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ORIGINAL ARTICLE

Involvement of abscisic acid on antioxidant enzymes activity and gene expression in *Lavandula angustifolia* cv. Munstead under drought stress

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Abstract

Climate change combined with drought stress, as a consequence of this global phenomenon, is among the most unfavorable factors that could limit the growth and production of crops worldwide and pose a serious threat to the production of sustainable crops. Since several plant physiological and biochemical relationships under drought conditions remain unknown, it may be possible to obtain more drought-tolerant plants by knowing these details. An effective management strategy involves the use of exogenous substances, increasing the expression of resistant genes, and increasing the production of enzymes that help the plant to withstand damaging environmental conditions such as drought. Experimental treatments consisted of four levels of irrigation regimes and foliar application of abscisic acid (ABA) at two concentrations, with three replicates. The results showed that drought in 30–40% field capacity increased the antioxidant activity by DPPH, ABTS, FRAP, and TAOC methods; and the antioxidant enzymes of catalase (CAT), superoxide dismutase (SOD), peroxidase (POX), and ascorbate peroxidase (APX), significantly. Foliar application of ABA on *Lavandula angustifolia* cv. Munstead improved plant metabolism under stress conditions, mildly. So that, the interaction between drought and ABA had a synergistic effect on antioxidant activity and related enzymes. The expression of *CAT*, *POD*, and *APX* genes decreased under drought stress, while spraying ABA ameliorated this decrease. The expression of the *SOD* gene tended to decline, and no drought treatment along with 15 µM ABA spraying revealed the highest expression. Hormone spraying, on the other hand, had a positive effect on this expression under stress compared to the control (no spraying). Overall, despite the unknown details of how radical scavenging functions in plants and the associated expression, these results suggest that ABA treatment reduced the inhibitory effects of drought stress and increased the tolerance of lavender under stress conditions.

Keywords Drought stress · Abscisic acid · Antioxidant activity · Antioxidant enzymes · Gene expression

Abbreviations

ABA	Abscisic acid
F.C.	Field capacity
I_1	90–100% Field capacity
I_2	70–80% Field capacity
I_3	50–60% Field capacity
I_4	30–40% Field capacity
A_1	0 µM
A_2	15 µM
A_3	30 µM
CAT3	Catalase3
APX	L-ascorbate peroxidase
SOD	Superoxide dismutase
POX	Peroxidase
ROS	Reactive oxygen species
APX	Ascorbate peroxidase
CAT	Catalase
ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid
DPPH	1-Diphenyl-2-picrylhydrazyl

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FRAP Ferric reducing antioxidant potential
TAOC Total antioxidant capacity

Introduction

Climate change and global warming are currently global issues leading to limited water resources and affecting biodiversity around the world (Resende et al. 2019). One of the biggest consumers of water in the world today is agriculture, which makes it one of the most vulnerable sectors in the water crisis (Kalamartzis et al. 2020). Agricultural productivity is typically affected by drought (Qi et al. 2018) and it is considered one of the major constraints to crop growth, yield, and crop production worldwide (González-Chavira et al. 2018; Abboud et al. 2020), especially in arid regions, which account for about 40% of the globe (Schlaepfer et al. 2017).

All plant physiological processes can be affected by drought stress such as photosynthesis, respiration, and assimilation (Basu et al. 2016; Qi et al. 2018). In addition to limiting the availability and uptake of nutrients on growth, other factors such as phenology, morphology, dry matter production, and secondary metabolites are also affected (Deikman et al. 2012; Modaresi Rad et al. 2016; Gorgini Shabankareh et al. 2018). Thereby, these adverse effects made drought an important factor in plant growth and development (Sun et al. 2018). Drought causes a significant increase in reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), superoxide (O_2), and hydroxyl radicals (OH), which are potentially harmful to all cellular components and lead to oxidative stress and lipid peroxidation (Camejo et al. 2016; Ali et al. 2021). In water-deficient environments, the production of ROS is a major factor in weakening plant growth (Vurukonda et al. 2016) by damaging proteins, lipids, nucleic acids, and carbohydrates, causing DNA mutations and ultimately leading to cell death (Dietz and Pfannschmidt 2011; Ghanbarzadeh et al. 2019). To avoid damage caused by the accumulation of ROSs due to its toxic nature and high reactivity, it is important to prevent its accumulation in plant tissues and intracellular organs (Tanveer and Shabala 2018).

An important mechanism to control ROS under drought stress and other abiotic stresses is the induction of antioxidant enzymes (Zare et al. 2018; Mirajkar et al. 2019). In plant cells, enzymatic antioxidants are essential for inactivating oxygen free radicals, so that their accumulation and activity inhibit membrane proteins and lipid peroxidation (Qiu et al. 2014). These enzymes include superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX), which are involved in the detoxification of superoxide and hydrogen peroxide. They also maintain the homeostasis of ROS in plants under normal and stress conditions (Kadkhodaie et al. 2014; Chrysargyris et al. 2018). SOD is the first defensive factor and the most important known enzyme against the toxic effects of ROS and the conversion of superoxide radicals into H_2O_2 and then conversion to other enzymes such as CAT, POD, and APX, which play the role of decomposing H_2O_2 into water and O_2 molecules and detoxifying it (Parida and Das 2005; Chagas et al. 2008; Gill and Tuteja 2010; Swapna and Shylaraj 2017).

Since plants are immobile organisms, they use phytohormonal regulatory networks as survival mechanisms to reduce the adverse effects of environmental stress by controlling biochemical and physiological activities (Rezaei Ghaleh et al. 2020). There is often a correlation between hormonal changes and plant behavior. In other words, hormones provide the necessary conditions for stress resistance under such situations (Gupta et al. 2017). Plants respond to stress by producing ABA, a type of sesquiterpene and a phytohormone; it is a key biochemical regulator in response to abiotic stress. Under stress and during grain maturity, its production will be increased (Singh et al. 2020). This increase and the binding of hormones to receptors results in signal transduction in the plant, leading to genetic changes that allow it to respond to stress (Ng et al. 2014). In addition, it can stimulate the transcription and activity of antioxidant enzymes (Wang et al. 2020). The levels of ABA increase significantly under drought and salt stress conditions, leading to stomatal closure, changes in gene expression, and physiological changes, and ultimately, adaptation (Qi et al. 2018).

English lavender (*Lavandula angustifolia* L.) is a fragrant Mediterranean perennial of southern European origin and belongs to the Lamiaceae family (Khorasaninejad et al. 2016; Heral et al. 2020). The main reason why lavender is cultivated is its abundant essential oil (EO) content. Because lavender is adaptable and flexible that can be grown in poor and dry soils, as well as for its aromatic compounds, it is widely used in the pharmaceutical, food, cosmetic, and health industries (Khorasaninejad et al. 2015). There are more than 50 varieties of lavender (Erland and Mahmoud 2015; Gorgini Shabankareh et al. 2021a). The best-known cultivars are 'Hidcote' (Monaghan et al. 2004) and 'Munstead' (Whitman et al. 1996). For this study, we chose the cultivar 'Munstead'.

As the drought in the world is spreading and medicinal plants such as lavender are gaining attraction due to their EO, the main metabolite of this plant, with high antioxidant properties, and also their production is considered as a defense mechanism to adapt to environmental stresses. In addition, the molecular nature of ABA and the decrease in the production flux of internal ABA under abiotic stress leads to increased production of EO as well as increased inhibition of free radicals (Mansouri and Asrar 2013). Therefore, the specific aim of this study was firstly to evaluate the antioxidant

properties of Lavandula in response to drought stress and external hormone spraying. Second, to investigate the function of antioxidant enzymes under these conditions and the dependence of free radical scavenging on enzyme systems. In addition, the effect of exogenous ABA on gene expression producing antioxidant enzymes CAT, POX, APX, and SOD was investigated to improve the quantity and quality of plant performance in arid soils.

Materials and methods

Plant materials and growth conditions

This study was carried out in Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran (36° 30′ N, 53° 57′ E) with an elevation of 155 m above sea level during 2017–2021. The Lavender seeds of *Lavandula angustifolia* L. cv. Munstead were provided by the Renees Garden® company in the USA.

To break the seed dormancy, the seeds were placed in May 2017 in a completely humid medium (cocopeat and perlit in a 1:1 ratio) for three weeks at 4 °C and in June 2017, the seeds were planted in the seedling tray at day temperature 26 °C and night temperature 22 °C and photoperiod 16 h of light and 8 h of darkness. The seed cultivation stage was done in the greenhouse, and since the seeds of this plant need a lot of light for germination, white and yellow fluorescent lamps were used during the day. About three months after the planting in the seedling tray (September 2017) to prepare the seedling, one plant was planted inside each pot (pots of 6 cm diameter and 10 cm height). After about four months, in January 2018, the seedlings were transferred to the main pots (plastic pots with the opening diameter of 30 cm and height of 40 cm) in the ten-leaf stage at day temperature 28 °C and night temperature 24 °C and photoperiod 16 h of light and 8 h of darkness. After three months, once the seedlings were adapted, the pots were moved to the university campus in the mid-April 2018.

To assess the quantitative and qualitative characteristics of the soil used (a combination of field soil, leaf mold, and sand in a 2:1:1 ratio), a sample had chemically and physically analyzed previously (Gorgini Shabankareh et al. 2021a). The results of the soil parameters are shown in Table 1.

Drought stress and ABA treatment

The experiment was carried out as a factorial experiment in a randomized complete block design with three technical replicates. Experimental treatments consisted of four levels of irrigation regimes (D1: (90–100%), D2: (70–80%), D3: (50–60%), and D4: (30–40%) of field capacity as the main factor; foliar application of ABA at two concentrations (A_1: zero, A_2: 15, and A_3: 30 µM L^{-1}) and three growth stages (full vegetative growth, beginning, and end of flowering) as sub-factors. After applying three stages of ABA foliar spraying and at the end of flowering, the materials were collected only once.

The drought treatments were applied based on the weighting method (Mozaffari et al. 2017). The pots were consistently irrigated 20 days following planting (late April). The samples were exposed to drought stress, whereas the control samples were grown in pots with the similar status. The standard garden soil (9 kg to each pot using scales) was added to the pots. Then, the soil was saturated by adding water to the pots. They were located on the grid surface (48 h) to let excess water drain out for reaching to the field capacity (FC). Then, soil moisture percentage (%) was assessed in the FC of the farm. To perform various moisture treatments, the soil moisture was measured to let water deficit calculation by weighing the sample pot in each block. In the next step, the pots were added with the required water.

Table 1 The soil physical and chemical analysis

Lab. no[a]	pH[b]	EC*10[c]	SP[d]	TNV[e]	N[f]%	OC[g]%	P(ava)[h] ppm	K(ava)[i] ppm[j]	%Clay	%Silt	%Sand	texture
947	7.4	4.076	141.86	5.59	0.09	0.9	24.8	256	12	42	46	Loam

[a]Laboratory number
[b]Power of hydrogen
[c]Electrical conductivity
[d]Saturation percentage
[e]Total neutralizing value,
[f]Nitrogen
[g]Organic carbon
[h]Phosphorus
[i]Potassium
[j]Part per million (Gorgini Shabankareh et al. 2021a)

For controlling the samples' dry weight, one additional pot was considered for the moisture treatments (totally four pots for index) and was used for adding their dry weights to the weights obtained from the pots as well as for allocating an appropriate amount of water to the pots while moisture treatments. Index pots were weight every day and moisture deficiency were calculated for assessing the needed level of irrigation to perform drought stress treatments. The regimes, by which the moisture weight percentage of the soil water content was close to the drought condition (filed capacity percentage), were considered in treatments (Khorasaninejad et al. 2018; Gorgini shabankareh et al. 2021a). The pots were irrigated with the same irrigation regimes until about 3 months after the main pots were transferred to the field (late June 2018). Then, to determine irrigation regimes on a daily basis, these systems were set according to the level of field capacity deficiency to achieve a desired soil moisture content after each irrigation (Khorasaninejad et al. 2018).

ABA was purchased from Sigma Aldrich®. ABA foliar application was applied at three growth stages, including completed vegetative growth, the beginning and end of flowering. To prepare the solutions, ABA was first dissolved in 0.5 mL of normal sodium hydroxide solution and made up to the desired volume with distilled water (depending on the amount of water required for each level) (Gorgini shabankareh et al. 2021b).

10 g of leaf sample was collected at the end of flowering, after the last foliar application of ABA (late June 2018). For the measurement of biochemical properties (antioxidant activities and enzymes) and molecular tests, the samples were immediately placed in liquid nitrogen and kept at −80 °C.

Antioxidants

Measurement of antioxidant activity by the DPPH radical scavenging method

The method of Fidrianny et al. (2013) was used to evaluate the potential to inhibit DPPH free radicals. First, a methanolic extract (1 mL) was mixed with 1 mL of DPPH at a concentration of 0.1 mmol. For the control sample, 1 mL of pure methanol was replaced with ethanolic extract at the same concentration. Pure methanol was used as a blank solution. After 30 min of darkness, the absorbance of the samples was measured at 517 nm using a spectrophotometer (UNICO 2800). The Eq. (1) was used to measure the percentage of radical scavenging activity (RSA):

$$\text{Percentage of free radical scavenging} = \frac{(A_c - A_s)}{A_c} \times 100. \quad (1)$$

In this equation, A_s and A_c are equal to the absorbance of sample and control, respectively. The obtained values are equal to the percentage of free radical scavenging in the methanol extract of the samples.

Measurement of antioxidant capacity by ABTS radical deionization method

This method is based on the radical reduction of ABTS (2,2′-azino-bis3-ethylbenzothiazoline-6-sulphonic acid). According to You et al. (2010), the radical scavenging activity of hydrolyzed proteins was determined. $ABTS^+$ radical solution was prepared using the same volume ratio of ABTS at a concentration of 7 and 45.4 mM potassium persulfate. The mixture was kept at room temperature in the dark for 12–16 h before consumption. The $ABTS^+$ solution was diluted to 734 nm with PBS (0.2 M and pH 4.7) to achieve adsorption of 0.02 ± 0.7. 40 µL of each sample (aqueous extract) was then added to 4 mL of diluted $ABTS^+$ solution. After severely shaking the mixture for 30 s, it was kept in the dark for 6 min. The absorbance of the final solution was measured at 734 nm. To calculate the percentage of $ABTS^+$ radical inhibition in the samples, the Eq. (2) was used:

$$AA(\%) = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100. \quad (2)$$

Measurement of antioxidant activity of leaves by FRAP method

This method is used to measure the antioxidant capacity of iron reduction according to Benzie and Strain (1996). To prepare the FRAP reagent, 300 mM acetate buffer (pH = 3.6) was mixed with 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in HCl (40 mmol) and ferric chloride solution in the ratio 1:1:1. The solution FRAP should be prepared daily and preheated in a water bath at 37 °C before use. An aqueous extract of 50 µLwas added to this solution (1.5 mL) and the adsorption rate at 593 nm was measured after 4 min at 37 °C. The standard curve was plotted using ferrous sulfate II in the normal range of 100–200 µM and the results were expressed as the equivalent µM of ferrous II per gram of the sample.

Measurement of total antioxidant capacity (TAOC)

Total antioxidant capacity was evaluated by Sun et al. (2011). Ammonium molybdate $(NH_4)_2MoO_4$, (4 mM), sulfuric acid (H_2SO_4) 0.6 M, and sodium phosphate (Na_3PO_4) 28 mM were used to prepare the TAOC reagent. One mL of the TAOC reagent was added to 100 µLof the methanolic extract. After vortexing, the test tubes were incubated at 95 °C for 90 min. Immediately after the samples were

cooled, their absorbance value compared to the control (all compounds except the extract) was recorded at 695 nm using a spectrophotometer. Ascorbic acid ($C_6H_8O_6$) was used to draw the standard curve in this method and values were expressed based on the mg ascorbic acid per gr of plant dry weight.

Activity of the antioxidant enzymes measurements

To prepare the enzyme extract, fresh leaves (0.2 g) were crushed in liquid nitrogen; then, phosphate buffer 50 mM (1 mL) (pH = 7.8) containing 0.2 M EDTA and 1% polyvinylpyrrolidone (PVPP) was added. Next, it was centrifuged at 5000 rpm for 20 m at 4 °C. The supernatant was used to determine the activity of the enzymes SOD, CAT, POX, and APX.

Peroxidase (POX) enzyme activity

POX activity was measured according to the method of Chu et al. (2016). The extract (33 µL) was mixed with 1 mL of POX solution consisting of 28 mM guaiacol, 5 mM H_2O_2 and 50 mM phosphate buffer (pH = 7) for 1 min at 10-s intervals at 470 nm. The absorbance of the enzyme was measured at 470 nm (in micromole per hydrogen peroxide consumed per minute). To make 100 mL of potassium phosphate buffer, 39 mL of 50 mM monobasic potassium phosphate was mixed with 61 mL of 50 mM dibasic potassium phosphate.

Ascorbate peroxidase (APX) enzyme activity

APX activity was determined according to the protocol of Nakano and Asadam (1981). A solution containing 50 mM potassium phosphate buffer (pH 7), 0.1 mM EDTA, 0.5 mM ascorbic acid, and 0.15 mM H_2O_2 was mixed with 50 µL of the extract. A spectrophotometer was used to measure the absorbance at 290 nm after one minute.

Catalase (CAT) enzyme activity

One mL of the CAT reaction solution containing 50 mM phosphate buffer (pH = 7) and 15 mM hydrogen peroxide was rapidly mixed with 50 µL of the enzymatic extract. The reaction started with the addition of the enzymatic extract and after one minute, its absorbance at 240 nm was recorded using a spectrophotometer (Chu et al. 2016).

Superoxide dismutase (SOD) enzyme activity

SOD activity was measured based on its ability to inhibit NitroBlue Tetrazolium (NBT) light conduction (Chu et al. 2016). The enzyme measuring solution consisted of 50 mM phosphate buffer (pH = 7.8), 14.5 mM L-methionine, 75 µM NBT, 4 µM riboflavin, 0.1 µM EDTA, and enzyme extract. The reaction solution was placed in a fluorescent light chamber at 4000 lx for 20 min. A tube without enzyme extract was used as a control, and a sample on which the fluorescent light had no effect served as a blank. Light absorption at 560 nm was measured using a spectrophotometer.

Real-time PCR

RNA extraction and cDNA synthesis

Total RNA extraction was performed using the RNeasy Plant Mini Kit, "Qiagen"® manufactured by Germany, according to the appropriate instructions. The quality of the extracted RNA was assessed using a thermo nanodrop device, and its integrity and quantity were evaluated using the 28S rRNA and 18S rRNA fragments on a 1% agarose gel. RNA samples (1 µg) were treated with Thermo DNase kit (RNase-free), Thermo Fisher® to remove genomic DNA. DNase-treated RNA (10 µL) was then treated with 1 µL oligo-dT, 1 µL dNTPs, U20 Rnase inhibitor, and U200 reverse transcriptase enzyme for first-strand cDNA synthesis (Thermo Kit), resulting in a final volume of 20 µL. The cDNA samples were stored at −20 °C until use.

qRT-PCR

The nucleotide sequences of the PCR primers used in this study were designed using Primer3 online software according to the desired features for use in the Q RT-PCR assay, based on the sequence of the desired genes on the NCBI website (Table 2). Real-time PCR reaction using RealQ Plus 2 × Master Mix Green without ROX in 10 µL reaction mixture containing RealQ plus 2 × Master Mix, forward and reverse primers, double-distilled water and cDNA in real-time PCR (German Qiagen model), capable of real-time evaluation in two biological repetitions and in three technical repetitions, was performed. The beta-actin gene was used as a housekeeping gene. The target DNA sequence was amplified under the following conditions: 95 °C for 15 min, 45 cycles at 95 °C for 20 s, and 60 °C for 40 s. These conditions were applied to all tested genes and the reference gene. The data obtained from real-time PCR were analyzed using the software REST, and the corresponding graphs were generated using the Excel software. The relative expression of the genes for *catalase* (*CAT1*), *CAT3*, *peroxidase* (*POX*), *ascorbate peroxidase* (*APX*) and *superoxide dismutase* was calculated using Eq. (3) of pfaffl et al (2002):

$$\text{Ratio} = \left(E_{\text{target}}\right)^{\Delta \text{CP}}_{\text{target(control}-\text{sample)}} / \left(E_{\text{ref}}\right)^{\Delta \text{CP}}_{\text{ref(control}-\text{sample)}} \quad (3)$$

Table 2 Oligonucleotides used in this study

Target gene	Primers	GC%	TM
CAT3	F-GCAATTATCCCGAATGGAAAC	42.86	55.92
	R-CAACAAGAGCAGGGCAAAAC	50	57.30
Superoxide dismutase	F-CAATGGTGAAGGCTGTCGTAG	52.38	59.82
	R-CCAGCAGGATTGAAGTGAGG	55	59.35
Catalase	F-TGGCTTGTAGTTTGGAGGAA	45	55.25
	R-TTGAGGAGGAATTGGCAGAG	50	57.30
Peroxidase	F-ACGAAGCAGTCGTGGAAGAG	55	59.35
	R-ATGGGGTTTTCGGACATTAG	45	55.25
L-ascorbate peroxidase	F-ATCAGTTGGCTGGTGTTGTG	50	57.30
	R-AATCCAGATCGCTCCTTGTG	50	57.30
Beta-actin (Demissie et al. 2012)	F-TGTGGATTGCCAAGGCAGAGT	52.38	59.82
	R-AATGAGCAGGCAGCAACAGCA	52.38	59.82

Statistical analysis

Experimental data were analyzed using SAS software. The significant differences among treatments were compared using the LSD test at a probability level of 1% and 5%. Ct analyses were performed using Relative Rest 2009 software and charts were generated using Excel.

Results

Antioxidant activity was different in ABA-treated lavender under drought stress

Medicinal plants have complex and efficient antioxidant systems to counteract the harmful effects of ROS. These systems include enzymes and non-enzymes that can scavenge, neutralize, or refine free radicals. Therefore, it seems essential to study how antioxidant activity changes in response to stress to better understand drought tolerance.

DPPH (1-Diphenyl-2-picrylhydrazyl)

The irrigation systems and ABA treatments, as well as their interaction, differed significantly in terms of DPPH antioxidant activity ($P \leq 0.01$) (Table 3). The results showed that drought stress initially decreased the antioxidant activity of DPPH compared with the control, so that the lowest value (42.16% free radicals) was recorded in the non-foliar spraying of ABA under 50–60% of field capacity (Fig. 1). However, under high-stress conditions, the percentage of free radical scavenging increased significantly. Moreover, a 30 µM solution of ABA increased the antioxidant activity of DPPH at all drought levels, so that the highest DPPH level was obtained in this treatment. In other words, it increased DPPH's antioxidant activity most effectively.

ABTS (2,2′-azino-bis3-ethylbenzothiazoline-6-sulphonic acid)

The interactions between the treatments of drought and ABA revealed a significant effect on the percentage of radical inhibition of ABTS (Table 3). Mean comparison

Table 3 Analysis of variance for the effects of irrigation regimes and foliar application of ABA on the studied traits

Traits	Replication	Drought (*D*)	Abscisic acid (*A*)	$D \times A$	Error	CV (%)
df	2	3	2	6	22	
DPPH	0.096^{ns}	3806.17**	843.03**	99.94**	0.95	1.38
ABTS	1.62^{ns}	369.92**	601.71**	192.18**	1.40	7.15
FRAP	6.58^{ns}	3516.28**	6501.94**	907.99**	12.24	4.48
TAOC	0.483^{ns}	17.034**	0.64**	1.85**	0.142	3.39
CAT	0.4016^{ns}	0.0020**	0.0005**	0.000023 *	0.000020	1.28
APX	0.045^{ns}	981.83**	9.77 **	3.04**	0.45	1.68
SOD	1319.44^{ns}	244,869.44**	84,252.77**	7097.22**	825.50	9.58
POX	0.00000022^{ns}	0.000022**	0.0000081**	0.0000011**	0.00000011	4.51

DF degrees of freedom; *ns* non-significant

*Significant at $P \leq 0.05$, **significant at $P \leq 0.01$

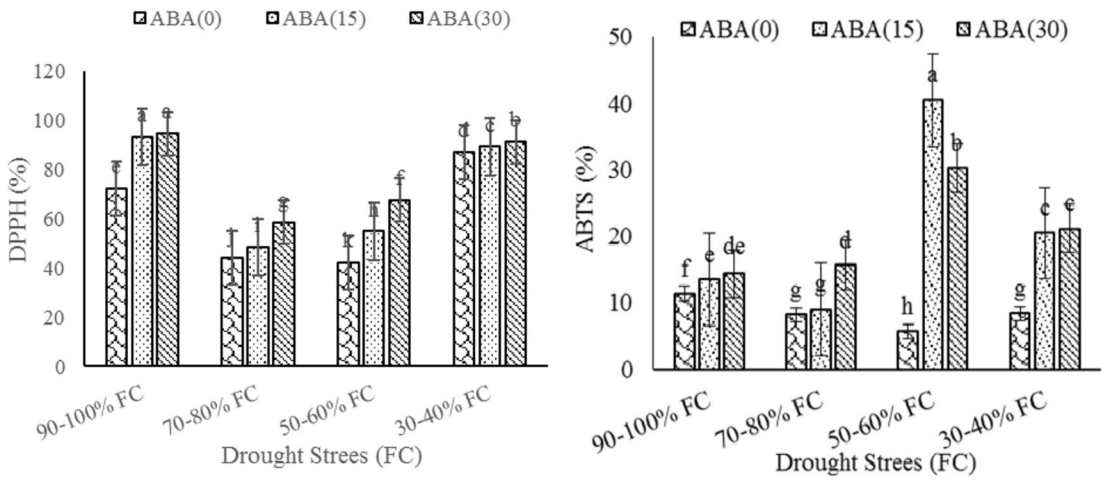

Fig. 1 Antioxidant activity DPPH (left) and ABTS (right) of *Lavandula angustsifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels 90–100, 70–80, 50–60, and 30–40 percent) and ABA (Levels 0, 15, and 30 μM L^{-1}) treatments. Different letters on top of column indicate significant different at $P \leq 0.01$ according to the LSD test

results (Fig. 1) demonstrated that the percentage of radical inhibition of ABTS was slightly reduced by drought stress. While, ABA spray hormone under stress conditions compensated this reduction, so that under 50–60% of field capacity, application of 15 μM L^{-1} ABA was most effective (40.55% free radicals) in terms of increased activity. Furthermore, the percentage of radical inhibition of ABTS increased at all irrigation levels by spraying with ABA compared with no hormone treatment.

FRAP (ferric reducing antioxidant potential)

FRAP was affected by different amounts of drought, ABA, and their interaction (Table 3). As shown in Fig. 2, the antioxidant activity of FRAP method was recorded by 44.78, 59.75, 61.52, and 74.21 mg.g, respectively, at various lowest to highest levels of drought stress, indicating that the amount of FRAP increased with enhancing irrigation level. By contrast, the foliar application of ABA significantly affected the antioxidant activity of FRAP method under irrigation conditions (Fig. 2). Therefore, the interaction of 30 μM ABA in 30–40% of drought capacity recorded the highest antioxidant

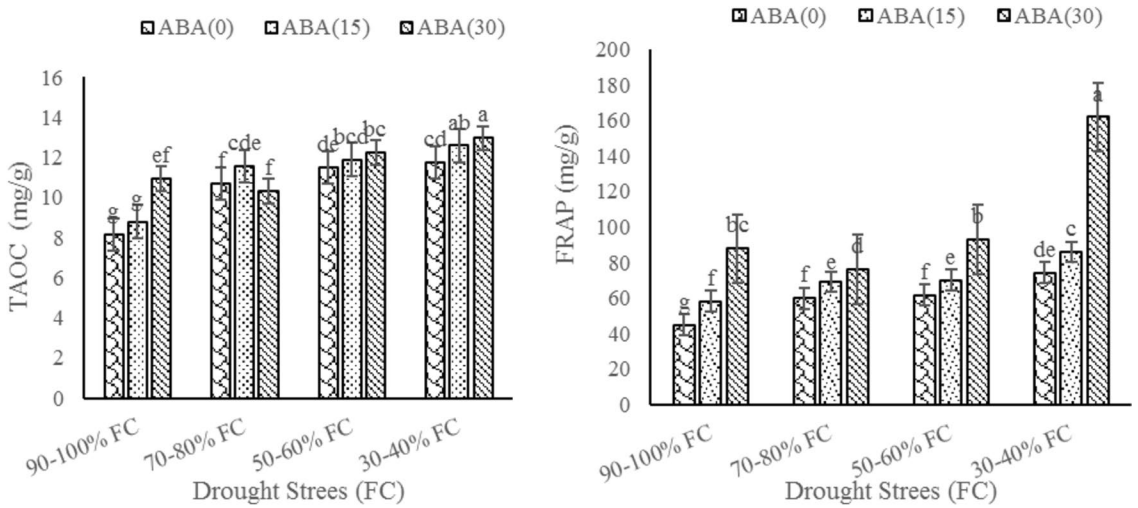

Fig. 2 Antioxidant activity TAOC (left) and FRAP (right) of *Lavandula angustsifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels 90–100, 70–80, 50–60, and 30–40%) and ABA (Levels 0, 15, and 30 μM L^{-1}) treatments. Different letters on top of column indicate significant different at $P \leq 0.01$ according to the LSD test

activity(161.82 mg. g), while the lowest one (44.78 mg g) was associated with the non-application of ABA and drought control.

TAOC (total antioxidant capacity)

ANOVA results in Table 3 showed that the interactions of drought stress and ABA spray hormone significantly affected leaf TAOC with ($P \leq 0.01$). Figure 2 illustrates that plant TAOC increased under drought conditions compared with well-watered plants. Moreover, spraying ABA increased this parameter under drought stress, even in plants without stress compared to non-sprayed plants. Thus, the highest amount of TAOC (12.97 mg. g dry weight) was obtained at the severest drought level (30–40% of field capacity) and hormone application of 30 µM L^{-1} ABA.

Activity of antioxidative enzymes

Drought stress increased antioxidant activity in response to exogenous ABA treatment, so measuring enzyme activity was essential for detecting changes. Enzyme activity in response to drought stress gradually increased, so that with increasing drought levels, all observed enzymes became more active. Under both drought stress and control, the observed enzyme activities were higher in the plants treated with ABA than in the untreated plants.

CAT enzyme

The interaction effects of the different drought levels and ABA treatments were significantly different according to the analysis of variance concerning CAT ($P \leq 0.01$) (Table 3). The lowest CAT activity (0.323 µmol hydrogen peroxide min^{-1}) was recorded under non-stress and hormone application conditions, while with increasing drought severity, enzyme activity increased. Moreover, hormone improved this increase compared with plants without ABA treatment. At all drought levels, 30 µM L^{-1} ABA had revealed the highest increased activity. By contrast, 30 µM L^{-1} ABA foliar applications along with 30–40% of field capacity increased in CAT activity (0.374 µmol hydrogen $peroxide^{-1}$) up to the highest (Fig. 3).

APX enzyme

As shown in Table 3, the interaction effects of drought and ABA treatments had a significant effect on the APX activity ($P \leq 0.01$). The results of the mean comparison showed that enzyme activity increased with higher drought levels, while, foliar application of ABA at 15 and 30 µM L^{-1} enhanced the enzyme activity slightly. The highest activity (46.78 µmol hydrogen peroxide min^{-1}) was recorded in the plants treated with 30 µM L^{-1} ABA, while 90–100% of field capacity along with no hormone application resulted in the lowest enzyme activity (23.50 µmol hydrogen peroxide min^{-1}) (Fig. 3).

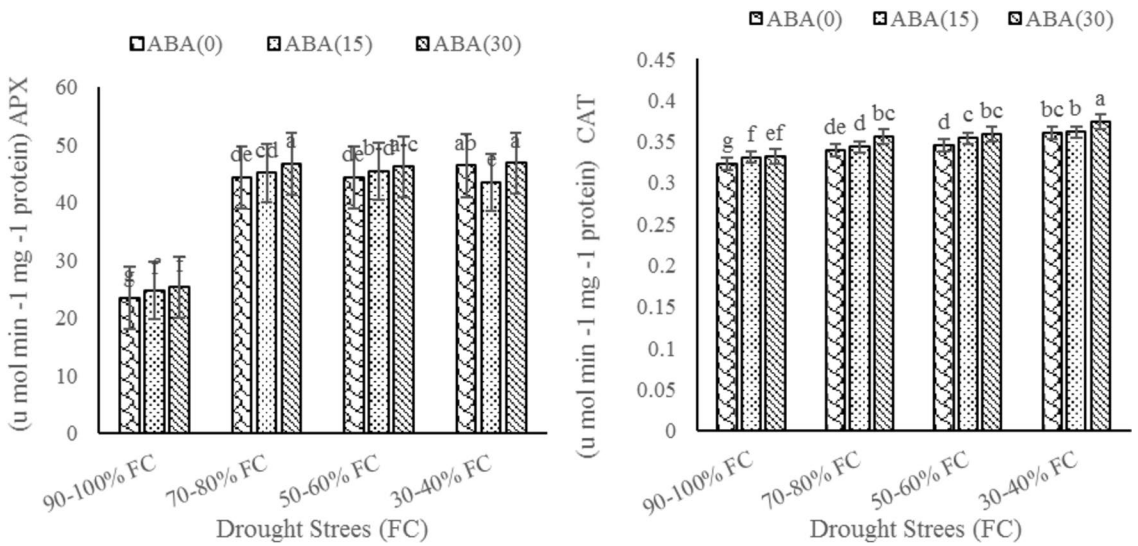

Fig. 3 Enzymatic activity APX (left) and CAT (right) of *Lavandula angustsifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels D1: 90–100%, D2: 70–80%, D3: 50–60%, and D4: 30–40%) and ABA (Levels A_1: 0, A_2: 15, and A_3: 30 µM L^{-1}) treatments. Different letters on top of column indicate significant different at $P \leq 0.05$ and $P \leq 0.01$ according to the LSD test, respectively

SOD enzyme

SOD was significantly affected by the interaction of drought stress and ABA ($P \leq 0.01$) (Table 3). As shown in Fig. 4, SOD activity was significantly higher at stress levels of 70–80%, 50–60%, and 30–40% of field capacity compared with the control treatments. The application of ABA also showed higher enzymatic activity in leaves. In addition, under all drought stress and control levels, 15 and 30 µM L^{-1} ABA had a significant effect on increasing enzyme activity. Whereas the application of ABA positively affected this enzyme activity under drought stress treatments, and the highest enzyme content (620 µmol hydrogen peroxide min^{-1}) was observed in leaves with 30–40% of field capacity sprayed with 30 µM per liter ABA. The lowest activity (70 µmol hydrogen peroxide min^{-1}) was observed in the control treatments.

POX enzyme

Table 3 shows that the combined effects of drought and ABA treatments were significant on POX ($P \leq 0.01$). POX enzyme activity increased to 50–60% of field capacity of drought stress; while under severe drought stress (30–40% of field capacity), its activity decreased that was higher than drought control (Fig. 4). Exogenous treatment with ABA ameliorated the reduced enzyme activity in plant leaves under severe stress and the highest enzyme activity (0.00988 µmol hydrogen peroxide min^{-1}) was observed under the most severe drought stress with the application of 30 µM L^{-1} ABA. Generally, spraying ABA at both 15 and 30 µM L^{-1} increased POX compared to not spraying.

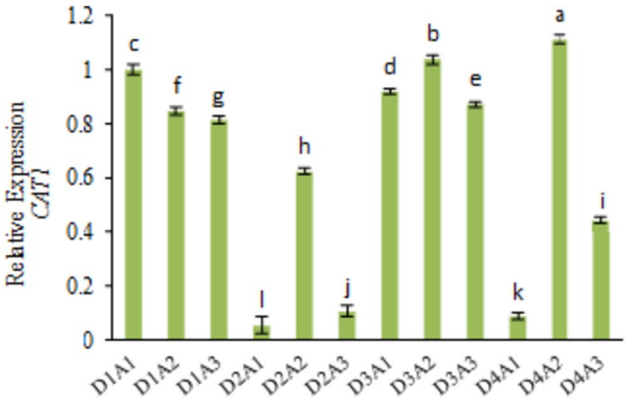

Fig. 5 Gene-relative expressions of *CAT1* of *Lavandula angustifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels D1: 90–100%, D2: 70–80%, D3: 50–60%, and D4: 30–40%) and ABA (Levels A_1: 0, A_2: 15, and A_3: 30 µM L^{-1}) treatments. RT-PCR analyses were performed on the total RNA extracted from *Lavandula angustifolia* cv. Munstead leaves and data were normalized as described. Different letters indicate significant differences at $P \leq 0.01$

Gene expression of antioxidant enzymes

The expression of *CAT1*, *CAT3*, *POX*, *APX*, and *SOD* genes was estimated quantitatively by PCR (Figs. 5, 6, 7, 8, 9). The results illustrated that drought stress in the absence of ABA reduced the expression of *CAT1* gene (Fig. 5), while application of ABA (at the level of 15 µM L^{-1}) regulated gene expression and significantly reduced these increases. Also, the results for *CAT3* and *APX* genes (Figs. 6, 7) showed that the medium stress of 50–60% of field capacity and 30–40% of field capacity, respectively, combined with 15 µM L^{-1}

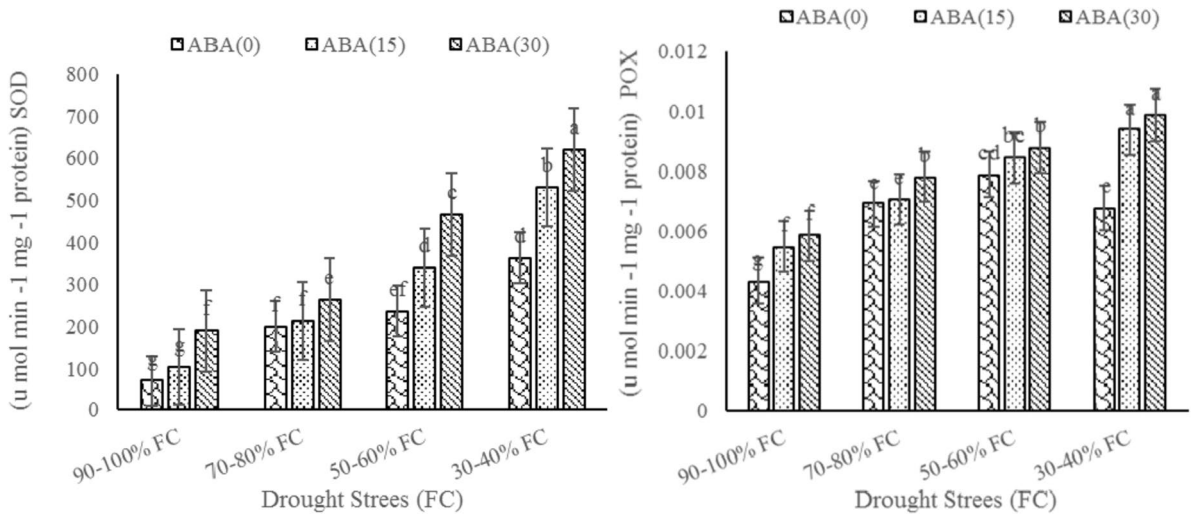

Fig. 4 Enzymatic activity SOD (left) and POX (right) of *Lavandula angustsifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels D1: 90–100%, D2: 70–80%, D3: 50–60%, and D4: 30–40%) and ABA (Levels A_1: 0, A_2: 15, and A_3: 30 µM L^{-1}) treatments. Different letters on top of column indicate significant different at $P \leq 0.01$according to the LSD test

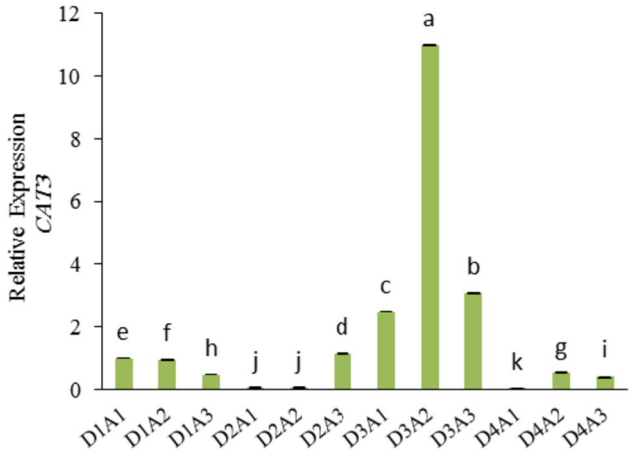

Fig. 6 Gene-relative expressions of *CAT3* of *Lavandula angustifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels D1: 90–100%, D2: 70–80%, D3: 50–60%, and D4: 30–40%) and ABA (Levels A_1: 0, A_2: 15, and A_3: 30 µM L^{-1}) treatments. RT-PCR analyses were performed on the total RNA extracted from *Lavandula angustifolia* cv. Munstead leaves and data were normalized as described. Different letters indicate significant differences at $P \leq 0.01$

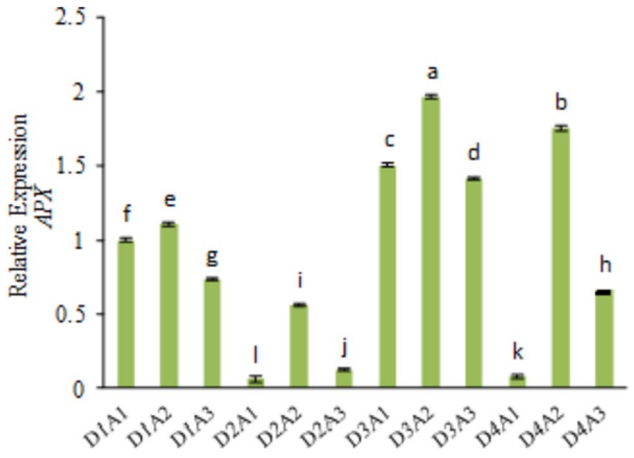

Fig. 7 Gene-relative expressions of *APX* of *Lavandula angustifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels D1: 90–100%, D2: 70–80%, D3: 50–60%, and D4: 30–40%) and ABA (Levels A_1: 0, A_2: 15, and A_3: 30 µM L^{-1}) treatments. RT-PCR analyses were performed on the total RNA extracted from *Lavandula angustifolia* cv. Munstead leaves and data were normalized as described. Different letters indicate significant differences at $P \leq 0.01$

ABA increased the expression to its maximum, while the expression of *CAT3* decreased in other levels of irrigation regime. The only exception was the gene *SOD* (Fig. 8), which revealed a decreasing trend along with increasing drought stress and the foliar spraying of ABA. Furthermore, its expression level decreased under drought stress, and the use of the hormone ABA had little effect on it compared

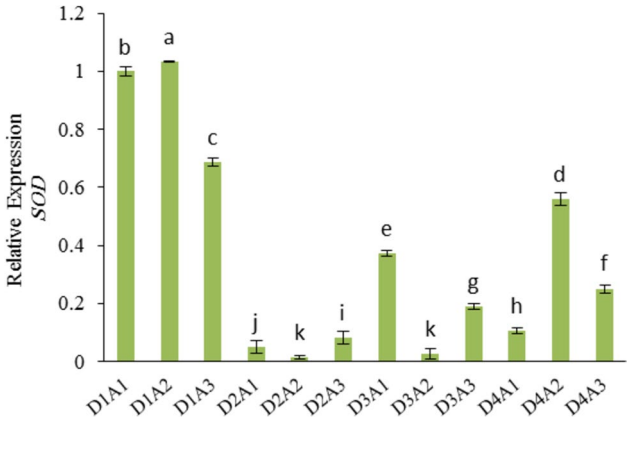

Fig. 8 Gene-relative expressions of *SOD* of *Lavandula angustifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels D1: 90–100%, D2: 70–80%, D3: 50–60%, and D4: 30–40%) and ABA (Levels A_1: 0, A_2: 15, and A_3: 30 µM L^{-1}) treatments. RT-PCR analyses were performed on the total RNA extracted from *Lavandula angustifolia* cv. Munstead leaves and data were normalized as described. Different letters indicate significant differences at $P \leq 0.01$

to no spray hormone. By contrast, gene *POX* expression (Fig. 9) increased under low (70–80% of field capacity) and moderate (50–60% of field capacity) drought compared with drought control, while its expression decreased under severe drought stress (30–40% of field capacity). Hormone spraying did not affect expression in the drought control, while it slightly improved the expression of *POX* under stress conditions.

Discussion

According to recent research, plants treated with ABA foliar application showed a greater increase in antioxidant activity than plants not treated with foliage.

In this study, it was found that the antioxidant activity of English lavender increased with enhancing drought stress, which is a defense mechanism that activates under stress. Under such conditions, spraying ABA can close stomata and prevent gas exchange, leading to the production of ROS. These destructive compounds act as secondary messengers and are responsible for increasing antioxidant activity to counteract ROS (Li et al. 2011). In this way, many methods can measure antioxidant capacity and efficacy under different conditions, but rarely is there a strong relationship between capacities measured by different methods on the same material and between capacities measured by one method in different laboratories (Magalhaes et al. 2008). To date, there is no simple and universal method for the evaluation and determination of antioxidants due to the wide variety of active ingredients, different mechanisms, and reaction

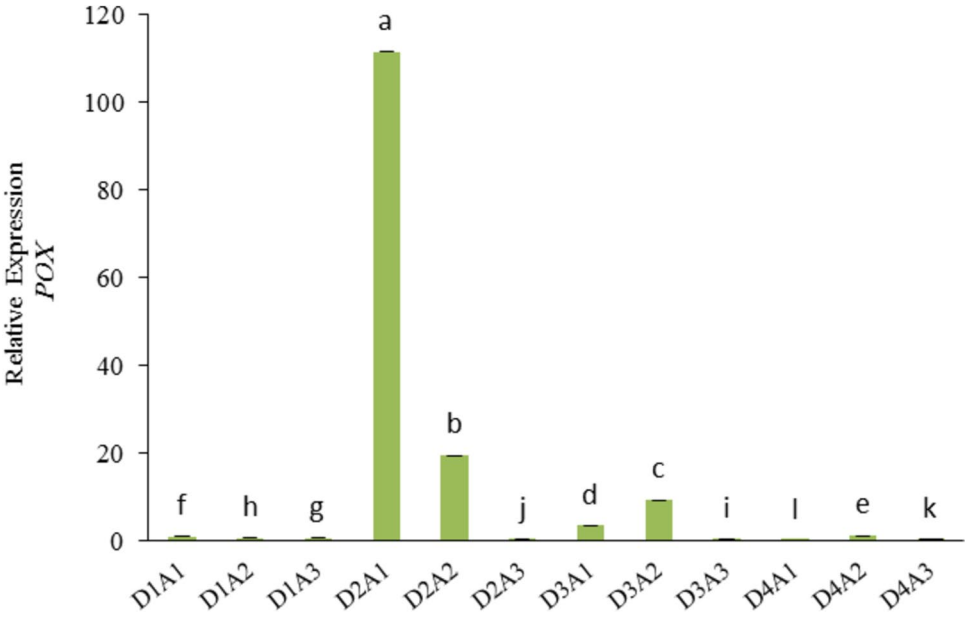

Fig. 9 Gene-relative expressions of *POX* of *Lavandula angustifolia* cv. Munstead in response to irrigation regimes based on field capacity (Levels D1: 90–100%, D2: 70–80%, D3: 50–60%, and D4: 30–40%) and ABA (Levels A_1: 0, A_2: 15, and A_3: 30 µM L^{-1}) treatments. RT-PCR analyses were performed on the total RNA extracted from *Lavandula angustifolia* cv. Munstead leaves and data were normalized as described. Different letters indicate significant differences at $P \leq 0.01$

properties, such as different antioxidants, the presence of other interfering substances in the sample, the absence of all sample's antioxidants in the reaction (Zulueta et al. 2009); Therefore, the method is not conclusive. Since antioxidants inactivate free radicals through both electron transfer and hydrogen transfer mechanisms, different methods of determining antioxidant capacity are often required to validate the results of a study and measure antioxidant potential (Niki 2010). Different results may be due to the variability of free radicals, the inefficiency of some antioxidants in reacting with free radicals, and differences in the type of compounds (water soluble or alcohol soluble) and pH range of the reaction medium (Deng et al. 2011). Khorasaninejad et al. (2018) studied the effects of drought stress on Echinacea (*Echinacea purpurea*) and reported that the stress greatly increased proline content and antioxidant activity in both leaves and roots to cope with stress conditions. Also, the evaluation of caraway seed extract (*Carum carvi* L.) showed that drought positively affected antioxidant indices.

According to Ghassemi et al (2018), foliar applications of ABA and salicylic acid can be a practical method to improve drought tolerance due to their increased antioxidant capacity. It has been shown that the antioxidant capacity of DPPH radical scavenging in *Mentha piperita* was significantly increased under drought stress (Chiappero et al. 2019). Chrysargyris et al. (2018) reported a decrease in antioxidant activity of DPPH, ABTS and FRAP of *Lavandula angustifolia* under salt stress. According to our results, a decrease in the radical scavenging activity of ABTS was observed, which this reduction was compensated by the hormone ABA. Researchers have reported that extracts of *Bunium persicum* and *Cuminum cyminum* seeds had higher DPPH and FRAP antioxidant activity than the control group under drought conditions (Saeidnejad et al. 2013). Since different compounds dissolve differently and some are soluble in water while others in alcohol, different methods are used to measure the antioxidant capacity of aqueous and alcoholic extracts.

This study focused on the evaluation of enzymatic pathways and the use of exogenous ABA to induce homeostasis through the indirect production of oxygen species (ROS). This process activates the antioxidant enzymes CAT, APX, POX, and SOD, which can increase plant resistance to water deficiency. Das and Kar (2018) found that the use of exogenous ABA and water stress significantly increased extracellular ROS production in roots and induction of antioxidant defense potential, while the activity of several antioxidant enzymes such as SOD, CAT, APX, and POD increased simultaneously. The results of our study were in agreement with those of Ghassemi et al. (2018) who found that antioxidant enzymes are activated and increased as a defense system to resist oxidative stress under various environmental stresses (Sierla et al. 2016). Similarly, Xiaolu et al. (2016) reported that in the early stages of mild drought stress, the activity of the enzymes CAT and POD in *Dendrobium moniliforme* increased significantly, suggesting that plants can generate antioxidant enzymes to protect themselves during early mild drought stress. In addition, they found that POX activity increased under severe drought stress after re-irrigation. CAT decomposes H_2O_2 produced during light respiration in peroxisomes and during beta-oxidation of fatty acids in glyoxysomes, breaking it down into the water and the O_2 molecule. CAT activity is therefore increased as an adaptive mechanism to cope with the damage caused by cellular metabolism. In addition, POXs can degrade H_2O_2 by oxidizing phenolic compounds or other antioxidants, such

as ascorbate. Our study revealed that both CAT and POX enzymes increased during drought stress, and application of ABA was effective in further increasing their activity. This phenomenon can be explained by the fact that ABA increases the content of single oxygen and hydrogen peroxide, which in turn increases the activity of antioxidant enzymes (Figs. 3 and 4).

Our findings illustrated that the activity of SOD increased in English lavender shoots under drought stress and foliar application of ABA (Fig. 4). It can be inferred that under water stress, the increase in hydrogen peroxide concentration induced by SOD can increase CAT and APX activity to degrade hydrogen peroxide. While under normal conditions, as there is not enough hydrogen peroxide, the production of peroxide hydrogen by superoxide ions will be reduced, which will decrease the activity of those enzymes. APX activity rose with increasing drought stress in this study, and 30 µM L^{-1} ABA had a greater effect on this increase than other concentrations (Fig. 6). 24-Epibrazinolide was found to increase the activity of antioxidant enzymes, including APX, in Echinacea under drought stress (Hosseinpour et al. 2020). In this study, English lavender plants under drought stress could maintain a highly efficient system by increasing the induction of antioxidant enzymes such as CAT, APX, POX, and SOD to ensure the homeostasis of ROS in different parts of the cell. The purpose of this defense mechanism is to keep the level of reactive oxygen free radicals as low as possible to protect the plant against drought damage. By inducing homeostasis, ABA indirectly generates the production of ROS and consequently increases the activity of the antioxidant enzymes, which increases the resistance of plants against water deficiency (Figs. 3, 4). In the same vein, many studies have found that drought stress and hormone application increase the activity of antioxidant enzymes such as SOD, POX, APX, and CAT in *Aloysia citrodora* (Dianat et al. 2016) and Ajowan (Ghassemi et al. 2018). According to Li et al. (2016), ABA activates ABA-responsive genes and regulates the activity of antioxidant enzymes. In addition, salicylic acid promoted the growth of Echinacea in a water-deficient environment by stimulating the antioxidant defense system, reducing lipid peroxidation, and increasing the enzymes SOD and CAT (Darvizheh et al. 2019). Mohasseli et al (2020) found that the activity of enzymes POX, CAT, and APX significantly enhanced with increasing water stress in *Melissa officinalis*. In the same stress condition, Ali and Hassan (2017) and Hassan et al. (2018) also reported an increase in antioxidant enzymes.

Stress tolerance in plants is determined by multiple factors and numerous genes, and considering the complexity of antioxidant systems and stress tolerance in plants, it cannot be argued that ROS inhibition is the only factor determining the degree of tolerance. Stress also often occurs in combination, so the relationship between ROS signaling mechanisms in response to different stress factors is complex (Miller et al. 2010). Examination of the expression of genes related to antioxidant enzymes revealed that the *CAT3, AXP,* and *POX* genes were somewhat consistent with the levels of the enzymes CAT, APX, and POX, respectively, such that as drought stress increased, the expression level also enhanced. However, the best performance was obtained under drought conditions of 50–60% of field capacity and application of 15 Mm L^{-1} ABA for CAT and APX, while the same POX activity was achieved under drought treatments of 70–80% of field capacity without hormone spraying. On the other hand, expression of the gene *SOD* was inversely related to the amount of this enzyme, such that enzyme activity increased during severe drought and ABA compared with controls. By contrast, the expression of the gene increased by drought and hormone treatment. The enzyme activity might play a regulatory role after transcription. Under drought stress conditions, gene expression may be reduced due to inhibition or changes in the accumulation of enzyme subunits. This result has also been reported previously. Lane et al. (2010) observed in *Lavandula angustifolia* that *LinS* transcription decreased during the latter stages of flower development, while linalool levels did not. It indicates that *LinS* transcription occurs before linalool synthesis and that linalool is stored when there is not at maximum level. Furthermore, it can be concluded that the enzyme activity may vary depending on the genotype, plant organ, growth stage, and experimental conditions. It can also be caused by posttranscriptional changes such as enzyme inactivation or degradation. In such a situation, enzyme activity is reduced despite increased gene expression. (Ara et al. 2013). To protect plant cells from oxidative stress caused by abiotic stresses, several genes encoding antioxidant enzymes are regulated. Therefore, analysis of transcript levels of antioxidant defense genes plays a key role in plant response to stress (Carvalho et al. 2013) (Table 4).

Conclusion

Our results showed that external application of ABA significantly reduced drought-induced oxidative stress by triggering potential antioxidant mechanisms in *L. angustifolia*. In other words, ABA increased antioxidant activity and enzymes CAT, APX, SOD, and POX to maintain cellular homeostasis. This phytohormone (especially at the level of 15 µM L^{-1}) also mitigated the reduction in gene expression of antioxidant enzymes under drought stress compared to control treatments. This study highlights the role of ABA in improving the drought tolerance of *L. angustifolia* due to its contribution in inducing antioxidant activity, antioxidant enzymes, and related genes. One of the aspects that emerge from this finding is that ABA foliar application enables

Table 4 Correlation between antioxidant activity and antioxidant enzymes of *Lavandula angustifolia* cv.Munstead under water stress and ABA application

Traits	FRAP	ABTS	TAOC	DPPH	CAT	APX	SOD	POX
FRAP	1							
ABTS	+0.31^{ns}	1						
TAOC	+0.66**	+0.37*	1					
DPPH	+0.42*	+0.08^{ns}	−0.001^{ns}	1				
CAT	+0.70**	+0.42*	+0.78**	+0.10^{ns}	1			
APX	+0.35*	+0.22^{ns}	+0.74**	−0.44**	+0.79**	1		
SOD	+0.79**	+0.48**	+0.83**	+0.26^{ns}	+0.90**	+0.64**	1	
POX	+0.66**	+0.50**	+0.84**	−0.07^{ns}	+0.86**	+0.78**	+0.88**	1

plant metabolism to function more optimally under stress conditions. Overall, it can be concluded that to understand the response to drought stress in plants, it is necessary to identify genes and metabolic pathways involved in stress tolerance. Methods such as the use of exogenous substances, increasing the expression of resistant genes, and the expression of enzymes that help the plant to resist stress can be tolerated stress, including drought in plants to achieve high performance.

Author contribution statement M. S. collected data and wrote the main manuscript text. S.Kh. conceived the idea and edited the main manuscript text. H.S. reviewed the collected data. All authors reviewed the manuscript.

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Declarations

Conflict of interest There is no conflict of interest.

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