Effect of Water Stress on some Quantitative and Qualitative Traits of Valerian (*Valeriana officinalis* L.) Plants

Seyed Hamid MUSTAFAVI^{*1}, Fariborz SHEKARI², Hamid HATAMI-MALEKI³, Yousef NASIRI³

¹Ph.D Student, Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh- Iran. P.O. Box 55181-83111

² Associate Professor, Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh.

³ Assistant Professor, Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh.

^{*}Corresponding author, e-mail: ha.mustafavi@gmail.com

BulletinUASVM Horticulture 73(1) / 2016

Print ISSN 1843-5254, Electronic ISSN 1843-5394

DOI:10.15835/buasvmcn-hort:11681

Abstract

In order to investigate the effects of water stress on some physiological and biochemical traits of valerian, a pot experiment was conducted using randomized complete block design with 3 replications. Drought stress levels were measured using control plants with 100% water availability, 70%, 50% and 30% of available water. The results showed that water stress significantly affected most biochemical characteristics of valerian plants. Leaf relative water content, chlorophyll a and b contents decreased while carotenoids and electrolyte leakage increased with the increase of water stress. For the ameliorating the adverse effect of water stress, among the investigated defense traits, plants increased proline levels and catalase and ascorbate peroxidase activities. Potassium, zinc and iron content in leaves was gradually increased by decreasing water depletion from 100 to 70% but further increase in drought stress decreased their values compared with control. Plant aerial biomass as well as root biomass was severely inhibited under water stress, but essential oil percentage increased with decreasing the water content from 100 to 50%. From this study, it was observed that lowering the soil water from 100 to 50% AWC is the best irrigation levels for valerian production, but as subterranean organs growth and essential oil accumulation were differently affected by water stress, therefore growers should be careful when increasing the irrigation intervals.

Keywords: *Antioxidant enzymes, cell membrane, essential oil, nutritional status, valerian, water deficit.*

INTRODUCTION

Water deficit is a major constraining plant photosynthesis and productivity in arid and semi-arid areas. Under water stress, growth and function suppression in plants involves many morphological, physiological and molecular changes including oxidative stress, metabolic disturbance either temporarily or permanently (Li et al. 2013). Responses of plants to stress may be revealed at a whole plant level as an integrated tissue system while some of the responses occur at the cellular level. However,

later changes mainly occur earlier than morpho-physiological traits. When the available water is limiting, plants tend to close their stomata (Cornic and Massacci 1996), resulting in reactive oxygen species (ROS) production. The plant cell membrane is one of the first targets of ROS, so maintenance of their integrity and stability under water deficit conditions is a major component of drought tolerance in plants (Bajji et al. 2002). Organelles such as chloroplasts and mitochondria are the main sites of ROS production in the plant cells (Kar 2011), for this reason, photosynthetic

pigments, primary source of plant growth, are most important sites that may adversely affected. From other point of view, under conditions of reduced soil moisture, the stomata may close, transpiration decreases, and thus the water and nutrient flow diminish due to a reduced diffusion rate of nutrients in the soil to the absorbing root surface (Pinkerton and Simpson 1986) resulted in nutritional imbalance. Therefore, Leaf nutritional status and demolition of growth sites (i.e. cell membrane and photosynthetic pigments) are two important events that primarily affected by water stress which lead to the greater changes in morphology and crop growth.

Plants respond to adverse conditions by altering their morphology, physiology, and biochemistry. Plant regulation of water utilization and loss is important in determining the drought tolerance in crop plants. It was also shown that a plant's ability to cope with abiotic stress is mainly related to an altered biochemical profile and produces a varied range of secondary metabolites. Osmotic adjustment and antioxidant enzymes activity are two important defense strategies which help to ameliorating the adverse effect of unfavorable conditions (Farooq *et al.*, 2009). Osmotic adjustment allows the cell to decrease osmotic potential, which attracts water into the cell, thereby minimizing the harmful effects of drought. Soluble sugars and proline play an important role in osmotic adjustment and may protect plants against oxidative stress (Molinari *et al.*, 2007). It was also shown that plants mainly change range of secondary metabolites as another defense system when exposed to stress conditions. Environmental stresses, such as drought stress often increase the accumulation of secondary metabolites (Ramakrishna and Ravishankar 2011). Saeidnejad *et al.*, (2013) reported that drought stress increased essential oil content of *Bunium persicum* (Boiss.) B. Fedtsch. plants.

Valerian (*Valeriana officinalis* L.) which belongs to the family Valerianaceae is an important medicinal plant that is, due to its mild sedative and sleep aid, widely used in traditional medicine (Bos *et al.* 1996). Valerian is the 8th top-selling herbal supplement in North America (Blumenthal 2001), making it very attractive to improve agronomical practices and in general its production increases. Iran is climatically regarded as an arid and semi-arid region in the world, where

the lack of precipitation and its inappropriate distribution, high temperature and extensive evaporation makes the irrigation the main way for meeting plants water demand. Understanding the physiological mechanisms providing drought stress tolerance is very important in terms of developing selection and breeding strategies. To date, there is no published and comprehensive research related to drought impacts on *Valeriana officinalis* characteristics. Therefore, the present investigation was conducted with the aim of evaluating drought impacts on quantitative and qualitative properties of *Valeriana officinalis*.

MATERIALS AND METHODS

A pot experiment was conducted in greenhouse condition at the University of Maragheh, Maragheh, Iran. Pots with 8 L content were filled with 12 kg soil and clean sand (2:1, v/v). The soil had been passed through a mesh number 10. Then, 20 days-old *valerian* seedlings were obtained from medicinal plants institute, Tehran, Iran. Uniform seedlings were collected and then transplanted into each pot. The pots were placed under a rain-shelter based on completely randomized design. During the next 30 days after transplantation, plants were irrigated with tap water, and then divided into four lots subjected to different water levels. Drought stress levels were measured using control plants with 100% water availability, 70%, 50% and 30% of available water. The pots were weighed daily and water was added to maintain soil moisture content. The physiological and biochemical measurements were carried out at 75 days after transplanting. Traits related to above ground and subterranean organs and also essential oils content were measured at the end of experiment.

Water status of leaves was determined by measuring relative water content (RWC). The leaves were subsequently rehydrated in distilled water for 4 h to obtain the turgid weight (TW), and dried for 48 h at 72 °C to obtain the dry weight (DW). RWC was calculated by the ratio: $RWC = [(FW - DW)/(TW - DW)] \times 100$. For measurement of electrolyte leakage, 20 leaf discs from the young fully expanded leaves were placed in 50 mL glass vials, rinsed with distilled water to remove electrolytes released during leaf disc excision. Vials were then filled with 30 mL of distilled water and allowed to stand in the dark for 24 h

at room temperature. Electrical conductivity (EC_1) of the bathing solution was determined at the end of incubation period. Vials were heated in a temperature-controlled water bath at 95°C for 20 min, and then cooled to room temperature and the electrical conductivity (EC_2) was measured. Electrolyte leakage was calculated as percentage of EC_1/EC_2 .

Chlorophyll-a, -b, and carotenoid were determined according to the method of Arnon (1949). Fresh leaves were taken from the plants and triturated in 80% acetone. The absorbance of the extracts was measured at 663, 642 and 472 nm using a spectrophotometer (BioTek, PowerWave, USA). Proline content of leaf tissues was estimated spectrophotometrically following the ninhydrin method described by Bates *et al.*, (1973). Total soluble sugars were extracted and determined by the anthrone method of Riaz *et al.*, (1985).

The shoot tissues (0.5 g fresh weight) were homogenized in 2 mL of 100 mM potassium phosphate buffer, pH 7 containing 1 mM of EDTA and 1% (w/v) polyvinylpyrrolidone (PVP). The extract was then centrifuged at 4°C for 15 min at 12,000 ×g in a cooled centrifuge. This supernatant was used to measure the activities of guaiacol peroxidase (GPX), ascorbate peroxidase (APX) and catalase (CAT). Activity of GPX was determined at 25°C with guaiacol (Bergmeyer, 1974). Activity of APX was measured by following the rate of hydrogen peroxide-dependent oxidation of ascorbic acid (Nakano and Asada 1981). One unit was defined as the amount of enzyme needed for the oxidation of 1 µmol of substrate per min. Finally, the activity of CAT was assayed following H_2O_2 decomposition. One unit of catalase activity is defined as that amount of enzyme which breaks down 1 µmol of H_2O_2 /min under the assay conditions described. Potassium and sodium concentrations were determined in the shoots after digesting 100 mg powder of the oven-dried tissues in a mixture of concentrated nitric acid and perchloric acid (3:1; v/v) at 175°C. The potassium and sodium contents of the digested extracts were quantified using a flame photometer (model Jenway PFP7, UK). Zn and Fe contents were measured using atomic absorption spectrophotometer (AAS) (Faithfull, 2002). Finally, 50 g of root was taken for determination of essential oil percentage by Clevenger (European Pharmacopoeia, 2005). Essential oil yield was determined using the

following formula: Essential oil yield = Essential oil percentage × Root yield. Analysis of variance appropriate to the experimental design was conducted, using SPSS software (version 16). The experiment was based on a Complete Randomized Design (CRD) with three replicates. Means of each trait were compared according to Duncan multiple range test at $P < 0.05$ by means of MSTATC software.

RESULTS AND DISCUSSION

Stress indicators Leaf relative water content (RWC) and electrolyte leakage (El) are recognized as important index for plant stress tolerance (Lakshmi *et al.*, 2012). RWC is closely related with cell size and it may strongly reflect the balance between water supply to the leaf and transpiration. It has been suggested that plant water status, rather than plant function, controls crop performance under drought. In other words, plant having higher yields under drought stress should have high RWC. Regarding Table 1, relative water content (RWC) was significantly decreased with decreasing the irrigation from 100% to 30% AWC. levels of 70%, 50% and 30% the available water content (AWC) lead to 13%, 27.4% and 33.5% of RWC, respectively (Table 1) Leaf RWC reflected the metabolic activity in tissues and it significantly declined due to water stress. It seems that decrease in leaf RWC could have been due to unavailability of water in the root zones, which is not able to compensate for water, lost by transpiration. Similar results were reported by Bolat *et al.* (2014) who found that leaf RWC decreased with intensifying stress.

Cell membrane stability is considered to be one of the best physiological indicators of drought stress tolerance. Results showed that water stress induced a marked increase in membrane permeability, so, the injury index of leaves increased with increasing the water shortage (Table 1). El level increased from 20 to 43%, when AWC dropped from 100 to 30%. This might be due to the production of reactive oxygen species (ROS) which damage cell membranes. Sairam and Srivastava (2001) reported that drought stress causes membrane lipid peroxidation and produce certain compounds, such as malondialdehyde and ethylene, which leads to declination of cell membrane stability.

Defense mechanisms To cope with the drought, plants initiate defense mechanisms

against water deficit. In this section, the main mechanisms of drought tolerance at physiological level are investigated. From this point of view, Osmotic adjustment, and a scavenging defense system have been the most important bases responsible for drought tolerance.

Osmotic adjustment (OA) is a biochemical mechanism that helps plants to acclimatize to dry and saline conditions by maintaining water uptake. OA results in a net increase of the number of osmotically active substances in the cell that leads to a more negative osmotic potential, which in turn can improve maintaining turgor in leaf tissue and in other metabolically active cells. Proline and soluble sugars are considered to act as compatible osmoticum materials and are perhaps involved in drought tolerance. In the present study, in response to water stress, proline concentration was gradually increased reaching a peak value at 50% AWC and then commenced to reduce (Table 1). The lowest proline content was obtained with normal irrigation (control), while the highest was obtained at irrigation with 50% AWC. Several possible roles have been attributed to this accumulation of proline: osmoregulation, detoxification of free radicals, conservation of nitrogen and energy for the post-stress period and regulating the stress protective proteins (Khedr *et al.*, 2003). Many studies have shown the accumulation of proline in response to water stress (Ghobadi *et al.*, 2013; Yadav *et al.*, 2014). Results revealed that total soluble sugars concentration was slightly increased at mild water stress (70% AWC), but further increase in water shortage induced soluble sugar losses in leaves. Reduction in soluble sugar content may be attributed to photosynthesis-reduction induced by chlorophyll degradation. These results support the findings of D'áz-Lo'pez *et al.* (2012) however; an opposite trend was obtained by Rahimi *et al.* (2013).

Antioxidant enzymes being the most efficient mechanisms against oxidative stress, which detoxifies reactive oxygen species (ROS), and keeps an adequate cellular redox balance. Among the enzymatic antioxidants, superoxide dismutase (SOD) scavenges O_2^- free radicals converting them into H_2O_2 . The H_2O_2 is then further scavenged by catalase (CAT) and peroxidase (POD) into H_2O and O_2 (Wang *et al.*, 2009). Peroxidases and catalases also play an important role in the fine regulation of reactive oxygen species in the cell through

activation and deactivation of H_2O_2 (Sairam *et al.* 2005). Results indicated that water stress had a significant effect ($P < 0.01$) on antioxidant enzymes activity in leaves of valerian plants. The activities of CAT and ascorbate peroxidase (APX) were increased with the increase of water stress from the control (100% AWC) to 50% AWC. However, further increase in water shortage reduced CAT activity, but maximum value of APX activity was achieved at 30% AWC. In contrast to CAT and APX, the activity of Guaiacol peroxidase (GPX) tended to decrease by increased water deficit. Maximum GPX (10.2 Units/min.mg.pro) contents were noted from well-watered valerian plants, which were decreased significantly upon exposure to drought stress (Table 1). Liu *et al.* (2011) reported that catalase activity was higher under mild drought stress than severe drought stress and well-watered treatments. The increase of CAT and APX activity in plants under water stress was also reported in other studies (Huseynova 2012; Ghobadi *et al.* 2013). These results were different from the reports of Csiszár *et al.* (2007) who found that with water stress, antioxidant enzymes activity remained the same or decreased, which was possibly due to the variation among the many experiments with differences in deficit severity and timing as well as the species used.

Physiological disorders Drought stress significantly affected chlorophyll a, b, and carotenoids contents (Table 1). The results revealed that Chlorophyll "a, b" contents decreased by decreasing the soil moisture content. Drought stress up to 70% AWC induced a slight effect in the chl-a of valerian leaf, then it reduced them markedly by the further increase in the level of drought stress. Severe (50% AWC) and very severe drought stress (30% AWC) decreased chl-a as much as 24.8 and 62.1% compared to the control, respectively. Stress-induced chl-b reduction was stable among the stress levels. Contrary to these results, plants carotenoid values was not significantly affected by water shortage up to 50%, but under very severe conditions (30% AWC), its value increased by 10.2% (Table 1). Our results about chl-a and chl-b changes in response to drought stress are in agreement with reports of Liu *et al.* (2011). Reduction in chlorophyll pigments concentration can be as a drought response mechanism in order to minimize the light absorption by chloroplasts (Pastenes *et al.*, 2005). Bahreininejad (2013)

reported that photosynthetic pigments and proline are both synthesized from the same substrate. Therefore, an increase in the synthesis of proline leads to a decrease in the synthesis of photosynthetic pigments under water deficit. Another reason for decrease in chlorophyll content as affected by water deficit is that drought stress by producing reactive oxygen species (ROS), can lead to lipid peroxidation and consequently, chlorophyll destruction. Since carotenoid plays an important role in photo-protection, the increased carotenoid content under drought conditions, indicate a higher need of photo-protection by carotenoid (Farooq et al., 2009)).

The results in Table 2 showed that potassium content in leaves was gradually increased by decreasing water depletion from 100 to 70% (mild drought stress) but further increasing drought stress caused low K content compared with control. Na content in leaves was gradually decreased due to increasing drought stress, however, differences were not statistically significant. The lowest Na content was obtained at irrigation with 30% AWC, while, the highest was obtained with irrigated with normal irrigation (100%). These findings are generally in line with those previously reported by Mohsenzadeh et al. (2006). Shaheen et al (2011) also reported that the leaves of olive contained a lower content of K when these plants grown under severe water stress. Under water stress conditions, K⁺ plays an important role in osmoregulation that resulted in water stress tolerance. Of all the micronutrient elements, the concentrations of Fe and Zn are of particular interest because Fe and Zn deficiency are afflicting over three billion people worldwide (Cakmak, 2008). Data presented in Table 2 indicated that highest and lowest zinc and iron contents in leaves were achieved at 70% and 30% AWC, respectively. Similar results were also reported by (Sanchez-Rodriguez et al. 2010). Since the transport of micronutrients to the plant roots occurs via diffusion, low soil moisture content will reduce micronutrient uptake. However, since plants require much smaller quantities of micronutrients, the effects of drought stress on micronutrient uptake are not as great as that on P and K uptake.

Quantitative yield Plant aerial biomass as well as root biomass was severely inhibited under water stress, and both of them decreased with increasing the water shortage (Table 2).

Aerial parts growth decreased by 32-60% while subterranean organs growth decreased by 22-40%. Therefore, roots were less affected by water stress than aerial parts (Table 2). Root is the first organ to come into contact with stresses in the rhizosphere, thus supplying assimilate for this organ may be a best strategy for their resistance. Drought stress frequently enhances allocation of dry matter to the roots, which can enhance water uptake (Leport et al. 2006). One of the first signs of water shortage is the decreasing the dry weight under water deficit could be a result of a reduction in turgor which causes a decrease in both growth and cell development and also may be due to reduction in chlorophyll content as our data indicated (Table 1 and 2). The growth reduction can be considered as a morphological adaptation of the plant to water stress to reduce transpiration and to induce a lower consumption of water. Growth reduction as a result of water deficit has been widely reported (Hassan and Ali 2014).

Qualitative yield Plant secondary metabolites are often referred to compounds that have no fundamental role in the maintenance of life processes in the plants, but they are important for the plant to interact with its environment for adaptation and defense. Secondary metabolites are involved in protective functions in response to both biotic and abiotic stress conditions. Essential oil percentage increased when soil AWC dropped to 50%, and then it declined with drought stress intensifying (Table 1). The high essential oil content was achieved at 50% AWC, accumulated two-folds when compared with control. As it is shown in Table 1 increasing of water stress level from 50% to 30% increased essential oil content by 7.8% in valerian. Bernatb (1999) reported that sufficient and continuous water supply not only could not enhance essential oil content of valerian plant also it could decrease essential oil content. Our results are in agreement with was previously documented by Rebey et al. (2012) on *Cuminum cyminum* L. and Hassan and Ali (2014) on *Coriandrum sativum* L. Saeidnejad et al. (2013) reported that drought stress increased essential oil content of *Bunium persicum* plants. Essential oil yield is a dependent variable determined by seed yield and essential oil percentage. The highest essential oil yield (334 mg) was achieved in plants exposed to 50% AWC. Water shortage up to 70% AWC had not significant effect on essential oil

yield (Table 2). The lowest value for essential oil yield was achieved when plants exposed to very severe water stress (30% AWC).

CONCLUSION

In view of findings of the present study, it can be concluded that water stress strongly decreased

Tab. 1. Relative water content (RWC), Electrolyte leakage (El), photosynthetic pigments contents and antioxidative enzymes activity of valerian plants under different drought levels (100%, 70%, 50% and 30% available water content). Data are the mean values of 3 replications $\pm$ SE

AWC (%)	RWC (%)	El (%)	Chlorophyll		Car	CAT	APX	GPX
			a	b				
			(mg/g FW)					
						Units/min.mg.protein		
100	77.3± 4.8a	20.1±0.8d	38.6±1.2a	38.0±0.5a	1.27±0.02b	11.4±0.6b	1.09±0.008c	10.2±0.3a
70	67.2 ±1.4a	33.8±0.8c	35.0±1.5a	29.3±1.4b	1.26±0.03b	17.3±1.5a	1.42±0.02b	10.3±0.3a
50	56.1±2.5b	40.0±1.0b	29.0±2.0b	28.6±1.4b	1.26±0.05b	19.4±1.2a	1.78±0.01a	7.59±0.1b
30	51.4±3.0b	43.4±1.1a	14.6±1.4c	26.3±2.9b	1.40±0.02a	11.6±1.1b	1.81±0.03a	6.77±0.2b
	**	**	**	**	*	**	**	**

Different letters indicating significant difference at $p < 0.05$. Carotenoid (Car), Catalase (CAT), Ascorbate peroxidase (APX), Guaiacol peroxidase (GPX).

Tab. 2. Proline, Soluble sugars, Sodium (Na⁺), potassium (K⁺), Zinc (Zn), Iron (Fe) contents and aerial and root biomass of valerian plants under different drought levels (100%, 70%, 50% and 30% available water content). Data are the mean values of 3 replications $\pm$ SE.

AWC (%)	Proline (μ mol/g FW)	Soluble sugars (mmol/g DW)	Na ⁺ (mg.g ⁻¹ dry weight)	K ⁺ (mg.g ⁻¹ dry weight)	Zn (ppm)	Fe (ppm)	Aerial biomass (g)	Root biomass (g/plant)
100	0.37 $\pm$ 0.06d	100 $\pm$ 10a	5.70 $\pm$ 0.2a	5.33 $\pm$ 0.2b	0.33 $\pm$ 0.05ab	9.6 $\pm$ 1.1b	116 $\pm$ 8.8 a	59.4 $\pm$ 6.6a
70	1.58 $\pm$ 0.1c	101 $\pm$ 4a	5.46 $\pm$ 0.08a	5.85 $\pm$ 0.12a	0.37 $\pm$ 0.02a	12.4 $\pm$ 0.01a	79.3 $\pm$ 2.9b	46.3 $\pm$ 3.0ab
50	2.41 $\pm$ 0.05a	82 $\pm$ 11b	5.36 $\pm$ 0.1a	4.36 $\pm$ 0.05c	0.29 $\pm$ 0.01bc	11 $\pm$ 0.8ab	59.6 $\pm$ 3.1c	44.7 $\pm$ 2.7b
30	1.93 $\pm$ 0.04b	79 $\pm$ 8b	5.17 $\pm$ 0.2a	3.12 $\pm$ 0.1d	0.25 $\pm$ 0.02c	6.8 $\pm$ 0.3c	47.6 $\pm$ 4.3c	35.8 $\pm$ 2.3b
	**	ns	ns	**	**	**	**	**

Different letters indicating significant difference at $p < 0.05$.

Fig. 1. Essential oil content and yield of valerian plants under different drought levels (100%, 70%, 50% and 30% available water content). Data are the mean values of 3 replications $\pm$ SE, represented by the vertical bar in each graph.

valerian growth parameters by decreasing aerial and root biomass. With respect to the achieved results, *Valeriana officinalis* plants are relatively tolerant to drought conditions and mild water stress mainly had not significant effects on their growth. Essential oil percentage increased with decreasing the water content from 100 to 50%, so this aspect of water stress is economically useful. As subterranean organs growth and essential oil accumulation were inversely affected by water stress, therefore growers should be careful when increasing the irrigation intervals. In the current research, regulation of irrigation intervals in which soil available water content was about 70% to 50% is the best practical way for valerian production.

REFERENCES

- Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol, 24, 1–15.
- Bahreinejad B, Razmjoo J, Mirza M (2013). Influence of water stress on morpho-physiological and phytochemical traits in *Thymus daenensis*. International Journal of Plant Production 7 (1), 151–166.
- Bajji M, Kinet JM, Lutts S (2002) The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. Plant Growth Regul. 36, 61–70.
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant and Soil, 39: 205–207.
- Bernatb J (1997) Cultivation of valerian. In: Houghton, P.J. Valerian. Harwood academic publishers.
- Blumenthal M (2001) Herb sales down 15% in mainstream market. Herbal Gram. 59, 69.
- Bolat I, Dikilitas M, Ercisli S, Ikinici A, Tonkaz T. (2014). The Effect of Water Stress on Some Morphological, Physiological, and Biochemical Characteristics and Bud Success on Apple and Quince Rootstocks. The Scientific World Journal. <http://dx.doi.org/10.1155/2014/769732>.
- Bos R, Woerdenbag HJ, Hendriks H, Zwaving JH, De Smet PAGM, Tittel G, Wikström HV, Scheffer JJC (1996) Analytical aspects of phytotherapeutic valerian preparations. Phytochem Anal. 7: 143–151.
- Cakmak I. 2008. Enrichment of cereal grains with zinc: Agronomic or genetic biofortification?. Plant Soil. 302: 1–17.
- Cornic G, Massacci A. (1996) Leaf photosynthesis under drought stress, in: Baker N.R., (Ed.), Photosynthesis and the Environment, Kluwer, Dordrecht.
- Csiszár J, Lantos E, Tari I, Madoşă E, et al. (2007). Antioxidant enzyme activities in Allium species and their cultivars under water stress. Plant Soil Environment. 53, (12): 517–523.
- Dr'az-Lo'pez L, Gimeno V, Simo'n I, Mart'inez V, Rodr'iguez-Ortega WM, Garc'a-Sa'nchez F. (2012). *Jatropha curcas* seedlings show a water conservation strategy under drought conditions based on decreasing leaf growth and stomatal conductance. Agric. Water Manage. 105, 48–56.
- Faithfull NT (2002). Methods in agricultural chemical analysis: a practical handbook. CABI Publishing
- Ghobadi M, Taherabadi S, Ghobadi ME, Mohammadi GR, Jalali-Honarmand S (2013). Antioxidant capacity, photosynthetic characteristics and water relations of sunflower (*Helianthus annuus* L.) cultivars in response to drought stress. Industrial Crops and Products. 50: 29– 38.
- Hassan FAS, Ali EF (2014). Impact of different water regimes based on class-A pan on growth, yield and oil content of *Coriandrum sativum* L. Plant. Journal of the Saudi Society of Agricultural Sciences (2014) 13, 155–161.
- Huseynova, I.M. (2012): Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought. Biochemistry and Biophysics Acta: Bioenergy. 1817, 1516–1523.
- Isettes, B. J. (2010): valerian. In: Tracy T. S. Kingston R. L. Herbal Products: Toxicology and Clinical Pharmacology. Humana Press Inc., Totowa, NJ.
- Kar RK (2011). Plant responses to water stress: Role of reactive oxygen species. Plant Signaling & Behavior 6: 1741–1745.
- Khedr AA, Abbas MA, Abdel Wahid AA, Paul, Quick W, Abogadallah GA (2003). Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Panocratium maritimum* L. to salt-stress. Journal of Experimental Botany, 54, 2553–2562.
- Li Z, Peng Y, Ma X (2013). Different response on drought tolerance and post-drought recovery between the small-leaved and the large-leaved white clover (*Trifolium repens* L.) associated with antioxidative enzyme protection and lignin metabolism. Acta Physiologia Plantarum, 35:213–222.
- Liu C, Liu Y, Guo K, Fan D, Li G, Zheng Y, Yu L, Yang R (2011). Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China. Environmental and Experimental Botany, 71, 174–183.
- Mohsenzadeh S, Malboobi MA, Razavi K, Farrahi-Ashtiani S (2006). Physiological and molecular responses of *Aeluropus lagopoides* (Poaceae) to water deficit. Environmental and Experimental Botany, 56(3):314–322.
- Nakano Y, Asada K (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidases in spinach chloroplasts. Plant Cell Physiology, 22, 867–880.
- Pastenes C, Pimentel P, Lillo J. (2005). Leaf movements and photoinhibition in relation to water stress in field-grown beans. Journal of Experimental Botany, 56, 425–433.
- Pinkerton A Simpson JR (1986). Interactions of surface drying and subsurface nutrients affecting plant-growth on acidic soil profiles from an old pasture. Australian Journal of Experimental Agriculture. 26:681–689.
- Rahimi A, Sayadi F, Dashti H, Tajabadi pour A. (2013). Effects of water and nitrogen supply on growth, water-use

- efficiency and mucilage yield of isabgol (*Plantago ovate* Forsk). Journal of Soil Science and Plant Nutrition, 13 (2), 341-354.
27. Ramakrishna A. Ravishankar G. A. 2011. Influence of abiotic stress signals on secondary metabolites in plants. Plant Signaling & Behavior 6:11, 1720-1731.
 28. Rebey IB, Jabri-Karoui I, Hamrouni-Sellami I, Bourgou S, Limam F, Marzouk B (2012). Effect of drought on the biochemical composition and antioxidant activities of cumin (*Cuminum cyminum*L.) seeds. Ind Crop Prod 36: 238–245.
 29. Saeidnejad AH, Kafi M, Khazaei HR, Pessarakli M 2013. Effects of drought stress on quantitative and qualitative yield and antioxidative activity of *Bunium persicum*. Turkish Journal of Botany. 37: 930-939.
 30. Sairam RK, Srivastava GC, 2001. Water stress tolerance of wheat (*Triticum aestivum* L.): variations in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotypes. J. Agron. Crop Sci. 186, 63–70.
 31. Sanchez-Rodriguez E, Rubio-Wilhelmi MD, Cervilla LM, Blasco B, Rios JJ, Leyva R, Romero L, Ruiz JM 2010. Study of the ionome and uptake fluxes in cherry tomato plants under moderate water stress conditions. Plant Soil, 335:339–347.
 32. Shaheen MA, Hegazi AA, Hmnam ISA (2011) Effect of Water Stress on Vegetative Characteristics and Leaves Chemical Constituents of Some Transplants Olive Cultivars. American-Eurasian Journal of Agricultural and Environmental sciences, 11 (5): 663-670.
 33. Yadav RK, Sangwan RS, Sabir F, Srivastava AK, Sangwan NS (2014) Effect of prolonged water stress on specialized secondary metabolites, peltate glandular trichomes, and pathway gene expression in *Artemisia annua* L. Plant Physiology and Biochemistry 74: 70-83.