



IMPACT OF DROUGHT STRESS ON SOME GROWTH, BIOCHEMICAL AND ANATOMICAL PARAMETERS OF *Thymus vulgaris* L.

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Received 19 September, 2018, Accepted 2 October, 2018

ABSTRACT

Two pot experiments were conducted on the 17th and 19th of march during 2015 and 2016 growth seasons respectively at the greenhouse, Dept. Agric. Botany, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt to investigate the impact of drought stress on some growth, biochemical and anatomical parameters of *Thymus vulgaris* L. Plants were exposed to two different irrigation levels: 70-80% and 30-40% of water holding capacity (WHC) as well-irrigated and drought stressed plants respectively. The results indicated that all investigated growth parameters and leaf photosynthetic pigments were decreased significantly by exposing to drought stress. There were significant increase in the concentration of reactive oxygen species (ROS) as indicated by measuring of H₂O₂ and malondialdehyde (MDA). A similar trend was observed in respect to osmolytes including proline, total soluble sugars and free amino acids. Also, drought stress increased significantly total soluble phenols and the specific activity of Phenylalanine ammonia-lyase (PAL) and Polyphenol oxidase (PPO), whereas, no significant differences were detected in peroxidase (POD). The anatomical examination showed that there were several significant changes associated with water limited supply in the stem and leaf of plants.

Keywords: *Thymus vulgaris*, drought, pigments, ROS, osmolytes, antioxidant enzymes and anatomical parameters.

INTRODUCTION

Thyme (*Thymus vulgaris* L.) is considered one of the most ubiquitous cultivated plants worldwide.

It has several of aromatic and medicinal properties. The leaves could be used fresh or dried as a flavoring agent in many culinary applications. They own a high ratio of minerals (K, Ca, Mg, Fe, Mn, and Se), antioxidants (flavonoids, phenolic compounds like pigenin, naringenin, luteolin, thymonin, lutein, and zeaxanthin) and vitamins (A, B6, B9, C, E and K) (Alireza et al 2015 and Dauçan & Abdullah, 2017). Moreover, the volatile oil of common thyme (obtained by distillation of the fresh leaves and flowering tops) contains 20-54% thymol (monoterpene) which is considered the main active ingredient with numerous pharmacological properties (Fachini-Queiroz et al 2012 and Nikolić et al 2014).

Drought Stress is considered the most environmental challenges which represent a serious threat to agriculture worldwide, it cause a considerable loss in the yield and quality for many economical crops. Plants can respond to drought stress at morphological, anatomical, physiological, biochemical and molecular levels with modifications that allow the plant to avoid the stress or to increase its tolerance (Smirnoff, 1993 and Ibrahim et al 2015). These responses include substantially changes in the content of reactive oxygen species (ROS) and the activity of antioxidant systems (enzymatic and non-enzymatic) that minimize or preventing the occurrence of oxidative damage under stress conditions (Ibrahim et al 2016). Among these antioxidants, phenolic compounds and their related enzymes (PAL, PPO and POD) have received considerable attention for being potentially protective factors against drought stress (Ibrahim et al 2015). Moreover, drought stress affects leaf water status, pigments and stomatal conductance leading to inhibit photosynthesis

(Farooq et al 2009 and Saraswathi & Paliwal, 2011).

Increasing plant resistance to drought stress would be the most economical approach to improving agricultural productivity and reducing agricultural use of fresh water resources (Xiong et al., 2006). Therefore the purpose of the present study was to evaluate the mechanism/s of drought tolerance in thyme plants by investigation of several parameters including growth, biochemical compounds and anatomical features.

MATERIALS AND METHODS

Plant materials and growth conditions

Two pot experiments were conducted on the 17th and 19th of march of 2015 and 2016 respectively at the greenhouse, Dept. Agric. Botany, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Egypt to investigate the impacts of drought stress on some growth, biochemical and anatomical parameters of *Thymus vulgaris* L. Plants were exposed to two different irrigation levels initiated after 20 days after planting (DAP). The well-irrigated plants had kept at 70-80% of water holding capacity (WHC) whereas; the drought stressed ones subjected to 30-40% of WHC. Water holding capacity of the experimental soil was determined using different calibrated tensiometer to each level of irrigation.

Uniform thyme plants (*Thymus vulgaris* L.) were purchased from a private garden in Qalubia governorate, Egypt; after that plants were identified at Flora and Phytotaxonomy Researches, Horticultural Research Institute, Agricultural Research Center; Egypt to know their systematic position according to Engler's system as represented by Melchior (1964). Uniform thyme plants were transplanted in 20 cm x35 cm plastic pots (one plant/pot) filled with 8 kg sandy- loam soil. The experimental design was Completely Randomized Block Design (CRBD) with three replicates, each one contains 7 pots.

Growth parameters

The growth parameters including root, shoot fresh weight and root/shoot fresh weight ratio were recorded at 60 days after planting (DAP).

Biochemical constituents

Leaf pigments

Leaves sample at 60 (DAP) were collected to determine plant pigments according to the method of Lichtenthaler and Buschmann (2001) with some modifications. 0.2 g of fresh leaves was homogenized with acetone 80% in dark at room temperature. Absorbance was measured in a UV/ VIS spectrophotometer. The concentrations were calculated using the following equations:

$$\text{Chl a } (\mu\text{g/ml}) = 12.25 A_{663.2} - 2.79 A_{646.8}$$

$$\text{Chl b } (\mu\text{g/ml}) = 21.50 A_{646.8} - 5.10 A_{663.2}$$

$$\text{Total Chl} = \text{Chl a} + \text{Chl b}$$

$$\text{Carotenoids } (\mu\text{g/ml}) = (1000 A_{470} - 1.82 \text{ Chl a} - 85.02 \text{ Chl b}) / 198$$

After that the calculations were done as mg. g⁻¹ f.wt

Reactive oxygen species (ROS)

a- Lipid peroxidation (MDA)

The level of lipid peroxidation of thyme plants at 60 (DAP) under the stressed and the well-irrigated plants was measured in the leaves as malondialdehyde (MDA). Content was determined by reaction with 2-thiobarbituric acid (TBA) reactive substances as described by Giannakoula et al (2008), according to Heath and Packer (1968). Tissue was homogenized in 0.3% TBA in 10% trichloroacetic acid (TCA) at 4 °C. The concentration of MDA was calculated from the difference of the absorbance at 532 and 600 nm spectrophotometrically using the extinction coefficient of 155 mmol⁻¹ cm⁻¹ and expressed as nmol (MDA) g⁻¹ fresh weight.

b- Hydrogen peroxide (H₂O₂) concentration

The colorimetric determination of hydrogen peroxide concentration at 390 nm in leaf tissues using potassium iodide was assayed as described by Loreto and Velikova (2001). A half gram of leaves at 60 (DAP) was homogenized in 3 mL of 1% (w/v) tri-chloroacetic acid (TCA). The homogenate was centrifuged at 10,000 rpm at 4°C for 10 min. Subsequently, 0.75 mL of the supernatant was added to 0.75 mL of 10 mM K-phosphate buffer (pH 7.0) and 1.5 mL of 1M KI. A Standard curve of H₂O₂ was done to calculate its concentration (μmol g⁻¹ f.wt) in plant tissues.

Determination of osmolytes

a- Proline

Leaves from both irrigation treated plants at 60 (DAP) were cut into small pieces, weighed, placed separately in glass vials containing 10 mL of 80% (v/v) ethanol, and heated at 60 °C for 30 min. The extract was then filtered and diluted with 80% (v/v) ethanol up to 20 mL. The concentration of free proline ($\mu\text{ mol.g}^{-1}$ f.wt) was determined in this extract as described by **Giannakoula et al (2008)**, following the acid-ninhydrin reagent method (**Bates et al 1973**).

b- Free amino acids

Free amino acids (mg.g^{-1} f.wt) were determined according to **Hamilton and Van Slyke (1943)**. One ml of each sample extract was treated with 1ml of 10% pyridine and 1 ml of 2% ninhydrine solution. The optical densities of these colored solutions were then read at 570 nm. Glycine solution were prepared as standard.

c- Total soluble sugars

Ethanol extracts from each treatment of thyme leaves as used for proline assay, were diluted 10 times with 80% (v/v) ethanol for the assay of soluble sugars (mg.g^{-1} f.wt) as described previously by **Giannakoula et al (2010)**. Briefly, the diluted extracts were added, drop-by-drop, in 2 mL anthrone reagent test tubes in an ice bath and left to mix the content. Fully mixed samples were incubated in a water bath at 90 °C for 15 min and cooled, and absorbance was read at 625 nm using a UV spectrophotometer.

Determination of total soluble phenols

The extraction of dry leaves in cold MeOH 85% was used to determine total soluble phenols (mg.g^{-1} f.wt) according to the method of Folin-Denis as described by **Shahidi and Naczki (1995)** by reading the developed blue color at 725 nm using spectrophotometer.

Enzyme assays

To prepare the extraction of enzymes and soluble proteins, fresh leaves were homogenized in 5 mL phosphate buffer (0.1 M, pH 7.8), centrifuged at 10,000 \times g for 20 min at 4 °C and then the su-

pernatant was used for assays. Soluble proteins were evaluated by the method of **Bradford (1976)**.

a- Polyphenol oxidase (PPO)

Polyphenol oxidase (PPO) (EC 1.14.18.1) activity was measured according to **Oktay et al., (1995)**. The reaction mixture consisted of 100 μL crude enzyme, 600 μL catechol and 2.3 ml phosphate buffer (0.1 M, pH 6.5). The absorbance at 420 nm was recorded at zero time and after 1 min. One unit of PPO activity was defined as the amount of enzyme that caused an increase in absorbance of 0.001 per min at 420 nm. The enzyme activity was expressed as unit. mg^{-1} protein.

b- Phenylalanine ammonia-lyase (PAL)

The activity of phenylalanine ammonia-lyase, PAL (E.C 4.3.1.5) was assayed as described by **Lister et al (1996)**. The PAL assay reaction consisted of 100 μL crude extract and 900 μL of 6 μmol phenylalanine in 500 mM tris-HCl buffer (pH 8.5). The mixture was incubated at 37 °C for 1 h and measured spectrophotometrically at 290 nm. Trans- cinnamic acid was used as standard.

c- Peroxidase (POD)

The activity of peroxidase, POD (EC 1.11.1.7) was assayed according to the method of **Dias and Costa (1983)** with some modifications. The following reaction mixture (3 ml) consisted of 10 mM sodium phosphate buffer, pH 7.0, 50 μL enzyme extract and guaiacol 1% (v:v) aqueous solution. The reaction was started by adding H_2O_2 at 100 mM. The changes of optical density at 470nm were recorded in a spectrophotometer. Unit of enzyme (IU) equal $0.01\Delta\text{ OD. min}^{-1}$. The specific activity expressed as $(\text{IU.min}^{-1}.\text{mg}^{-1}\text{ protein})$.

Anatomical study

Specimens of the unstressed and stressed plants (leaves and stems) were collected. Plant samples were fixed in FAA (formalin, acetic acid and 70% ethyl alcohol, 5:5:90/100 ml) for 24 h at room temperature. Then it dehydrated and processed using the schedule of the paraffin method according to **Johansen (1940)**. Transverse sections (12 μm) in thickness were made by LEICA rotary microtome model RM 2125 RTS and fixed on slides by means of Haupt's adhesive **Sass (1951)**. Double stained with Safranin-Fast green combination were made and then mounted in Canada balsam **Sass (1951)**. Anatomical exami-

nation and measurements were achieved using a LEICA light research microscope model DM-2500 supplied with a digital camera

Statistical analysis

Data of the two seasons were arranged and statistically analyzed using **SAS Institute Inc. (1988)** software. The comparisons among means of the different treatments were determined as illustrated by **Snedecor and Cochran (1982)**. Standard deviation of the means (SD) was calculated and Duncan's test based on a probability of $P \leq 0.05$ was used to determine significant differences between means.

RESULTS AND DISCUSSION

Changes in the growth parameters

As shown in **Figure (1)** water deficit caused significant ($P \leq 0.05$) decreases in all studied growth parameters of the thyme plants including shoot fresh weight, root fresh weight and root/shoot fresh weight ratio compared to the well-irrigated ones in both seasons. These results were confirmed in many species that belong to family Lamiaceae including *Thymus vulgaris* (**Aziz et al 2008**); *Majorana hortensis* Moench (**Massoud et al 2010**); *Ocimum Sp.* (**Dos Santos et al 2016**) and *Satureja hortensis* (**Miranshahi and Sayyari, 2016**).

Drought stress is considered one of the most limiting factors for plant growth and development because it affects cell turgor, enzyme activities and energy supply which required for different phases of plant growth and development including cell division, elongation and differentiation (**Taiz and Zeiger 2002**).

Furthermore, drought stress can trigger multiple of molecular, biochemical and physiological responses that affect negatively plant growth and development; among these effects: (1) increasing the level of reactive oxygen species (ROS) in plant tissues leading to a series of oxidative damage for most plant cell components like proteins, lipids and nucleic acids (**Impa et al 2012 and Noctor et al 2014**), (2) inhibiting the photosynthesis through impairing the electron transport systems inside the chloroplast and destroying its pigments. Moreover, reducing water supply limits the entry of CO_2 and the rate of its carboxylation reaction by inducing the partial stomatal closure and affecting the effi-

ciency of RuBisCO (**Smirnov, 1993**), (3) changing the level of endogenous phytohormones such as ABA, ethylene, GA_3 , cytokinins and auxins and altering their complex relationships with each other and consequently their dynamic and complementary actions which related to plant growth and development (**Taiz and Zeiger 2002; Bielach et al., 2017**).

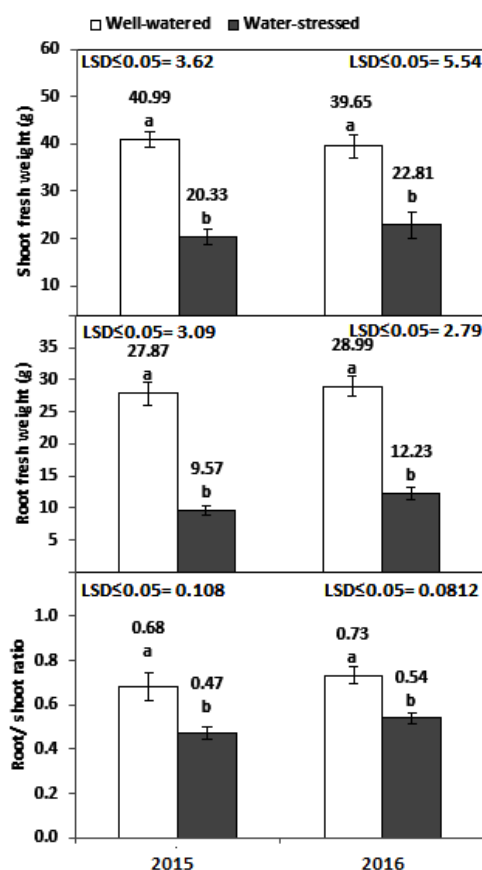


Fig. 1. Effect of drought stress on some growth parameters of thyme plants during the seasons of 2015 and 2016.

Changes in the leaf pigments

Data in **Figure (2)** illustrate that water stressed plants reduced significantly ($P \leq 0.05$) the concentration of chlorophyll a, b and total chlorophyll in both seasons compared to the well-irrigated plants in both seasons.

This reduction might be attributed to increase the production of reactive oxygen species (ROS),

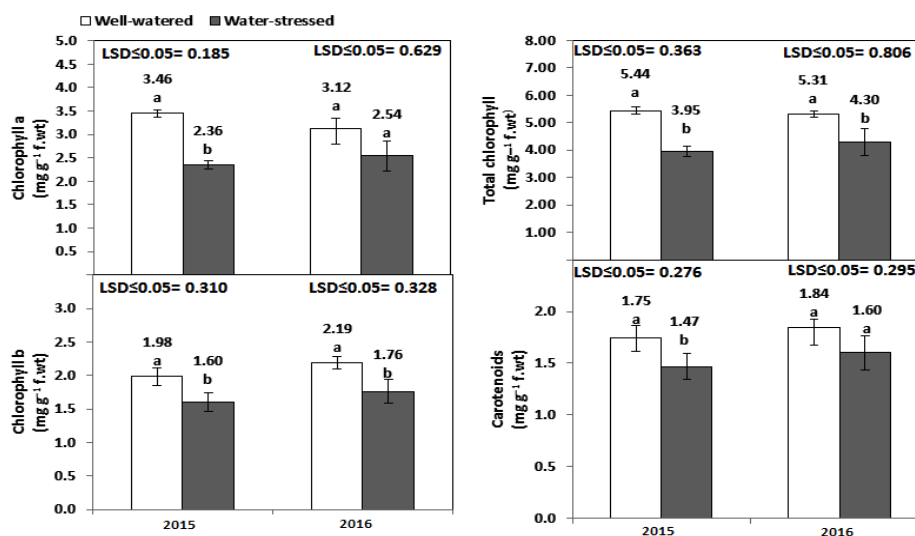


Fig. 2. Effect of drought stress on leaf pigments of thyme plants during the seasons of 2015 and 2016.

which in turn displayed oxidative stress of chloroplast membranes during water deficit. These results were in agreement with **Smirnov (1993)**. Another explanation could be suggested in this respect; this reduction may be considered as an important regulatory step to avoid the exceeding light absorbance and to restrict the over reduction of photosynthetic electron transport chain and consequently the generation of ROS. (**Mazars et al 2010**).

As well as thyme plants under water stress showed an obvious reduction in the concentration of carotenoids compared to the unstressed ones in both seasons. This effect reached the level of significance in the first season compared to the control. This response could be occurred through highly uses of carotenoids to build up more ABA particularly under water stress, which it is considered the major signal to drought stress (**Taiz and Zeiger, 2002**).

Changes in the Hydrogen peroxide (H₂O₂) and lipid peroxidation

Reactive oxygen species (ROS) has been reported as being products of both biotic and abiotic stresses (**Reddy et al 2004**). These molecules are responsible for most the oxidative damages in the biological structures including DNA, RNA, amino acids, protein and lipid (**Johnson et al 2003**). Therefore plants under stresses possess efficient

antioxidant systems that minimize or preventing the occurrence of oxidative damage.

Data presented in **Figure (3)** show that there were significant increases in the concentration of H₂O₂ and the level of Lipid peroxidation as indicated by increasing the concentration of malondialdehyde (MDA) in leaf tissues of the drought-stressed plants compared to the well-irrigated ones in both seasons.

Hydrogen peroxide (H₂O₂) is one of the most stable ROS which produced in plant cell during different physiological processes including photosynthesis, photorespiration and relatively to a lesser extent in respiration; it plays an important role as a signaling molecule under stressful conditions (**Ślesak et al 2007**). This response could help plants to develop their defense mechanisms against various stressful factors either biotic or abiotic ones. It can induce defense genes which required in the induction of systemic acquired resistance (**Kuzniak and Urbanek, 2000**).

Malondialdehyde (MDA) is also widely known as a biochemical marker to increase the activity of ROS and the oxidative stress in plant tissues under adverse conditions. It is considered the most final product of lipid peroxidation and an important indicator to the oxidative damage which could be occurred to the cellular membranes under different stresses conditions (**Angel Catala, 2012**). In this study, the increases in the concentration of MDA in the drought-stressed plants could be attributed to increase ROS especially H₂O₂.

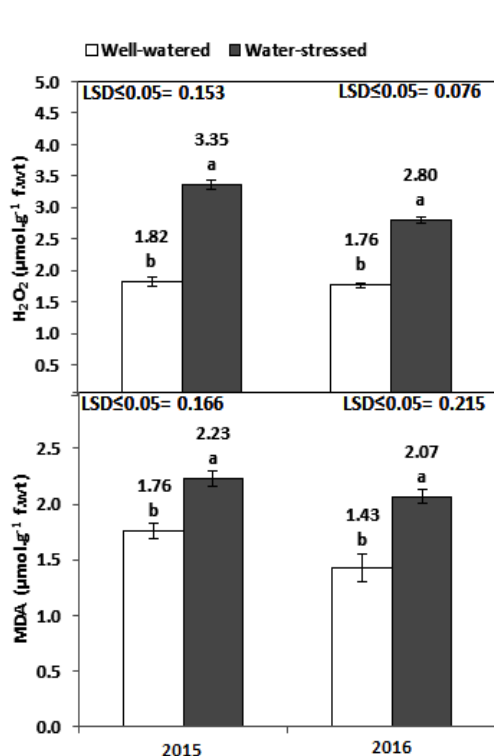


Fig. 3. Effect of drought stress on the concentration of H₂O₂ and malondialdehyde (MDA) of thyme plants during the seasons of 2015 and 2016.

Changes in the osmolytes

Data in **Figure (4)** show that plants under drought stress revealed a considerable and significant ($P \leq 0.05$) increases in the soluble sugars, proline and free amino acids concentration compared to those of the well-irrigated ones in the two seasons.

Generally, it is well established that accumulation all of these organic substances which are known totally osmolytes or compatible solutes is very an essential and a common key response for plants to counteract the adverse effects of water deficit (**Oraki et al 2012 and Ibrahim, 2014**). They can adjust the osmotic potential leading to increase the absorption of water and improve tolerance capacity of plants to drought stress (**Misra & Gupta 2005 and Naz et al 2016**). Moreover, it can protect the vital enzymes and the other different plant cell components from the fatal oxidation by scavenging ROS (**Farooq et al 2012**)

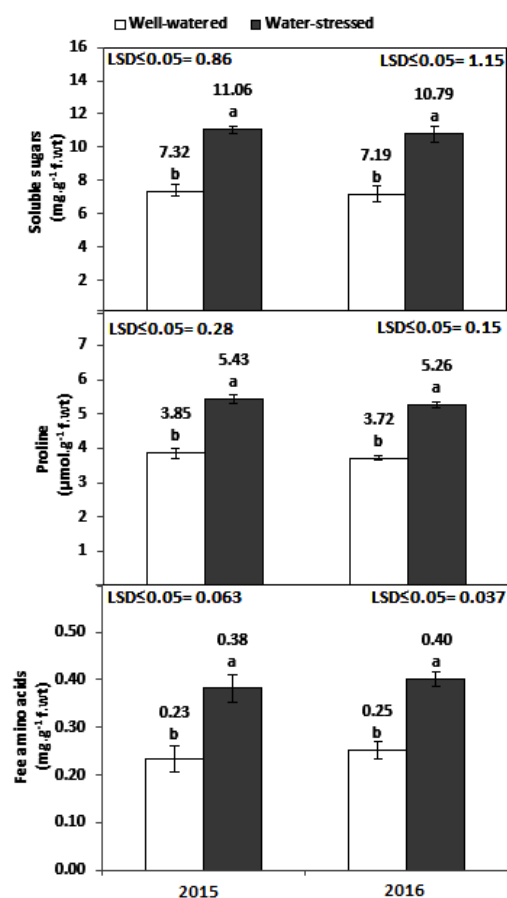


Fig. 4. Effect of drought stress on the concentration of total soluble sugars, proline and free amino acids of thyme plants during the seasons of 2015 and 2016.

Changes in the Phenolic compounds and their related enzymes

Data presented in **Figure (5)** show that total soluble phenols revealed a significant increase in the water- stressed plants compared to the well-irrigated ones in both seasons. A similar trend was observed in the specific activity of phenylalanine ammonia-lyase (PAL) in the first season whereas, in the second season, these increases did not reach the level of significance.

The increases in the total soluble phenols could improve the antioxidant capacity, scavenging systems for ROS which act as a carbon sink in plants under water stress (**Weidner et al 2009**) The substantial progress in the PAL activity of the stressed plants is considered an important regulatory step in the formation of many phenolic compounds and secondary metabolites in plants which play many

considerable roles under stress conditions. This reaction occurs via the elimination of an ammonia molecule from phenylalanine to form cinnamic acid (Taiz, and Zeiger, 2002).

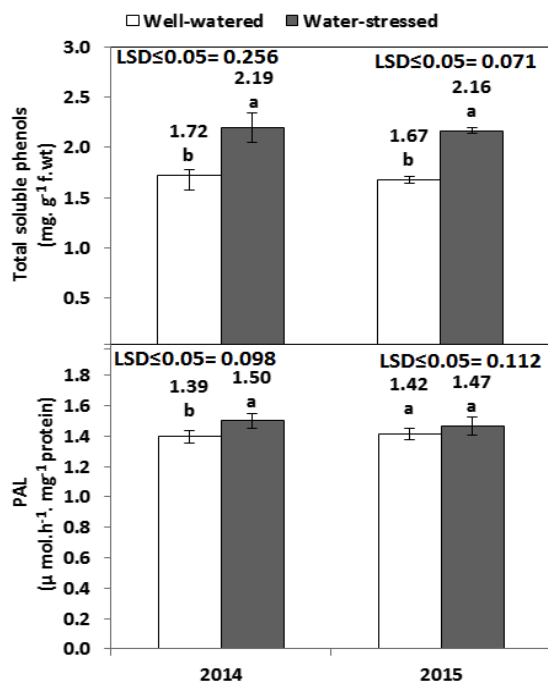


Fig. 5. Effect of drought stress on the concentration of total soluble phenols and the specific activity of phenylalanine ammonia-lyase (PAL) of thyme plants during the seasons of 2015 and 2016

Moreover, data presented in Figure (6) indicate that the specific activity of polyphenol oxidase (PPO) in the leaf tissue of *Thymus vulgaris* L was increased significantly by exposing to drought stress compared to the well-irrigated plants in both season. PPOs are ubiquitous group of enzymes in angiosperms, they are inducible by both biotic and abiotic stresses, and have been implicated in several physiological processes including plant defense against different stresses. They are involved in the mehlner reaction, photoreduction of molecular oxygen by PSI, regulation of plastidic oxygen levels and deriving of the phenylpropanoid pathway (Thipyapong et al 2007). Also, in this study, increasing the specific activity of PPO under drought stress may be related to the considerable synthesis of total soluble phenols (Figure, 5), many others phenolic compounds and secondary metabolites which are considered the substrate for PPO.

Although, it is well documented that peroxidase (POD) has been implicated in several metabolic

processes related to induce resistance to various stress conditions by including lignin synthesis, phenol oxidation (Sancho et al 1996), increasing cross-linking proteins (Perez et al 2006) and another plant cell wall cross-linking reactions (Ralph et al 2004); but In this study, no significant differences were detected in the specific activity of peroxidase (POD) between the both investigated levels of irrigation in both seasons. This effect might indicate that *Thymus vulgaris* L as an aromatic plant may only depends on PAL and PPO in its secondary metabolites pathways more than POD.

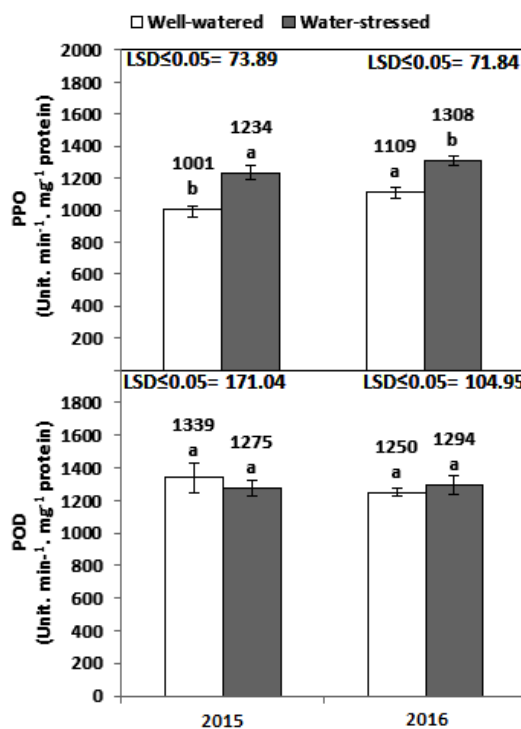


Fig. 6. Effect of drought stress on the specific activity of polyphenol oxidase (PPO) and peroxidase (POD) of thyme plants during the seasons of 2015 and 2016

As shown in Table (1) and Plate (1), it can be observed that all investigated anatomical characteristics of the leaf of thyme plants except % palisade tissue area and the cuticle thickness were decreased significantly by exposing to drought stress. These decreases reached 75.8% in the cross section area, 80.7% in the mid vein vascular bundle area, 72.5% in the average of xylem diameter and 45.8% in the number of xylem vessels compared to the well-irrigated plants.

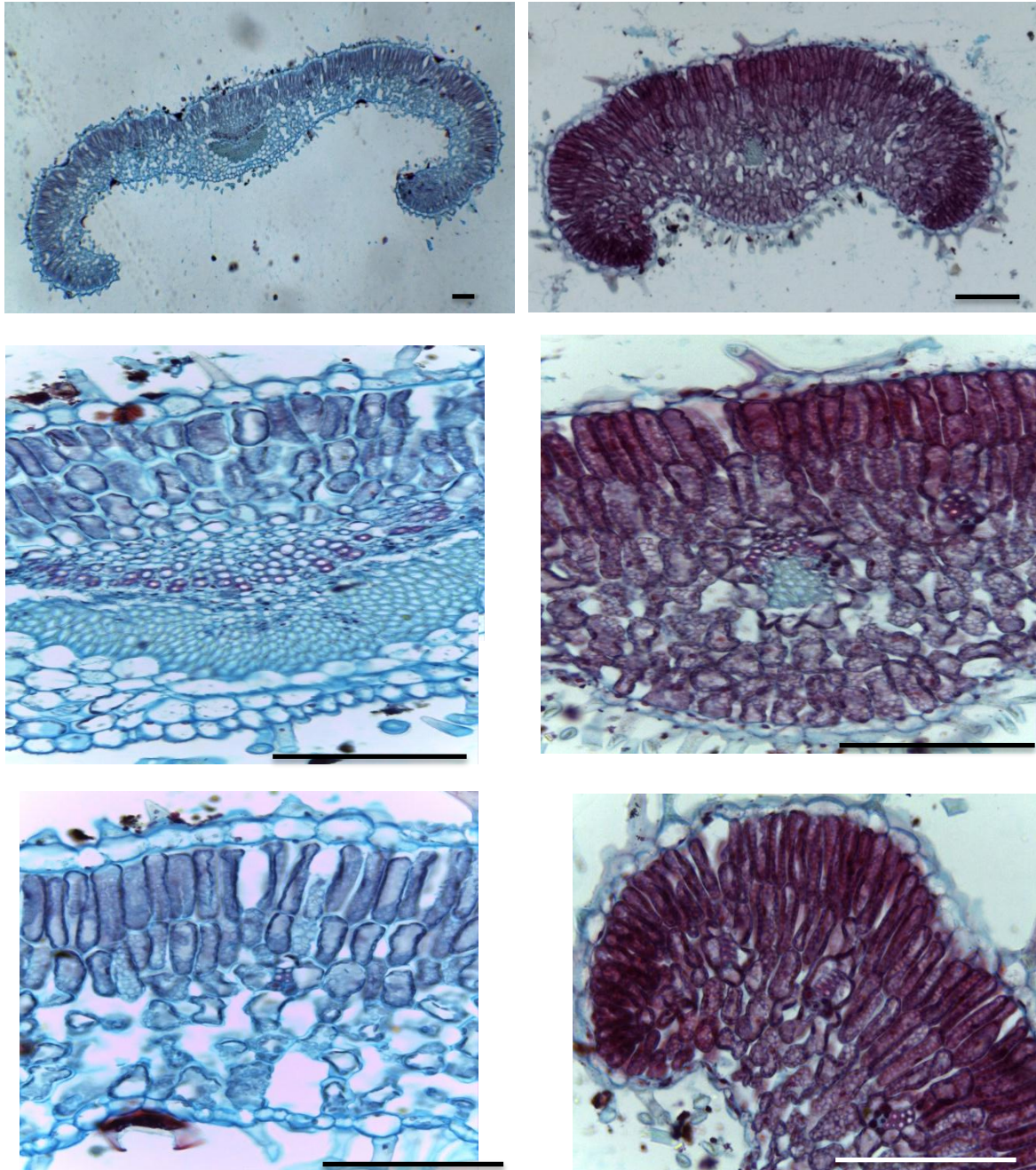


Plate 1. Transverse sections of thyme leaf plants illustrating the anatomical changes resulting from water stress: (A,B) cross-section area , (C,D) midvein vascular bundle, (E,F) palisade tissue. (Well-watered plants on the left and water-stressed plants on the right respectively). (Bar= 100 μ m).

Table 1. Effect of drought stress on some anatomical features of leaf of thyme plants at 60 DAP.

Variable	Well-watered	water-stressed	LSD \leq 0.05
Cross section area (mm ²)	0.653 \pm 0.019 a	0.158 \pm 0.029 b	0.0562
Mid vein vascular bundle area (μ m ²)	8467 \pm 343 a	1637 \pm 87 b	568
% palisade tissue area (μ m ²)	50.25 \pm 3.92 b	63.08 \pm 1.33 a	6.64
Average of xylem diameter (μ m)	4.29 \pm 0.27 a	1.18 \pm 0.09 b	0.449
Number of Xylem vessels	29.5 \pm 0.5 a	16.0 \pm 1.0 b	1.79
Cuticle thickness (μ m)	0.79 \pm 0.01 b	1.70 \pm 0.12 a	0.193

Means followed by different letters are significantly different at $P \leq 0.05$ according to LSD's multiple range test

Respecting the effect of drought stress on the anatomical characteristics of the stem of *Thymus vulgaris* (Table 2 and Plate 2), it can be observed that drought stress caused significant decrease in all studied parameters including cross section area (30.2%), vascular bundle area (36.7%), xylem area (27.6 %) and average of xylem diameter (40.6%) compared to the well-irrigated plants.

Table 2. Effect of drought stress on some anatomical features of stem of thyme plants at 60 DAP.

Variable	Well-watered	water-stressed	LSD \leq 0.05
Cross section area (mm ²)	0.652 \pm 0.009a	0.455 \pm 0.01b	0.0213
Vascular bundle area (mm ²)	0.308 \pm 0.002a	0.195 \pm 0.003b	0.0564
Xylem area (mm ²)	0.217 \pm 0.005a	0.157 \pm 0.007b	0.0138
Average of xylem diameter (μ m)	8.37 \pm 0.44 a	4.97 \pm 0.24b	0.799

Means followed by different letters are significantly different at $P \leq 0.05$ according to LSD's multiple range test

Generally, plants have three different strategies to respond to the abiotic stresses at the anatomical level: (1) inhibition of cell elongation, (2) localized stimulation of cell division and (3) alterations in cell differentiation status (Patakas, 2011).

Based on the above mentioned results, it could be claimed that drought stress affects negatively plant growth and alters many of biochemical components including leaf pigments, ROS, osmolytes, phenolic compounds and some of phenolic related enzymes. These effects could explain the changes that occurred in the leaf tissues at the anatomical level, For instance, enhancing the production of ROS as indicated by increasing the concentration of MDA and H₂O₂ (Figure,3) could serve as signal-

ing molecules and may contribute in controlling some of developmental processes (Gapper and Dolan 2006). In particular, ROS is reported to influence cell development (e.g., xylem vessel formation; (Ros-Barcelo et al 2002), cell division (i.e., temporarily inhibiting cell cycle activity; Reichheld et al 1999), cell elongation (Schopfer, 2001).

Another possibility could be suggested to explain the effect of drought stress on plants at the anatomical level, several previous studies on many plant species have reported that drought stress causes diverse and complex changes in the hormonal homeostasis between plant growth promoters (IAA, GA3 and CKs) and inhibitors (ABA, ethylene) in the stressed plants. These responses are tightly linked to altering cell elongation, cell division, and reorientation of growth (Schradler et al 2003)

In addition, the cortex of stem consists of 5-7 thin-walled parenchyma cell layers with small inter-cellular spaces. The innermost cortex (endodermis) consists of thin-walled compacted of parenchyma without any specialized feature of casparian bands. Drought causes a well-developed endodermis with noticeable casparian bands on the radial walls. It is known that the casparian bands increase underdrought conditions and may help in avoid the desiccation of steller tissue (plate 2 Fig. C, D).

The above mentioned data showed the stressed plants, have an increasing into phenolic compounds which considered as intermediate compounds in the biosynthesis of lignin. This result reflected in the stem internal structure. Through increasing lignification of stellar cells consequently prevent the stem from the collapsing and reducing the risk of mechanical damage during water deficit.

Drought stress causes significant decrease in the average of inner diameter of xylem vessels in stems and leaves.

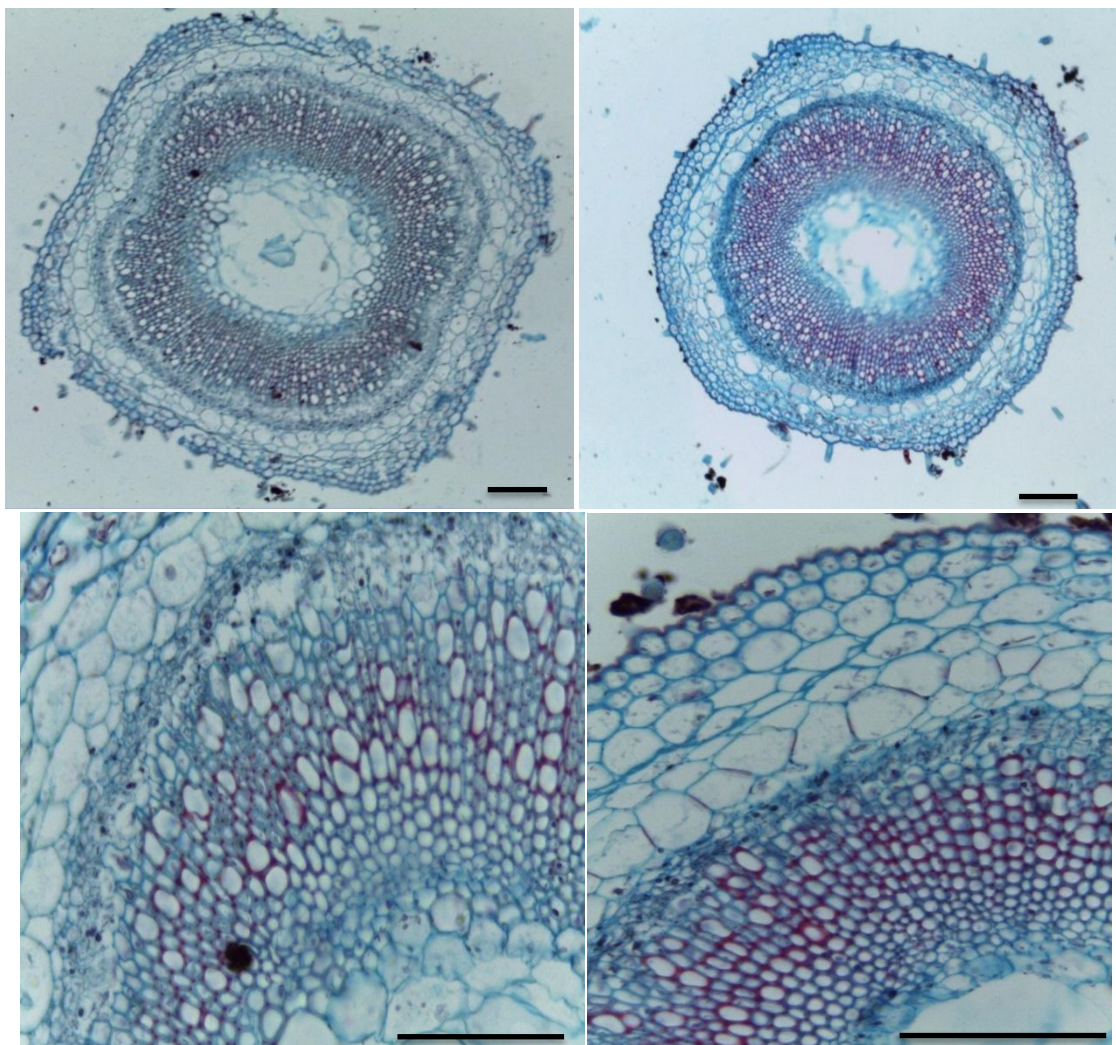


Plate 2. Transverse sections in the thyme stem plant showing the anatomical changes resulting from water stress: (A,B) cross-section area and (C,D) reveals the xylem area and average of xylem diameter (Well-watered plants on the left and water-stressed plants on the right respectively). (Bar= 100 μ m).

This result may be benefit under drought condition because the narrow vessels allow slow water flow since they guarantee water transport also when larger vessels are embolised (**Abdelbar, 2015**).

Our result indicated that the phloem and xylem tissue areas were decreased under water stress conditions. Similar results were reported by in many previous studies (**Kuutlu et al 2004 and Abdelbar & Abd.El-Maboud 2013**). These differences may be conceived as an adaptation to stress and related to loss of water.

In addition, in this study, the significant increase in % palisade tissue area under drought

stress may help plants to adapt to inadequate water supply and keep suitable rate of photosynthesis because the thicker palisade parenchyma could contain larger numbers of CO₂-fixation sites (**En-najeh et al 2010**).

Cuticle is thought to play a critical role in plant drought tolerance through its ability to restrict transpiration or postpone the onset of cellular dehydration stress during drought (**Samuels et al 2008**). In this investigation, the increasing in the cuticle thickness of the leaf epidermal cells under drought stress could be attributed to induction more of cuticle lipids which was associated with reduced cuti-

cle permeability and may be important for plant acclimation to subsequent water-limited conditions (Kosma *et al.*, 2009).

Conclusion

According to the results of this study, it can be concluded that thyme plants (*Thymus vulgaris* L.) responded to drought stress by altering several of growth, biochemical and anatomical parameters which totally can help plant to tolerate and survive under water deficit condition.

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