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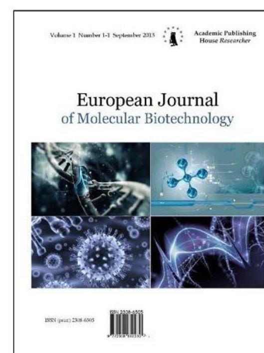

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The Effect of Drought Stress on the Essential Oil Content and Some of the Biochemical Characteristics of Anise Hyssop (*Agastache foeniculum* [Pursh] Kuntze)*

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Abstract

Plot trials were carried out in a research field in Tehran (Iran) to determine the effect of drought stress on the essential oil content and some of the plant biochemical characteristics of Anise Hyssop (*Agastache foeniculum* [Pursh] *Kuntze*), which is a valuable medicinal plant. Drought stress was conducted at different levels including: well-water (100% FC), mild drought stress (85% FC), moderate drought stress (70% FC), severe drought stress (55% FC), 100% FC (vegetative stage) 85% FC (reproductive stage), 100% FC (vegetative stage) 70% FC (reproductive stage), and 85% FC (vegetative stage) 100% FC (reproductive stage). The experiment was arranged as a RCBD with three replications. The output results showed that, water deficit stress significantly ($P \leq 0.05$) increased activities of antioxidant enzymes (Superoxide Dismutase, Catalase, Glutathione Peroxidase) as well as Essential Oil yield and Abscisic Acid content. Lipid and protein oxidation (malondialdehyde and dityrosine contents) also increased significantly under severe water deficit stress. According to the results, severe drought conduction (55% FC) is the optimum level of soil moisture to plant Anise Hyssop under water deficit stress.

Keywords: Anise Hyssop, drought stress, anti oxidative enzymes, biochemical characteristics.

Introduction

Iran is located in arid and semi-arid region. Having an average annual precipitation of 250 mm, Iran receives less than one third of global average precipitation (750 mm). In addition, the rainfall distribution pattern over the country is not the same everywhere. Bearing in mind such a climatic condition, many severe or mild droughts are inevitable to come up. Any drought can inflict a severe damage on the agricultural, domestic and industrial sectors of the country. Due to the growth of population and expansion of the agricultural, energy, and industrial sectors, the

*__Abbreviations:__ EO – Essential Oil; SOD – Superoxide Dismutase; GPO – Glutathione Peroxidase; MDA – Malondialdehyde; Di-Tyr – Dityrosine; ABA – Abscisic Acid; ROS – Reactive Oxygen Species

demand for water has increased extensively, and water scarcity has been occurring almost every year in many parts of the world [39].

Drought is known as a major abiotic factor that limits plant's growth and production. Although the general effects of drought on plant growth are fairly well known, the primary effects of water deficit at the biochemical and molecular levels are not well understood [11]. Furthermore, the physiologic and metabolic responses of crops to dry environments have been well studied, but similar studies are lacking in medicinal and aromatic plant. Stress is a factor outside plant's body which damages plant growth [27]. Among the abiotic stress, drought is the most important one which affects plants periodically in some growth stages, or permanently in all life cycle [46]. Drought stress usually occurs when available water in soil reduces and atmospheric conditions increase water loss through evapotranspiration [24]. A primary symptom of low available water to plants is the loss of turgor pressure and reduction of cell development especially in stems and leaves. Reduction of cell development makes the plant smaller in size, which is the characteristic of drought stressed plants. Moreover, drought stress disturbs nutrient absorption and reduces leaves growth. Lower leaf area means lower light absorption and photosynthesis. All these events finally decrease plant growth and yield [22]. Drought stress in induced when moisture at the rhizosphere falls below the permanent wilting point (PWP). So the plant is not able to take up sufficient water, resulting in cell dehydration. Dehydration is reversible until a certain point (elastic point); however, is irreversible if the water loss is too server (plastic point) [30]. However, the time duration and frequency of drought stress incident, soil properties and so many other factors affect plant tolerance to drought, and different genotypes may also respond differently [50]. Drought stress induces some morpho–physiological responses in plant such as the reduction of leaf area, shoot growth, enhancement of root growth, stomata closure, reduction of growth rate, sudden antioxidants and soluble compounds accumulation, and activation of some enzymes [23].

Stephanie et al. [54] and later, Asadi [6] reported that drought stress reduced stem length and root length of *Salvia splendens*. Lebaschy and Sharifi Ashoorabadi [31] concluded that higher drought stress levels reduced plant high and shoot weight in some medicinal plants such as *Salvia officinalis* and *Achillea millefolium*. Sangwan et al. [49] reported that mild drought stress decreased lemon grass height, leaf area and leaf weight. Finally, Ardakani et al. [5] reported that drought stress affected shoot yield, essential oil percentage and yield, leaf yield, stem yield, height, the number of tillers, leaf area, stem diameter and the length of internodes in balm (*Melissa officinalis*). Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for normal plant grow and to complete the life cycle [60]. The lack of adequate moisture leading to water stress is common occurrence in rain fed areas, brought about by infrequent rains and poor irrigation [56]. In higher plants the oxygen toxicity is more serious under condition of water-deficit conditions. Water stress causes stomatal closure, which reduces the CO_2/O_2 ratio in leaves and inhibits photosynthesis [25, 41]. These conditions increase the rate of reactive oxygen species (ROS) like superoxide radical ($O_2^{\bullet -}$) hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$) particularly in chloroplast and mitochondria [40, 45], *via* enhanced leakage of electrons to oxygen. The superoxide radicals and their dismutation product, hydrogen peroxide, can directly attack membrane lipid and inactivate SH containing enzymes [48].

The hydroxyl radical, one of the most reactive oxygen species, is responsible for oxygen toxicity in vivo, causing damage to DNA, protein, lipids, chlorophyll and almost every other organic constituent of the living cell. Plants protect cellular and sub-cellular system from the cytotoxic effects of active oxygen radicals with anti-oxidative enzymes such as SOD, POX and CAT as well as metabolites like glutathione, ascorbic acid, tocopherol and carotenoids [3]. It has been reported which membranes are subject to damage rapidly with increasing water stress. This leakiness of membranes is caused by an uncontrolled increase in free radicals, which cause lipid peroxidation [53]. The stress induced burst in free radicals could also be partially related to the activity of lipoxygenase, which convert C18:2 and C18:3 to the corresponding hydroxyl peroxides [10]. Further damage to fatty acid could then produce small hydrocarbon fragments including malondialdehyde (MDA) [3]. It is hypothesized that modulation of the activities of these enzymes at early growth stage may be important in imparting resistance to a plant against environmental stresses. Therefore, in the present investigation the relative significance of antioxidative enzymes, MDA, H_2O_2 content, PRO, GB accumulation, photosynthetic activity and membrane permeability has been examined at seedling stage in drought-tolerant and susceptible maize cultivars [20].

Plants subjected to environmental stress evolved a complex and efficient antioxidant system, which includes enzymatic antioxidants and non-enzymatic antioxidants to counteract the detrimental effects of active oxygen species [59]. These are toxic intermediates that result from a reduction in molecular O_2, including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH) [15]. The role of antioxidative defence systems in plant responses to drought stress was comprehensively documented in *Gypsophila aucheri*, which is a xerophytic plant [51]. In another study, antioxidative and physiological responses of 2 sunflower (*Helianthus annuus*) cultivars under drought stress were evaluated, and the efficiency of antioxidative systems in coping with drought effects was clear [9]. 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. Secondary metabolite production is a critical part of the defence response to stress conditions. The role of lipid peroxidation in initiation of secondary metabolites has been documented by some researchers. Consequently, the accumulation of secondary metabolites is mainly related to membrane lipid protection from oxidative stress, and reactive oxygen species (ROS) are the mediators in the biosynthesis of particular secondary metabolites [59]. Water stress decreases growth of some medicinal plants, including *Hypericum brasiliense Choisy* [42] and *Bupleurum chinense DC* [59]. On the other hand, many studies have shown that drought enhances the amount of secondary metabolites in a wide variety of plant species, such as *Rehmannia glutinosa* (*Gaertn.) DC.* [14]. Conversely, drought caused a significant reduction in all growth parameters and essential oil yield and percentage in some medicinal plants such as peppermint (*Mentha piperita L.*) [29, 47]. With the looming prospect of global water crisis, the recent laudable success in deciphering the early steps in the signal transduction of the "stress hormone" – abscisic acid (ABA) has ignited hopes that crops can be engineered with the capacity to maintain productivity while requiring less water input. The involvement of ABA in mediating drought stress has been extensively researched. ABA plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins involved in cellular dehydration tolerance. Early work showed that ABA can act as a long-distance water stress signal in sensing incoming soil drying [58]. The built up of the hormone then triggers diverse adaptive pathways permitting plants to withstand temporary bouts of water shortage [18, 19, 32]. Two lines of evidences support this claim. The first is that ABA mediates many physiological responses that help plants to survive the abiotic stress. The second is that the stress-related production and catabolism of ABA are themselves delicately stress-regulated. Indeed such delicate regulations make it possible for ABA to act as a stress signal [58].

Anise Hyssop (*Agastache foeniculum (Pursh) Kuntze*) is an erect, branched perennial herb with opposite, toothed leaves and square stems. It is a member of the mint family (*Lamiaceae*), and has a pleasant anise-like aroma. The leaves are ovate-triangular, green above and with soft grey hairs below. Flowers are purple-blue, two-lipped, in a dense terminal spike 4–8 cm long. It blooms from June to September and grows up to 1 m tall. Anise Hyssop has been used by North American First Nations people as a breath-freshener, as a tea and as a sweetener. An infusion of the herb was used for chest pains, and the roots were used for coughs. *Agastache foeniculum* is used in Chinese prescriptions for heatstroke, headache, fever, and angina. Leaves are used as poultices for sores. It is used in dried flower arrangement, and the essential oils are used in perfumes and aromatherapy. It is also a good source of nectar. Anise Hyssop is found mostly in moist, open woods, along streams and lakeshores, and in wet ditches and prairies. It prefers sandy, moist, well-drained loam, in full sun or very light shade. The pH value should be 6.0–6.5. It requires lots of moisture, and wilts if it is too hot. Its natural distribution is from BC across the prairies (in MB north to the Porcupine Mountains) and into western Ontario and the adjacent states. It has been introduced into the northeastern North America [36].

The objective of this experiment was to determine the effect of different levels of drought stress on essential oil yield and some of the biochemical characteristics of Anise Hyssop (*Agastache foeniculum (Pursh) Kuntze*).

Material and Methods

The experiment was conducted in Randomized Completely Block Design with three Replications at the research field of Tarbiat Modares University (Peykan Shahr, Tehran, Iran) in April 2009. Average annual precipitation at the site was 122.2 mm, minimum air temperature was -

5°C and maximum air temperature was 40.4°C. The dominant winds at the area blow from Northeast. The properties of soil at the test site are listed in Table 1. Information about test site climate and meteorological data in 2009–2010 is listed in Table 2 and Table 3, respectively.

Table 1: Physical and chemical properties of the test site soil

B	Mn	Cu	Zn	Fe	Mg	Ca	K	P	N	% organic matter	Electrical conductivity	Acidity	Soil texture
ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	%	%	Ds/ms	pH	
0.6	10.63	0.87	3.37	9.08	381	5045	770	140	0.15	1.73	1.04	7.7	Sandy loam

Seeds were obtained from Zardband Company. Each replication consisted of seven treatments. Seeds were sown in rows with distance of 20 cm and depth of 0.5 cm. The distance of rows in each plot was 40 cm. TDR (Time Domain Reflectometry) apparatus was used to to measure volumetric water content in soil. The TDR technique is based on the measure of dielectric constant of a soil to estimate its volumetric water content. Drought stress was conducted according to percentage of weight moisture. The treatment consisted of 7 FC levels. Due to induce the drought stress, 100% of FC was considered as the well water and 85%, 70%, 55% of FC; 100% FC (vegetative stage), 85% FC (reproductive stage); 100% FC (vegetative stage), 70% FC (reproductive stage); 85% FC (vegetative stage), 100% FC (reproductive stage) were considered as the drought stress treatments. Vegetative organs and flowers were harvested in 3 stages (1 – floret formation, 2 – formation of adult florets, 3 – full flowering) to measure content of essential oil and enzymes activity. Leaf samples were frozen in liquid nitrogen (t = -80°C) to determine enzymes activity, biological markers and Abscisic acid content. EO was extracted by subjecting flowers and leaves together (25 g) to hydro-distillation for 4 h using an all glass Clevenger-type apparatus (Goldis, Tehran, Iran), according to the method outlined by the European pharmacopoeia [4]. EO yield was expressed as percentage (w/w) on dry matter basis.

Table 2: Profile of test site climate

Profile of climate research site	
35° 43 ‘ North	Latitude
51° 8 ‘ East	Longitude
1315 m	altitude
Semi-arid	climatic regime

Table 3: Meteorological Data in 2009–2010

2010 Statistics	2009 Statistics	Meteorology factors making up
17.3	17.5	Average Temperature, °C
41	40.4	Absolute maximum, °C
-16.25	-5	Least desirable, °C
41%	36%	Humidity
2685.4	3201.1	Sun Clock
***	2125.5	Evaporation
West	Northeast	Winds side
20 m/s	16 m/s	Wind speed
277.7 mm	122. 2 mm	Rainfall

Antioxidant Enzyme Activity:

Superoxide Dismutase (SOD) Activity

Frozen leaf segments (0.5 g) were homogenized into a fine powder with a mortar and pestle under liquid nitrogen. Soluble proteins were extracted by homogenizing the powder in 10 ml of 50 mM potassium phosphate buffer (pH=7.0) containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 12000×g for 20 min at 4°C and then the supernatant was used for the following enzyme assays. The amount of soluble proteins was quantified according to the Bradford method with bovine serum albumin as standard. Superoxide dismutase activity was measured spectrophotometrically by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT), with the modification as follows: the reaction mixture contained 50 mM phosphate buffer (pH=7.8), 0.1 mM EDTA, 130 mM methionine, 750 µM NBT, 20 µM riboflavin and 0.1 ml enzyme extract. Riboflavin was added at last, and the reaction was initiated by placing the glass test tubes under fluorescent lamps. The reaction was terminated after 25 min by removal from the light source. An illuminated blank without protein gave the maximum reduction of NBT, thus, the maximum absorbance at λ=560 nm. In this assay, one unit of SOD was defined as the amount of enzyme inhibiting the photo-reduction of NBT by 50%. The specific activity of SOD was expressed as units g^{-1} FW.

Catalase (CAT) Activity

CAT activity was estimated by the method of Cakmak and Horst[13]. The reaction mixture contained 100 crude enzyme extract, 500 µl of 10 mmol H_2O_2 and 1400 µl of 25 mmol of sodium phosphate buffer. The decrease in the absorbance at λ=240 nm was recorded for 1 min by spectrophotometer; model Cintra 6 GBC (GBC Scientific Equipment, Dandenong, Victoria, Australia). Enzyme activity of the extract was expressed as enzyme units (µmol min^{-1} substrate) per 1 milligram of protein.

Glutathione Peroxidase (GPX) Activity

GPX activity was measured by the Paglia method in which 0.56 mol (pH=7.0) of phosphate buffer, 0.5 mol of EDTA, 1 mmol of $NaNO_3$, and 0.2 mmol of NADPH were added to the extracted solution; GPX catalyses the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP. The decrease in absorbance at λ=340 nm and t=+30°C was measured with a spectrophotometer.

Estimation of Malondialdehyde Content

Oxidative damage to lipids was estimated by measuring the content of malondialdehyde (MDA) in leaf. Leaf segments (0.3 g) were homogenized in 10 ml of 10% trichloroacetic acid (TCA), and centrifuged at 12000×g for 10 min. Then 2 ml of 0.6% thiobarbituric acid (TBA) in 10% TCA was added to an aliquot of 2 ml from the supernatant. The mixture was heated in boiling water for 30 min, and then quickly cooled in an ice bath. After centrifugation at 10000×g for 10 min, the absorbance of the supernatant at 440, 532 and 600 nm was determined with a spectrometer (Unicam UV-330, Cambridge, UK). MDA content was calculated as described by Hodges et al. [21].

Estimation of Di-Tyrosine Content

Fresh tissue material (1.2 g) were homogenized with 5 ml of ice-cold 50 mM HEPES-KOH, pH=7.2, containing 10 mM EDTA, 2 mM PMSF, 0.1 mM p-chloromercuribenzoic acid, 0.1 mM *D,L*-norleucine and 100 mg Polyclar® AT. The plan tissue homogenate was centrifuged at 5000 g for 60 min to remove debris. *o,o*-di-tyrosine was recovered by gradient elution from the C-18 column (Econosil C18, 250×10 mm) and was analyzed by the reversed-phase HPLC with simultaneous UV-detection (λ=280 nm). A gradient was formed from 10 mM ammonium acetate, adjusted to pH=4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. A standard di-tyrosine sample was prepared according to Amado et al. (1984). Di-tyrosine was quantified by assuming that it's generation from the reaction of tyrosine with horseradish peroxidase in the presence of H_2O_2 was quantitative (using the molar extinction coefficient e_{315} = 4.5 $mM^{-1} \cdot cm^{-1}$ at pH=7.5) [38].

Estimation of Abscisic acid (ABA)

Abscisic acid was extracted, purified and assayed following the procedure described by Li et al. [33] with some modifications using GC–MS technique as reported earlier [38, 44].

Chemicals

Tris-Phosphate Buffer, photodynamic phosphate and EDTA were purchased from LabScan (Dubline, Ireland), $NaNO_3$ and NaDPH were purchased from Flula (Buchs, Switzerland), acetone and acetic acid were purchased from Sigma-Aldrich (Buchs, Switzerland).

Hydro-distillation

After recording their fresh biomass, 25 g of each *Agastache foeniculum [Pursh] Kuntze*) organs (leaves, flowers, stems, seeds) were air dried during 15 days. In order to extract their EO, they were subjected to conventional hydrodistillation for 4 h. Then, extraction of all replications belonging to each treatment was mixed, and final extractions were analyzed. The hydrodistillation was performed by a simple laboratory Quikfit apparatus which consisted of a 2000 ml distillation flask, a condenser and a receiving vessel. For the determination of the yield, the EO was weighed on an analytical scale.

Results and Discussion

Effect of drought stress on Essential Oil content (EO)

The data of this investigation (Table 4) showed that drought stress had a significant ($P \leq 0.05$) effect on the essential oil content. The most important characteristic of medicinal plants is the EO yield. Fortunately, EO of *Agastache foeniculum [Pursh] Kuntze* in this experiment enhanced significantly ($P \leq 0.05$) at 55% FC. The EO yield was 1.84% in well-water treated plants. It peaked 2.30% at 55% FC. In other words, the highest EO content was obtained at 55% FC. On the other hand, the lowest amount of EO was obtained at 100% FC (vegetative stage) and 70% FC (reproductive stage) (1.64%). EO yield decreased gradually from 2.30 at 55% FC to 1.64% at 100% FC (vegetative stage) and 70% FC (reproductive stage). EO yield had a small increase at 85% FC (vegetative stage) and 100% FC (reproductive stage) compared with 100% FC (vegetative stage) and 70% FC (reproductive stage). Generally, drought stress conduction increased the biosynthesis of the EO in the leaves of *Agastache foeniculum [Pursh] Kuntze*.

Drought stress increases the essential oil percentage of more medicinal and aromatic plants, because in case of stress, more metabolites are produce in the plants and substances prevent from oxidization in the cells, but essential oil content reduce under drought stress, because the interaction between the amount of the essential oil percentage and shoot yield is consider important as two components of the essential oil content and by exerting stress, increases the essential oil percentage but shoot yield decreases by the drought stress, therefore essential oil content reduces [1]. The effect of water stress on essential oil was studied in excised leafs of Palmarosa (*Cymbopogon martinii var. motia*) and Citronella Java (*C. winterianus*). Essential oil percentage was increased under water stress and essential oil content was decreased under this condition [17]. An experiment was carried out to study the influence of water deficit stress on essential oil of balm. The results of this experiment showed that essential oil yield was reduced under water deficit stress but essential oil percentage was increased under stress [1]. Also, Khalid [28] evaluated the influence of water stress on essential oil of two species of an herb plant that is *Ocimum basilicum L.* (sweet basil) and *Ocimum americanum L.* (American basil). For both species under water stress, essential oil percentage and the main constituents of essential oil increased. Seventy five percent of field water capacity resulted in the highest yield of herb and essential oil for both species. Also, three parsley cultivars (plain-leafed, curly-leafed and turnip-rooted) were grown under conditions of 35–40% and 45–60% water deficit in order to evaluate the effect of this form of stress on essential oil yield [2].

Table 4: Effect of drought stress on the measured traits*

Treatment	ABA (ppm)	Di-Tyr (mM/mg protein)	MDA (mM/mg protein)	GPX (mg/Unit protein)	CAT (mg/Unit protein)	SOD (mg/Unit protein)	EO Yield, %
1. T1	^{cd}31.07	^{b}11.43	^{c} 24.67	^{b}**36/3**	^{c}84.6	^{c}831.3	^{ab}1.844

2. T2	bcd39.9	b12.4	b39.23	b**39/97**	c85.27	c837	ab1.7316
3. T3	ab42.73	b12.33	b39.4	a**58/93**	b113	b1191	ab1.8597
4. T4	a52.57	a29.57	a2.47	a**60/83**	a135.5	a1515	a2.3077
5. T5	d30	b11.43	c25.53	b**37**	c89.47	c827.7	ab1.7249
6. T6	cd31.1	b11.9	c25.7	b**36/9**	c84.13	c839	b1.6449
7. T7	abc41.93	b12.63	b38.53	a**58/5**	c84.17	c833	ab1.7741

Notes* T1 – 100% FC, T2 – 85%FC, T3 – 70% FC, T4 – 55% FC, T5 – 100% FC (vegetative stage)–85% FC (reproductive stage), T6 – 100% FC (vegetative stage)–70% FC (reproductive stage), T7 – 85% FC (vegetative stage)–100% FC (reproductive stage); means in a column followed by the same letter are not significantly different at P≤0.05.

Effect of drought stress on Superoxide Disesmutase activity (SOD)

Drought stress significantly activated antioxidant system of leaves of Anise Hyssop. As regards SOD activity, it varied widely (Table 4). SOD activity was about 831.3 mg/Unit protein at well-water treatment. Mild water deficit resulted in a slight increase of SOD activity, but it increased significantly (P≤0.05) to 1515 mg/Unit protein when soil water content dropped to 55% FC. However, it decreased remarkably to 827.7 mg/Unit protein at 100% FC (vegetative stage) 85% FC (reproductive stage) compared with well-water treatment.

The activity of superoxide dismutase was assayed as given by Dhindsa et al. [16]. The superoxide dismutase activity enhanced continuously with the increase of drought stress levels. It is known that plants have a well-organized defense system against reactive oxygen species (ROS) under stress conditions and SOD constitutes the first line of defense via detoxification of superoxide radicals [20, 48]. It seems when the intensity of water deficit stresses increase too much in crops, the physiological damages will increase, too. Thus, they can not promote their antioxidant defensive mechanism along with the intense of water deficit in parallel manner. In other words, in severe water deficit stress condition, the antioxidant defensive mechanism of crops will be activated as well and the antioxidants content will increase as compared to the full-irrigated. But, due to the excessive physiological damages resulted of water deficit stress, the antioxidant activities are less than mild water deficit level. These findings can be related as the ability of the crops against different intensities of water deficit stress. Previously, an increase in the level of antioxidants was reported with an increase in stress intensity in maize and soybean by Vasconcelos et al. [55], Jiang and Zhang [26] which might be attributed to inhibitory effects of water stress on protein turnover causing depletion of antioxidants. Moreover, Masoumi et al. [38] reported a positive and significant correlation between SOD and CAT under both conditions of well irrigated and water deficit stress conditions.

Effect of drought stress on Catalase activity

Analysis of variance for catalase content showed that, there were significant differences (P≤0.05) among irrigation levels (Table 4). Drought stress conduction enhanced significantly (P≤0.05) catalase activity (mg/Unit protein). In other words, the enhancement of catalase activity was 1.6-fold when soil moisture reached the lower values of available soil water (55% FC) compared with well-watered plants. However, it showed a wide difference at 100% FC (vegetative stage), 85% FC (reproductive stage) and 100% FC (vegetative stage) 70% FC (reproductive stage) levels (about

89.47 and 84.13 mg/Unit protein, respectively) compared with well-water treatment (84.6 mg/Unit protein).

Nayar and Kaushal [43] also reported that the increased activity of CAT and POX enzymes constitute potential defense mechanism against chilling induced oxidative damage in germinating wheat grains. In other words, drought stress enhanced enzyme activity of Catalase in wheat. The activity of Catalase was determined as described by Siminis et al. [52]. The combined action of CAT and SOD converts the toxic O_2 and H_2O_2 into water and molecular oxygen, averting the cellular damage under unfavorable conditions like water stress.

Masoumi et al. [38] indicated that activities of the antioxidants (CAT) were increased in all levels of water deficit stress in soybean cultivars. Induction of oxidative stress in drought-stressed plants reported in the previous studies [12, 37]. They showed that enzymatic antioxidants content played an important role in scavenging harmful oxygen species and the activities of antioxidant enzymes were altered when plants were subjected to stress. These results are in agreement with findings of Masoumi et al. [38].

Effect of drought stress on Glutathione Peroxidase activity (GPX)

Regarding Glutathione Peroxidase activity (mg/Unit protein), the activity of well-watered plants was 36.3 mg/Unit protein, although the activity enhanced significantly ($P \leq 0.05$) to 58.93 mg/Unit protein under moderate drought stress (Table 4). In fact, the highest activity of Glutathione Peroxidase was obtained under severe drought stress (60.83 mg/Unit protein). At 100% FC (vegetative stage) and 85% FC (reproductive stage) level, Glutathione Peroxidase activity sank significantly to 37 mg/Unit protein.

Activities of various antioxidant enzymes are known to increase in response to drought. The hydrogen peroxide content enhanced linearly with increasing level of water stress in maize cultivars. It is known that H_2O_2 is a toxic compound that is produced as a result of scavenging of superoxide radicals. Its higher concentration is injurious to plant via lipid peroxidation and membrane injury. Water stress increased Glutathione Peroxidase activity in wheat grains [43]. Sairam and Saxsna [48] studies on three wheat cultivars indicated that water stress increased lipid peroxidation and reduced membrane resistance, the chlorophyll, carotenoid, antioxidant enzymes, ascorbate peroxidase, glutathione reductase and peroxidase significantly. The activity of peroxidase was determined according to Macheix and Quessada [35].

Effect of drought stress on Malondialdehyde (MDA) content

The effects of drought stress on the levels of lipid peroxides (i.e., MDA content) in leaves of anis hyssop are shown in Table 4. According to the results, the malondialdehyde levels were found to be significantly ($P \leq 0.05$) higher in the water deficit stress levels (mild, moderate and severe) than optimum condition of irrigation.

In other words, the lowest content of MDA was obtained at well-water treatment (24.67 mM/mg protein). However, it peaked under mild and moderate drought stress (39.23 mM/mg and 39.4 mM/mg proteins respectively). In fact, the highest MDA content was obtained under severe drought stress (62.47 mM/mg protein), but as drought stress levels increased [100% FC (vegetative stage) 85% FC (reproductive stage) and 100% FC (vegetative stage) 70% FC (reproductive stage)], the MDA activity sank significantly to 25.53 mM/mg protein for the former and 25.7 mM/mg protein for the latter. However, at 85% FC (vegetative stage) and 100% FC (reproductive stage), the activity of MDA rose widely to 38.53 mM/mg of protein.

Malondialdehyde (MDA), as a breakdown product resulting from lipid peroxidation, has been used as an index for the occurrence of oxidative stress [38]. The MDA content was significantly higher in maize cultivars both under non-stress and water-stress conditions. The rise in MDA content under stress conditions suggests that water stress could induce membrane lipid peroxidation by means of reactive oxygen species (ROS) [48]. Nayar and Kaushal [43] showed that drought stress enhanced MDA content in wheat grains. Moreover, our results are in agreement with Masoumi et al. [38], which showed drought stress significantly increased MDA content in all soybean cultivars.

Effect of drought stress on Di-Tyrosine (Di–Tyr) content

As regards di-tyrosine content (mM/mg protein), it varied widely (Table 4). In fact, drought stress increased significantly (P≤0.05) Di-Tyr content. The highest content of Di-Tyr was obtained under severe drought stress (29.57 mM/mg protein). However, well-watered plants had the lowest Di-Tyr content (11.43 mM/mg protein). There was a significant difference between Di-Tyr content at under severe drought stress and all other treatments, but there were no significant difference between Di-Tyr content under other drought stress levels. According to Nayar and Kaushal [43] results, Di-Tyr content in wheat grains enhanced under water stress condition. Furthermore, these results agreed with results of Masoumi et al. [38]; di-tyrosine levels were found to be significantly higher in the water deficit stress levels than optimum condition of irrigation in soybean cultivars. The elevation of MDA and di-tyrosine in our experiment could be a direct reflection of an oxidative injury of the cells after water deficit stress.

Effect of drought stress on Abscisic Acid content (ABA)

In this study, the ABA content increased significantly in the leaves of Anise Hyssop with an increase in intensity of water deficit stress. According to the biosynthesis of ABA (ppm), well-watered plants had the lowest ABA content (Table 5). However, ABA content peaked under moderate drought stress (42.73ppm). In other words, ABA content enhanced 1.6-fold under severe drought condition.

On the other hand, ABA content decreased to 30 ppm at 100% FC (vegetative stage) 85% FC (reproductive stage). There was a significant rise (P≤0.05) between ABA content at 100% FC (vegetative stage) 85% FC (reproductive stage) and 85% FC (vegetative stage) 100% FC (reproductive stage) levels (30 ppm and 41.93 ppm respectively).

The amount of abscise acid in plant leaves of maize increased under drought stress [7], 1975). ABA accumulated up 10 to 30-fold in plants under drought stress relative to unstressed conditions [19]. Heidari and Moaveni (2009) reported that, the increase in ABA content is suggested to be associated with maintenance of growth of roots and shoots under water stress due to suppression of ethylene in case of maize. Lobato et al. [34] have been found a positive and significant correlation between content of antioxidants with accumulation of ABA in soybean cultivars. Masoumi [38] reported that there is a positive and significant correlation between ABA, antioxidant enzymes, at the extreme severe deficit stress.

Table 5: Analysis of variance of the effect of drought stress on the measured traits

SOV	DF	EO Yield	SOD	CAT	GPX	MDA	Di-Ty	ABA
Replication	2	0.01^{ns}	1618.857^{ns}	25367.935^{ns}	3.939^{ns}	3.918^{ns}	0.438^{ns}	3.443^{ns}
Drought stress	4	0.142**	203751.667**	24444.241**	440.419**	243.183**	45.593**	222.248**
Error	12	0.006	2179.357	2655.270	17.170	13.103	6.146	1.713
CV%		1.21%	4.93%	12.37%	8.96%	14.67%	19.42%	11.19%

Notes: ns – non significant; * – significant at P≤0.05; ** – significant at P≤0.01

Conclusions

The results showed that, water deficit stress significantly (P≤0.05) increases activities of antioxidant enzymes (Superoxide Dismutase, Catalase, Glutathione Peroxidase) as well as Essential Oil yield and Abscisic Acid content. Lipid and protein oxidation (malondialdehyde and dityrosine contents) also increased significantly under severe water deficit stress. According to the results, severe drought conduction (55% FC) is the optimum level of soil moisture to plant Anise Hyssop under water deficit stress.

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