34.18, 29.18, 29.13 and 18.13%, respectively.

Hassani *et al*. (2003) reported the decrease in RWC from 77.72% in the 100%-stressed treatment to 69.05% in 55%-stressed treatment in basil.

Since membranes play critical role in different cell activities, particularly those membranes which have enzymes and water and ion transporters, pressure or stress on them is one of the most important effects which water deficit have on plant survival; a stable cell membrane which can function well under water deficit conditions play a pivotal role in adaptation to high temperature and resistance to drought (Chaves and Oliveira, 2004).

In a study on corn under water deficit condition, Valentovic *et al*. (2006) found that electrolyte leakage was used for stabilizing cytoplasm membrane; the stronger the electrolyte leakage, the weaker the membrane stability. They reported that in drought-resistant corn cultivar Nova, electrolyte leakage in leaf was 8% in stress treatment and 5% in no-stress treatment, whereas in drought-sensitive cultivar Ankora, leaf electrolyte leakage in stress and no-stress treatments was 15 and 5%, respectively.

Saneoka *et al*. (2004) reported that CMS can be used as a tool in measuring resistance to environmental stresses like drought and that by applying nitrogen fertilizer, drought stress increased CMS in bent compared with no-stress conditions.

By applying 45 t manure/ha on soybean, Ro[illegible]moosavi *et al*. (2007) reported that this crop produced the highest leaf moisture content in response to the fertilizer. At the end of the flowering stage, CMS improved due to intensive moisture stress and increased from 70.14% under optimum irrigation to 76.22% under severe stress.

In a study on the effects of water stress as well as applying natural zeolit, which acts like a manure, on quantitative and qualitative characteristics of dragonhead, Gholizadeh *et al*. (2006) reported that the interaction between zeolit and different levels of soil moisture depletion significantly affected leaf area, aerial dry weight, root dry weight, leaf number and essence percentage. Also, when the yield of dragonhead was evaluated, the application of 25 g zeolit/12 kg soil as well as 50% soil moisture depletion had produced the highest dry matter (2.767 g/plant) and 2% essence.

In a study on the effects of water deficit stress on yield and essence of dragonhead, Hassani (2006) reported that the biomass decreased from 233 g in no-stress treatment to 112.5 g in severe stress treatment, but essence reached from 0.34 ml of dry matter to 0.35 ml in moderate stress treatment.

In a study on the effects of drought stress on yield and morphological traits of dragonhead, Safikhani *et al*. (2007) found that the yield of branches with flowers decreased from about 4126 kg/ha in FC 100% treatment to 2477 kg/ha in FC

Table 1. Results of the analysis of soil used in experimental pots

Depth (cm)	pH	EC ($ds.m^{-1}$)	SP (%)	Total N (%)	AWP (ppm)	AWK (ppm)	Texture
0-30	8.1	0.89	25	0.03	12	220	Loamy sand

40% treatment.

Anomalous application of chemical fertilizers brings about environmental problems, too. Application of organic fertilizers such as manure along with chemical fertilizers can improve crop yield sustainability in addition to decreasing chemical fertilizer application (Khajueinejhad *et al*., 2004). Manure application is a method for avoiding soil moisture decrease. Despite the popularity of herb cultivation in different parts of Iran, little information is available about their responses to stresses. Therefore, the current study was carried out to investigate the effects of drought stress and manure on water relations and cell membrane stability.

Materials and Methods

To study the effects of manure application on dragonhead and to evaluate its resistance to drought stress as well as to study cell water relations and the physiology of drought resistance in the crop, a strip plot experiment was carried out based on a Randomized Complete Block Design with three replications as a pot experiment in the greenhouse of Islamic Azad University, Jiroft Branch, Iran in 2009. In this study, low irrigation by applying water stress in three levels – mild stress (irrigation at field capacity of 75%), moderate stress (irrigation at field capacity of 50%) and severe stress (irrigation at field capacity of 25%) – constituted the vertical factor and manure application in five levels of 0, 10, 20, 30 and 40 t/ha constituted the horizontal factor.

Firstly, the soil was sampled and its physical and chemical parameters were measured (Table 1). The pots were 28 cm high with the diameter of 30 cm. Each one was filled with about 10 kg soil on average. Ten pots received enough water to become saturated. They were covered by plastic sheet and after 24 hours when the redundant water leaked from the bottom hole due to gravity, their soils were sampled and dried in oven for 24 hours at 105°C. Then, the field capacity of the pots was determined. Manure application level was determined according to pot level. After weighing, cattle manure was used in fertilizer treatments. After preparing the pots, the seeds were planted with the rate of 15 seeds/pot at the depth of 0.5-1 cm. After emergence, the plants were thinned twice a month. Finally, four plants were left in each pot.

Since the fifth week (when plants had 8-12 leaves), water deficit treatment commenced. To determine irrigation time (stress treatments), moisture meter was used so that when it showed that the pot reached the intended field capacity, it was irrigated. To measure RWC of leaves, a sample leaf was selected and immediately weighed (fresh weight). Then, it was kept in aqua pura for 2-5 hours. Afterwards, it was dried and weighed by filter paper (turgid fresh).

Next, it was put in oven for 24 hours at 80°C. Afterwards, it was weighed and finally, its RWC was measured by the following formula (after Mata and Lamattina 2001):

$$RWC(\%) = \frac{FW - DM}{TW - DM} \times 100$$

where, FW, DM and TW are leaf fresh weight, leaf dry weight and leaf turgid weight, respectively.

To measure cell membrane stability, cell electrolyte leakage was measured. Plant sample was selected by punch (about 20 circular discs) and were put in aqua pura for 24 hours. Then, the electric conductivity (EC) of sample water was measured. In addition, aqua pura was used as control. Then, EC of aqua pura was subtracted from ECs of samples. Finally, all data were statistically analyzed by software MSTATC and treatments were compared by Duncan Multiple Range Test.

Results and Discussion

Soil analysis showed that it was loam-sandy, alkaline and had no limitation from salinity and minerals viewpoint. It was poor in nitrogen and good in absorbable phosphorous and potassium (Table 1).

The results of analysis of variance of the effects of drought stress and manure on cell RWC and cell membrane stability in dragonhead (Table 2) showed that the effect of manure, drought stress and manure × drought stress interaction on RWC was significant at 5% statistical level. It seems that the difference of this parameter was

Table 2. Analysis of variance for the effects of manure and water deficit stress on leaf relative water content and cell membrane stability

Source of variation	df	Means of squares	
		Relative water content	Membrane stability
Replication	2	63.85^{ns}	4656.62^{ns}
Water deficit stress	2	563.24*	48040.02**
Error a	4	64.62	1381.29
Manure	4	218.19*	62294.22**
Error b	8	60.05	4923.87
Manure × water deficit stress	8	175.85*	38432.94**
Error c	16	61.16	1841.41
C.V. (%)		10.91	22.13

*,** and ^{ns} show significance at 5 and 1% and non-significance, respectively.

caused by treatments with the probability of 95%.

Moreover, the effects of manure and water deficit stress and their interactions were significant on cell membrane stability at 1% level, and the differences of cell membrane stability were brought about by treatments with the probability of 99% (Table 2).

RWC decreased with the increase in water deficit intensity, so that the highest RWC was 77.69% under mild stress which decreased to 65.44% under severe stress (Fig. 1). No significant differences were observed in RWC between mild and moderate stresses, but it was highly significant with severe water deficit stress. Many researchers believe that the decrease in RWC due to water deficit stress is associated with stomatal closure which is in turn caused by the accumulation of abscisic acid (ABA) produced in roots under drought stress and accumulated in guard cells (Khan *et al*., 2007; Chaves *et al*., 2003). Some others (Chaves *et al*., 2002) believe that RWC values below 70-75% is caused by stomatal closure. It seems that there is a direct relationship

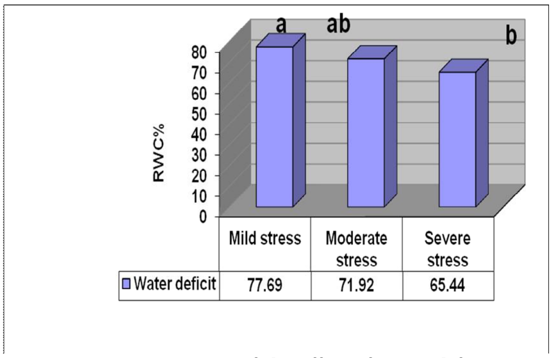

Fig. 1. Means comparison of the effect of water deficit stress on leaf relative water content of dragonhead (LSD 5% = 8.150).

between RWC and soil moisture content, so that RWC decreases with the decrease in soil moisture (under stress). Abbaszadeh *et al*. (2007) found similar results for *Melissa officinalis*, so did Bahernik *et al*. (2004) for savory and Hassani *et al*. ([illegible]) for basil.

As can be seen in Fig. 2, RWC increased with the increase in manure level, so that it was not statistically significant until the third manure level (20 t/ha). Also, there were not significant differences among different manure levels, but a significant difference was observed between 30 and 40 t/ha compared with control, so that application of 40 t manure increased RWC by 12.5% compared with control. RWC was 63.60% under no-fertilizer treatment which increased to 76.06% after applying 40 t manure/ha. Drought stress had a high correlation with soil moisture content (Nautiyal *et al*., 2002).

Root growth retardation and the decrease in its activity as well as the increase in evapotranspiration rate are known as the causes of the decrease in RWC (Tarumingkeng and Coto, 2003).

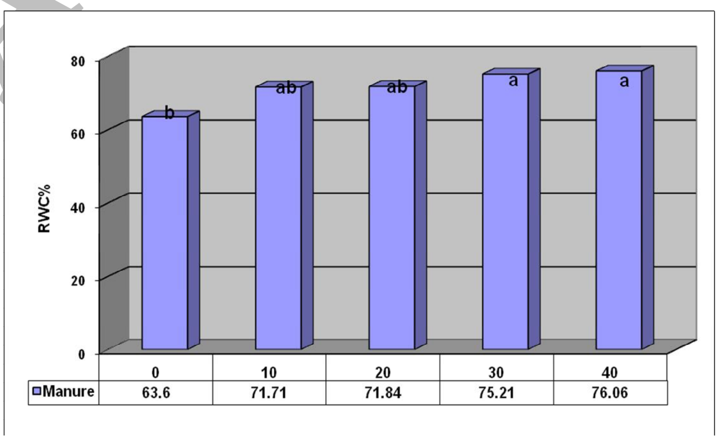

Fig. 2. Means comparison of the effect of manure levels on leaf relative water content in dragonhead (LSD 5% = 8.424).

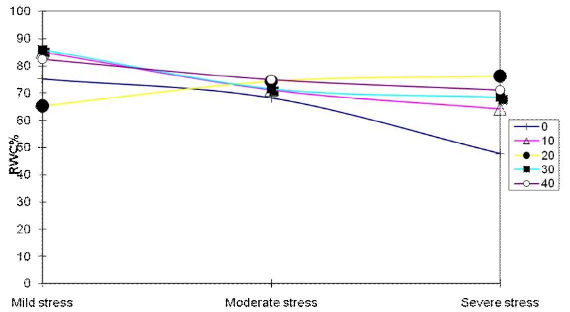

Fig. 3. Means comparison of effect of the interaction between drought stress and manure on leaf relative water content in dragonhead (LSD 5% = 13.54).

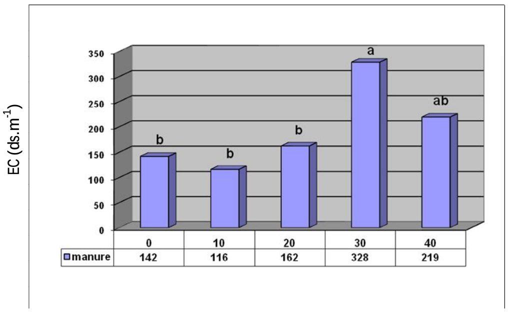

Fig. 5. Means comparison of effect of manure level on cell membrane stability in dragonhead (LSD 1% = 111).

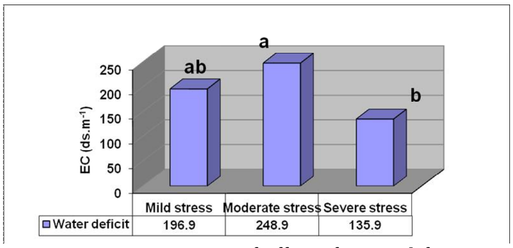

Fig. 4. Means comparison of effect of water deficit stress on cell membrane stability in dragonhead (LSD 1% = 62.48).

Leaf RWC was reportedly increased by the application of nitrogen (Saneoka *et al*., 2004) and potassium (Azizi and Rashed Mohasel, 1998). Therefore, considering the favorable effect of manure on soil water preservation and also because of having high nutrient content, it had positive effects on RWC. Poormoosavi *et al*. (2007) reported the highest RWC of 77.11% in soybean by applying 45 t manure/ha, which is consistent with the results of the current study.

As can be seen in Fig. 3, the highest RWC (85.88%) was obtained under mild stress by applying 30 t manure/ha. The lowest RWC (about 47.73%) was obtained under severe stress with no manure application. The point is that the mild stress with the application of 30 t manure/ha, severe drought stress with the application of 20 t manure/ha, moderate stress with the application 40 t manure/ha and moderate stress with the application of 20 ad 30 t manure/ha did not show significant differences in RWC. As mentioned, manure helps in preserving water in plant. The results are consistent with Poormoosavi *et al*. (2007).

With the increase in water deficit stress intensity, electrolyte leakage, which increased EC, was increased (Fig. 4) which damaged cytoplasm membrane. The EC of mild stress was 196.9 dS.m^{-1} which increased to 248.9 dS.m^{-1} under moderate stress. With further increase in stress intensity from moderate to severe, electrolyte leakage started to decrease again, likely due to the creation of resistance to drought which was consistent with Afsharmanesh (2007) and Poormoosavi *et al*. (2007) and Saneoka *et al*. (2004). Under moisture stress, plasma membrane is one of the first organs damaged (Levitt, 1980), by which cell membrane permeability increases and causes the leakage of electrolytes of cell to outside (Blum and Ebercon, 1980). High EC shows that cells are damaged (Inze and Montago, 1995). Jabbari *et al*. (2006) indicated that cell walls were destroyed under drought stress because stomatal closure under drought conditions decreased carbon dioxide fixation, while photo reactions and electron transfer went on in their normal manner. Under such condition, NADP availability will be limited for electron acceptance. Therefore, oxygen can be an alternative electron acceptor which leads to the accumulation of poisonous oxygen species such as superoxide radicals (O_2^-), peroxide hydrogen (H_2O_2) and hydroxyl radicals (OH). The accumulation of active oxygen species, which are produced under stress, damages many cell compositions like fats, proteins, carbohydrates and nucleic acids (Jiang and Huang, 2001). As a result, fatty peroxides destroy cell membrane (Liang *et al*., 2003).

Fig. 5 shows that with the increase in manure application level, cell membrane stability was improved except in no-manure treatment. Electric conductivity measurement showed that electrolyte leakage was the highest when 30 t manure/ha was applied with no significant difference with the application of 40 t manure/ha. Poormoosavi *et al*. (2007) reported that the damage to cytoplasm membrane under no-drought stress was 70.63% which decreased to 68.19% with the application of 45 t manure/ha. The likely reason, as he concluded, was the improvement of physical and chemical conditions of soil such as water storage capacity with the increase in manure ap-

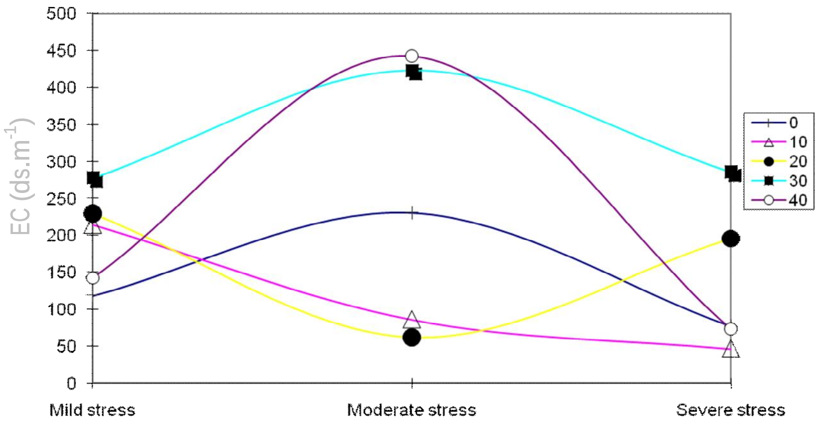

Fig. 6. Means comparison of effect of interaction between manure and water deficit stress on cell membrane stability in dragonhead.

plication level which helped plant not to face such a severe drought and not to invest on the increase in membrane stability. In addition, under favorable growth conditions such as water and nutrient availability, the plants do not tend to invest on integrity of cell walls and they try to provide the conditions for the expansion and growth of cells.

As can be seen in Fig. 6, moderate stress treatment with the application of 40 t manure/ha had the highest electrolyte leakage in terms of EC measurement of 443.0 $dS.m^{-1}$. It did not show statistically significant difference with moderate stress treatment with the application of 30 t manure/ha whose EC was 422.7 $dS.m^{-1}$. But it showed significant differences with other treatments. The lowest electrolyte leakage, i.e. the highest membrane stability, was resulted from the interaction between severe drought stress and the application of 10 t manure/ha with the EC of 46.67 $dS.m^{-1}$.

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