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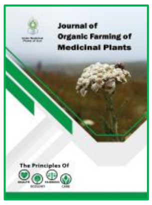

Original Article

Salicylic acid and biochar improve drought tolerance in *Borago officinalis* L. by enhancing antioxidant enzymes, leaf proline and soluble sugars

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ABSTRACT

Water scarcity has become an increasingly important factor contributing to crop yield decline in arid regions. Soil amendments and certain growth regulators are some strategies used to mitigate the negative effects of drought stress. This study aimed to investigate the effects of biochar and salicylic acid on some physiological and biochemical properties of *Borago officinalis* L. under water deficit conditions. This experiment was conducted as a split-plot factorial experiment based on a randomized complete block design with four replicates. The factors were irrigation regimes according to the percentage of water requirement (100% WR, 75% WR, and 50% WR) in the main plots, biochar application (0, 5, and 10 ton ha^{-1}) and foliar application of salicylic acid (0, 0.5 mM) in the subplots. This study was conducted in the research field of Shahrood University of Technology, Shahrood, Iran, in two cropping years, 2017 and 2018. The results showed that the total amount of soluble sugars decreased with an increase in the irrigation regime; however, the leaf antioxidant enzymes (SOD, CAT, and POX) and leaf proline concentration significantly increased in the 75% WR irrigation regime and slightly decreased in the 50% WR irrigation regime in both years. Applying salicylic acid (0.5 mM) significantly increased the leaf proline by 50% and 55% in irrigation regimes of 100% WR and 75% WR, respectively, in both years. It also modified the total amount of soluble sugars in both years. In addition, salicylic acid also stimulated leaf antioxidant enzymes under 100%WR under 75% WR and 50% WR irrigation regimes in both years. Biochar application reduced leaf proline concentration by 27% and 28% (10 ton ha^{-1}) under 100% WR and 75% WR irrigation regimes, respectively. An improvement of all antioxidant enzymes via biochar application was found in both years. Application of 5 ton ha $^{-1}$ biochar mitigated relative water content in 75% WR. Our results clearly indicated that applying exogenous salicylic acid in combination with biochar could be a promising approach to improve plant stress tolerance mechanisms under water deficit conditions.

1. Introduction

Water deficit affects various aspects of crops through anatomical, morphological, physiological, and biochemical changes. Drought can cause deleterious effects through cell membrane lipid peroxidation and promotes the production of reactive oxygen species (ROS), protein denaturation, and DNA breakdown, which eventually leads to yield reduction in plants (Du et al., 2021; Darvizheh et al., 2019; Hussain et al., 2018). Plants resort to various approaches against oxidative stress caused by drought to maintain their viability through enzymatic mechanisms – such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate peroxidase (APX) – non-enzymatic mechanisms - such as phenolic compounds, flavonoids, carotenoids, and anthocyanin (Gao et al., 2020; Chavoushi et al., 2020), and osmoprotectants – such amino acids like proline (La et al., 2019; Ghaffari et al., 2019), and regulating soluble carbohydrates and ammonium compounds (Huang et al., 2018). The implementation of agronomic methods is a complementary solution to drought stress. Some agronomic strategies can be used to mitigate the adverse effects of drought. These strategies include using soil additives and some growth regulators (Jovanovic et al., 2020).

Salicylic acid (SA) is considered a regular phytohormone that also has an elicitor effect on plants under numerous types of stress. Several studies have shown positive correlations between salicylic acid application and plant stress tolerance factors (Gorni et al., 2020; Estaji & Niknam, 2020), including increased germination (Huang et al., 2021), induction of flowering, improved plant growth and development, increased yield and fruit yield (Estaji & Niknam, 2020), inhibition of ethylene synthesis, opening and closing of pores, water relations, membrane stability (Ghassemi et al., 2019), nutrient uptake, and activation of defense mechanisms such as maintenance of higher levels of antioxidant activity (Gacnik et al., 2021; Shah Jahan et al., 2019; Farhadi & Ghassemi-Golezani, 2020).

Emissions from crop residue burning which result in the loss of soil organisms and organic carbon are a significant source of greenhouse gasses in the earth's atmosphere, contributing to global warming (Lehmann et al., 2011). Therefore, the decomposition of crop residues in fields can be a key factor in soil health in agricultural systems by promoting the soil's physical, chemical, and biological properties (Verheijen et al., 2010). Biochar is a carbon-rich product in a stable form that persists in the soil for hundreds or thousands of years (Guo et al., 2021). Plenty of research shows that treatments with biochar as a soil conditioner improve soil properties as long as own favorable adsorption characteristics (high cation exchange capacity and surface area), resulting in increased irrigation efficiency and improved plant physiological properties (Akhtar et al., 2014; Batool et al., 2015; Zhang et al., 2020; Guo et al., 2021). In addition, biochar amendment would confirm direct growth promotion by increasing the nutrient supply and nutrient availability to plants (Akhtar et al., 2014). Biochar also directly increases plant growth by providing minerals and nutrients. In addition, it increases irrigation efficiency, resulting in improved physiological characteristics of plants (Batool et al., 2015; Zhang et al., 2020; Guo et al., 2021). Moreover, it increases soil carbon stabilization and reduces greenhouse gas emissions (Borchard et al., 2019).

European borage (*Borago officinalis* L.) is an annual herbaceous medicinal plant considered indigenous to Europe and Asia (Abdelli et al., 2016). It is commonly known as the richest source of gamma-linoleic acid. It is widely used as a dietary supplement for the treatment of heart disease, diabetes, arthritis, and multiple sclerosis (Shahbazi et al., 2019; Karimi et al., 2018). In light of the side effects of using chemical drugs, medicinal plants have taken on an added significance all over the world nowadays. Apart from this, it is necessary to facilitate the production of medicinal plants in water-limited areas to address water scarcity.

Crop ecology conducts many water-saving approaches for optimal water supply and increased water use efficiency by the crop, which is related to the hydrological situation as influenced by climate and physiological response of the plant (Jovanovic et al., 2020). Despite many studies on the beneficial effects of exogenous salicylic acid and biochar amendment on drought, to our knowledge, few studies have examined the effects of these two factors on leaf antioxidant enzymes (SOD, CAT, and POX), and leaf proline content in European Borage under water deficit conditions.

2. Material and Method

2.1. Plant material and experimental design

This experiment was conducted as a split plot factorial based on a randomized complete block design with four replications. Factors included 100% water requirement (100% WR), 75% water requirement (75% WR), and 50% water requirement (50% WR) in the main plots, and the application of biochar (0, 5, and 10 ton ha^{-1}) and the foliar application of salicylic acid (0, 0.5 mM) in the sub-plots. This study was conducted in the research field of Shahrood University of Technology, Bastam, Shahrood, Iran (Longitude 55˚ 15', Latitude 36˚ 39', altitude 1445 m) in two growing seasons, 2017-2018. Soil preparation was carried out in winter 2016, deep plowing and furrowing were applied according to the cultivation plan, weeding and thinning were done manually, and seeds were planted by hand in wheat-fallow rotation soil. The experimental plot was 2×4 m^2, and each experimental unit included five planting rows with a length of 4 m, with the on-row spacing of 20 cm and between-row spacing of 50 cm. The biochar was prepared using walnut wood as feedstock.

The optimal range for pyrolysis temperature is generally 400-700 °C. In this study, 400 °C was used as slow pyrolysis with a residence time of 30 min because the functional groups were better retained at this temperature (Kambo & Dutta, 2015). Soil samples were also collected to determine the physicochemical properties of the soil before the experiments.

Sampling was done from a depth of 0 to 30 cm of the soil at the experimental site.

Some characteristics of the soil of the experimental site, chemical analysis of the biochar and the climatic conditions in the study area are presented in Table 1, Table 2 and Table 3, respectively.

Table 1. Some physicochemical characteristics of the soil

Soil texture	C (%)	P (ppm)	EC (dS/m)	pH	K (ppm)	N (%)
Loam-silt	0.4	19	0.41	8.30	149	0.04

Table 2. Some chemical characteristics of biochar

Volatile Organic (%)	Ash (%)	C (%)	P (ppm)	EC (dS/m)	pH	K (ppm)	N (%)
46.95	7.60	45.44	0.01	1.72	9.70	0.21	1.27

Table 3. Climatic condition in the area studied

Year	Tmin (°C)	Tmax (°C)	Min relative humidity (%)	Max relative humidity (%)	Rainfall (mm)	The number of frost days	The number of rainy days
2017	-2	39.5	10	98	87.8	97	48
2018	-1.5	37	10	100	85.2	95	24

Biochar was used only in the first year of cultivation, but salicylic acid treatments and irrigation regimes were applied in both years. Salicylic acid treatment was applied before flowering (flower bud development), and irrigation was applied at the time of full plant establishment (3-4-leaf stage and about two months after planting). The first irrigation was done immediately after the seeds were sown, and the irrigation regimes were carried out via a drip-irrigation system. The water requirement of the plants was met for all plots from sowing to seedling establishment, and subsequent irrigations were done according to water deficit treatment. Water requirement was based on the CROPWAT model, which contains a simple water balance model using the FAO Penman-Monteith to calculate evapotranspiration of the reference crops (Malamos et al., 2015). A total of 4670, 3502, and 2335 m^3 ha^{-1} of water was applied to the plots in Shahrood, which were 100% WR, 75% WR, and 50% WR, respectively, during the growing season of European borage, which lasted 99 days. Basal fertilization was applied according to the soil analysis (Table 2), which was carried out before the onset of experiment. The top 5 cm of the soil was removed with a hand hoe for biochar application and mixed with the soil in the top 15 cm of the sandy soil before cultivation.

European borage obtained from Pharmasaat Company was cultivated in the same plots in the first year of cultivation. Seeds were sown on 16 March in the first year of cultivation (2017) and 20 March in the second year (2018).

Leaf samples were collected at the beginning of flowering to measure the antioxidant enzymes, leaf proline content, and relative leaf water content. The samples were then immediately taken to the laboratory and stored at -70 °C after freezing with liquid nitrogen.

2.2. Antioxidant enzymes

First, 0.2 g of plant tissue was homogenized with the extraction solution – 1600 µl potassium phosphate buffer (pH = 6.8), 20 µl 0.1 M EDTA, and 380 µl distilled water. Then, the reaction mixture was centrifuged at 4000 rpm for 25 min at 4 ° C, and the upper phase of the extract was used to measure the enzyme activity. The activity of the CAT enzyme was measured using the spectrophotometric method proposed by (Aebi, 1984). Once the enzyme extract had been prepared, 2.5 ml of potassium phosphate buffer (pH = 7) and 0.3 ml of oxygenated water (3%) were mixed in an ice bath, and then 0.2 ml of enzyme extract was added immediately to measure the kinetic activity of the enzyme catalase. The absorption change curve at 240 nm was read with a spectrophotometer and expressed as mmol of decomposed H_2O_2 per min per mg fresh leaf weight.

For the activity of the POX enzyme, the following concentrations were used for the reaction mixture: 1400 µl 100 mM potassium phosphate buffer (pH = 7), 750 µl 10 mM double distilled water, 100 µl 70 mM H_2O_2 soluble potassium phosphate 100 mM (pH =7), and 750 µl distilled water. The following reaction mixture was placed in a spectrophotometer containing the enzyme extract, and the peroxidase activity was measured at 470 nm. Finally, the enzyme activity was calculated by mmol of tetraguaiacol produced per milligram of fresh leaf weight (Chance & Maehly, 1955).

The activity of the SOD enzyme was determined by its ability to inhibit the photochemical reduction of NBT (nitrotetrazolium blue chloride) at 560 nm, according to Beauchamp & Fridovich (1971). To this end, a 50-mM phosphate buffer solution with pH = 7.5 was first prepared. The mixture containing: 50 mM phosphate buffer (pH 7.5), 0.05 % NBT, 0.1 mM EDTA and 0.065 % NaN3. The mixture was then heated at 85 °C for 15 min and subsequently cooled. The NBT-reducing activity was presented as an increase in absorbance at 560 nm g^{-1} of fresh weight.

2.3. Leaf proline

The following procedure was used for the leaf proline concentration. According to Bates et al. (1973), 4 ml of sulfuric acid (3%) was added to 0.05 g of fresh leaf tissue, and the upper phase was separated with a centrifuge and mixed with 2 ml of glacial acetic acid and 2 ml of ninhydrin solution. The mixture was heated to 90 ° C for half an hour. After cooling, 4 ml of toluene was added to each tube, which was then shaken several times. The supernatant was removed, and the samples were read in the instrument at 520 nm. The proline concentration was determined from a standard curve and calculated as described in Eq.1.

$$\mu\text{moles}\ \frac{\text{proline}}{\text{g}}\ \text{of fresh weight} = \frac{\left[\frac{\mu g\,prolin \times ml\ Toluene}{115.5\mu g/\mu mol}\right]}{(g sample/5)} \quad \text{(Eq. 1)}$$

2.4. Measurement of relative water content (RWC)

The relative water content (RWC) was measured according to Barrs and Weatherley (1962). In this method, 5 samples were collected from the field at the capsule stage (seed filing) and immediately taken to the laboratory, then weighed (FW), placed in deionized water, and left in the dark for 48 h. They were then weighed to determine the leaf turgid weight (TW) and then placed in an oven at 60 °C for 48 h to determine the dry weight (DW) (Romano et al., 2013). The following equation (Eq. 2) was used for RWC calculation:

$$\text{RWC} = [(\text{FW} - \text{DW})/ (\text{TW} - \text{DW}) \times 100] \quad \text{(Eq. 2)}$$

In this equation, FW is the fresh weight of the leaf (in grams), DW is the dry weight of the leaf (in grams), and TW is the saturated weight of the leaf (in grams).

2.5. Total amount of soluble sugars:

The modified Sctilegel method (Chow and Landhäusser, 2004) was used to measure the total amount of soluble sugars in the leaves. First, the plant shoots were removed, followed by drying the samples in an oven at 70 ° C for 48 h. Then, the dried samples were used to measure the total amount of soluble sugars in the plant. The dried samples were ground to a fine powder. Next, 0.1 g of the sample was added to the falcon, and 15 ml of 80% ethanol (which was previously heated) was added to the Erlenmeyer, vortexed for 20 sec, and centrifuged at 3000 rpm for 10 min. The falcons containing the extract were kept in an oven at 50 °C for 24 h. The extract was then centrifuged. Afterward, 5 ml of 5% $ZnSO_4^{2-}$ and 4.7 ml of 3% N-$Bacl_2$ solutions were added to the falcon, and the falcons were centrifuged again at 3000 rpm for 10 min. Subsequently, 2 ml of the liquid phase extract was transferred to a 15-ml falcon tube, and 1 ml of 5% phenol solution and 5 ml of 98% H_2SO_4 were added to each test tube. It was then allowed to stand for 45 min, and the samples were read with a spectrophotometer at a wavelength of 485 nm. Finally, the amount of sample sugar was estimated using the standard curve.

2.6. Statistical analysis

Data analysis was performed using statistical analysis system (SAS 9.4) (SAS Institute, Cary, USA), and the means of the treatments were compared using the least significant difference (LSD) test ($P < 0.05$).

3. Results

3.1. Antioxidant enzymes (CAT, POX, SOD)

Analysis of variance showed that salicylic acid, biochar, and irrigation regimes significantly affected antioxidant enzymes ($P \leq 0.05$) (Table 4). Antioxidant enzymes were increased in 75% WR regime compared to 100%WR, but it was decreased sharply when the irrigation regime reached 50% WR. The highest amount of CAT, SOD, and POX enzymes was observed in the combined application of biochar (5 ton ha^{-1}) and salicylic acid in 75% WR regime, which increased them by 72%, 23%, and 103%, respectively, compared to the control (without biochar and salicylic acid application). In addition, the amount of CAT, SOD, and POX enzymes was also affected by the higher amount of biochar (10 ton ha^{-1}) and salicylic acid in this irrigation regime and by increasing the amount of CAT, SOD, and POX enzymes by 24%, 3.4%, and 28.5%, respectively, compared to the control (Table 5).

3.2. Leaf proline

The interaction effect of irrigation regimes and biochar on the leaf proline concentration was significant in the second year ($P \leq 0.05$) (Table 6). Decreased plant water requirement increased proline production in the plant leaves up to 75% WR. Biochar application decreased the leaf proline concentration under regular and 75% WR irrigation regimes. Application of 5 and 10 ton ha^{-1} of biochar decreased the leaf proline by 4.4% and 23%, respectively, compared to the control (without biochar) under regular irrigation conditions. Application of 10 ton ha^{-1} of biochar also caused a slight decrease in the leaf proline under 75% WR irrigation regime (7%) in the second year (Figs 1). Application of salicylic acid under regular and 75% WR irrigation regimes increased the leaf proline, but it did not make a significant difference under higher irrigation regime (50% WR) in both cropping years (Figs. 2 and 3).

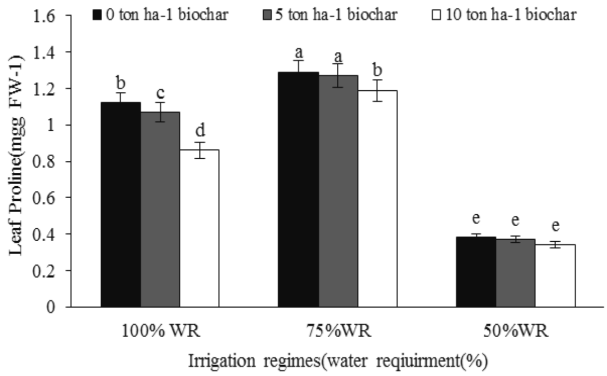

Fig. 1. Interaction effects of biochar and different irrigation regimes on leaf proline concentration (mg/g FW) in *Borago officinalis* L. in 2018.

3.3. Leaf RWC

According to the results (Table 6), the interaction effects of irrigation regime and biochar, and also interaction effects of irrigation regime and salicylic acid had a significant effect on the leaf RWC in both years. The results showed that reducing the irrigation regime reduced the leaf RWC (Figs. 4-7) in both years.

Table 4. Analysis of variance (mean of squares) for the effect of biochar and Salicylic acid on anti oxidative enzymes of *Borago officinalis* L. under different irrigation regimes in two cropping years

S.O.V	df	SOD		POX		CAT	
		2017	2018	2017	2018	2017	2018
Replication	3	0.00007^{ns}	0.00001^{ns}	0.0313^{ns}	0.00035^{ns}	0.00002	0.0002^{ns}
Irrigation regimes (A)	2	0.36188^{**}	0.55386^{**}	6.9026^{**}	0.43410^{**}	0.07444^{*}	0.5032^{**}
Error a (Ea)	6	0.00012	0.00018	0.0053	0.00003	0.00003	0.00062
Biochar (B)	2	0.01096^{**}	0.02156^{**}	0.0414^{**}	0.02362^{**}	0.00380^{**}	0.2257^{**}
A ×B	4	0.00307^{**}	0.01147^{**}	0.00146^{*}	0.01402^{**}	0.00193^{**}	0.00991^{**}
Salicylic acid (C)	1	0.05432^{**}	0.15061^{**}	0.1945^{**}	0.08682^{**}	0.03657^{**}	0.12669^{**}
A×C	2	0.08682^{**}	0.08086^{**}	0.0222^{**}	0.01873^{**}	0.01564^{**}	0.03701^{**}
B×C	2	0.00982^{**}	0.00956^{**}	0.00549^{ns}	0.00982^{**}	0.00209^{**}	0.00619^{**}
A×B×C	4	0.1675^{**}	0.00466^{**}	0.0300^{**}	0.1675^{**}	0.00014^{**}	0.00982^{**}
Total error	45	0.00035	0.00020	0.005	0.00035	0.00005	0.00020
CV (%)	-	1.80	11.08	8.35	1.80	8.78	11.02

ns, non-significant., * Significant at *P*≤0.05., ** Significant at P≤0.01.

Table 5. Mean comparisons of salicylic acid, biochar and different irrigation regimes on anti-oxidative leaf enzymes of *Borago officinalis* L. in two cropping years

Irrigation regimes (Water requirement (%)	Biochar (ton ha^{-1})	Salicylic acid (mM)	SOD (mg/g FW)		POX (mg/g FW)		CAT (mg/g FW)	
			2017	2018	2017	2018	2017	2018
100% WR	0	0	0.08^{gh}	0.048^{hi}	1.09^{d}	0.988^{fg}	0.05^{ij}	0.088^{gh}
	0	0.5	0.17^{ef}	0.137^{f}	1.18^{bc}	1.07^{e}	0.07^{fg}	0.177^{ef}
	5	0	0.97^{gh}	0.035^{ji}	1.09^{gh}	0.975^{hg}	0.05^{ij}	0.075^{hi}
	5	0.5	1.05^{e}	0.113^{g}	1.14^{c}	1.053^{e}	0.06^{gh}	0.153^{f}
	10	0	0.96^{ghi}	0.028^{ikj}	1.01^{d}	0.968^{ghi}	0.05^{ij}	0.068^{hi}
	10	0.5	1.0^{f}	0.068^{h}	1.09^{cd}	1.008^{f}	0.05^{hi}	0.108^{g}
75% WR	0	0	1.15^{c}	0.213^{d}	1.24^{b}	1.153^{c}	0.10^{d}	0.253^{d}
	0	0.5	1.15^{c}	0.461^{b}	1.19^{bc}	1.156^{c}	0.21^{b}	0.346^{b}
	5	0	1.14^{c}	0.204^{d}	1.18^{bc}	1.144^{c}	0.09^{e}	0.244^{d}
	5	0.5	1.42^{a}	0.496^{a}	1.35^{a}	1.426^{a}	0.23^{a}	0.436^{a}
	10	0	1.10^{d}	0.165^{e}	1.18^{bc}	1.105^{d}	0.08^{ef}	0.195^{e}
	10	0.5	1.19^{b}	0.275^{c}	1.26^{ab}	1.197^{b}	0.13^{c}	0.317^{c}
50% WR	0	0	0.91^{l}	0.027^{jk}	0.16^{g}	0.912^{l}	0.03^{kl}	0.012^{l}
	0	0.5	0.95^{hij}	0.015^{jkl}	0.48^{e}	0.955^{hij}	0.05^{ij}	0.055^{ij}
	5	0	0.95^{hij}	0.014^{kl}	0.16^{g}	0.954^{hij}	0.02^{m}	0.054^{ij}
	5	0.5	0.94^{jkl}	0.005^{l}	0.33^{f}	0.943^{jkl}	0.05^{ij}	0.043^{jk}
	10	0	0.92^{kl}	0.002^{jkl}	0.15^{g}	0.920^{kl}	0.02^{lm}	0.020^{kl}
	10	0.5	0.92^{jkl}	0.011^{kl}	0.19^{g}	0.928^{jkl}	0.04^{jk}	0.028^{kl}

Means followed by similar letters in each column do not significantly differ at α= 5% probability level based on LSD test.

Table 6. Analysis of variance (mean of squares) for the effect of biochar and Salicylic acid on leaf proline concentration, total amount of soluble sugars and leaf relative water content of *Borago officinalis* L. under different irrigation regimes in two cropping years

S.O.V	df	Leaf Proline Concentration		Total amount of soluble sugars		Leaf relative water content	
		2017	2018	2017	2018	2017	2018
Replication	3	0.001^{ns}	0.008^{ns}	197446^{**}	295533^{**}	13.94ns	16.29^{ns}
Irrigation regimes (A)	2	5.19^{**}	5.243^{**}	1075275^{**}	862562^{**}	287.54**	2879.58^{**}
Error a (Ea)	6	0.009	0.0120	20954	9633	5.54	8.03
Biochar (B)	2	0.080^{**}	0.086^{**}	119244^{ns}	81460^{ns}	15.08ns	15.42^{**}
A ×B	4	0.034^{ns}	0.036^{*}	18351^{ns}	6359	25.83**	25.94^{**}
Salicylic acid (C)	1	0.019^{ns}	0.0276^{ns}	299332^{**}	137149^{**}	682.40**	696.51^{**}
A×C	2	0.78^{**}	0.7347^{**}	41698^{ns}	26268^{ns}	27.5**	27.55^{**}
B×C	2	0.025^{ns}	0.018^{ns}	16311^{ns}	34727^{ns}	15.36ns	15.39^{ns}
A×B×C	4	0.018^{ns}	0.021^{ns}	57842^{ns}	31771^{ns}	5.26ns	5.07^{ns}
Total error	45	0.012	0.016	47455	30226	4.97	5.03
CV (%)	-	11.39	13.4	14.36	14.64	3.87	3.99

ns, non-significant., * Significant at *P*≤0.05., ** Significant at *P*≤0.01.

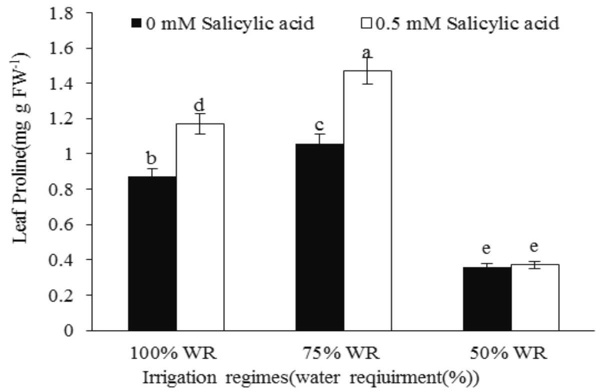

Fig. 2. Interaction effects of salicylic acid and different irrigation regimes on leaf proline concentration (mg/g FW) in *Borago officinalis* L. in 2017.

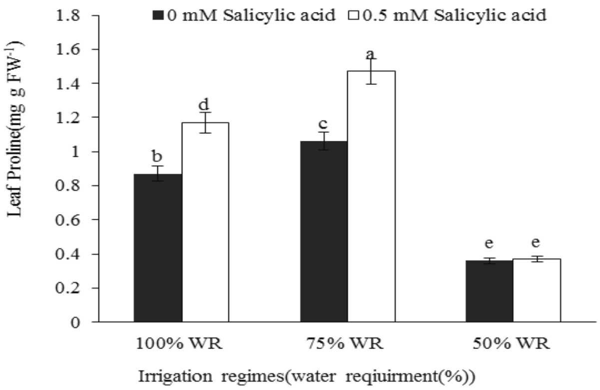

Fig. 3. Interaction effects of salicylic acid and different irrigation regimes on leaf proline concentration (mg/g FW) in *Borago officinalis* L. in 2018.

borage leaves compared to the control (0 ton ha^{-1} of biochar) (Figs. 4 and 5).

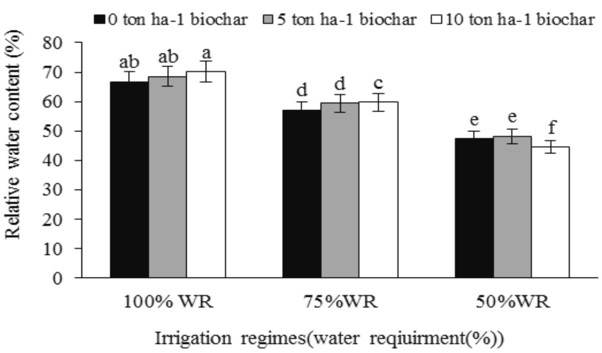

Fig. 4. Interaction effects of biochar and different irrigation regimes on relative water content (RWC) in *Borago officinalis* L. in 2017.

Also, the results of the interaction effects of irrigation regimes and salicylic acid showed that the application of salicylic acid increased the RWC of leaves in all irrigation regimes compared to the control (0m M SA) in both years (Figs. 6 and 7).

3.4. Total soluble sugars

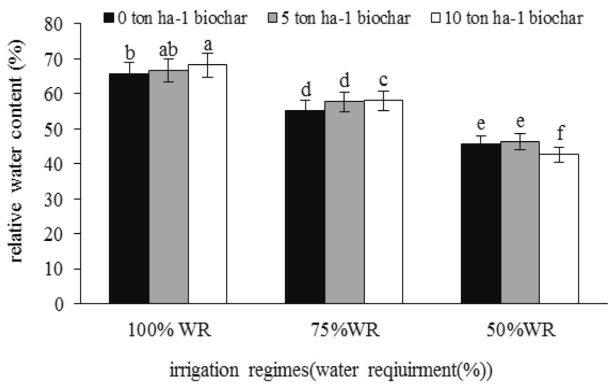

Fig. 5. Interaction effects of biochar and different irrigation regimes on relative water content (RWC) in *Borago officinalis* L. in 2018

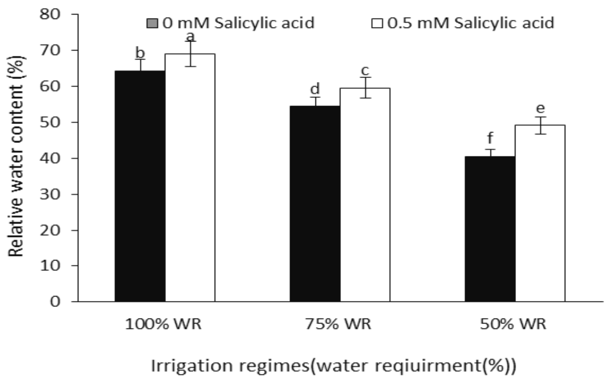

Fig. 6. Interaction effects of salicylic acid and different irrigation regimes on relative water content (RWC) in *Borago officinalis* L. in 2017.

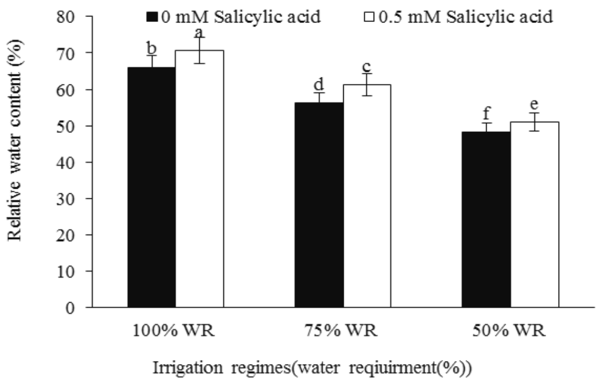

Fig. 7. Interaction effects of salicylic acid and different irrigation regimes on relative water content (RWC) in *Borago officinalis* L. in 2018.

The results of the analysis of variance showed that the effects of irrigation regimes and salicylic acid on the soluble sugars of European borage leaves were significant in both years (Table 6). Comparing the main effects of the mean values (Figs. 8 and 9) showed that as irrigation regimes increased to 75% WR, the amount of soluble sugars in the leaves increased in both years. However, it showed a sharp decrease as the irrigation regime dropped to 50% WR. Under this irrigation regime, the amount of soluble sugars decreased by 19% and 20% in the first and second year, respectively.

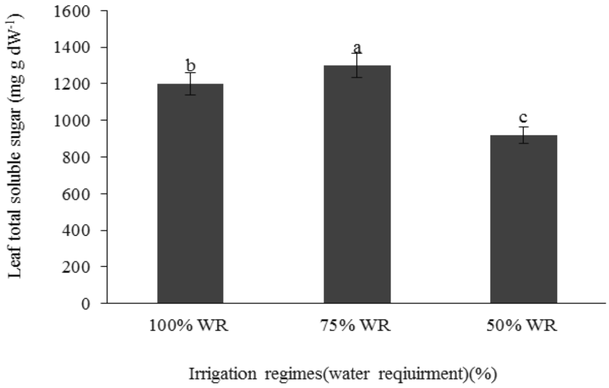

Fig. 8. Effects of salicylic acid on Leaf total soluble sugar in *Borago officinalis* L. in 2017.

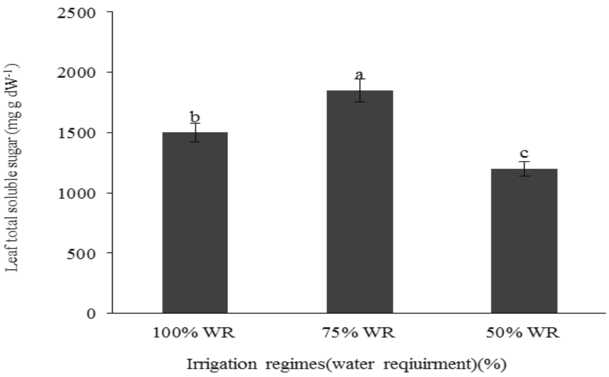

Fig. 9. Effects of Salicylic acid on Leaf total soluble sugar in *Borago officinalis* L. in 2018.

Salicylic acid treatments mitigated the negative effects of water deficit conditions. The use of salicylic acid was effective in improving the amount of soluble sugars in both years, increasing their amount by 8% in the first year (Fig 10) and 7% in the second year (Fig 11).

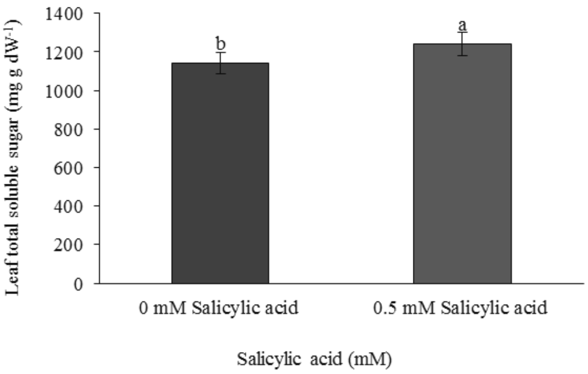

Fig. 10. Effects of salicylic acid on Leaf total soluble sugar in *Borago officinalis* L. in 2017.

4. Discussion

4.1. Antioxidant enzymes (CAT, POX, SOD)

The results showed an increasing trend of antioxidant enzymes by increasing water deficit at 75% WR, but by increasing water deficit conditions it decreased sharply when the regime of 50% WR was reached. Previous studies have found that drought stress can induce oxidative stress. In addition, the glycolate oxidase pathway, which produces H_2O_2, is activated by drought (Cao et al., 2017).

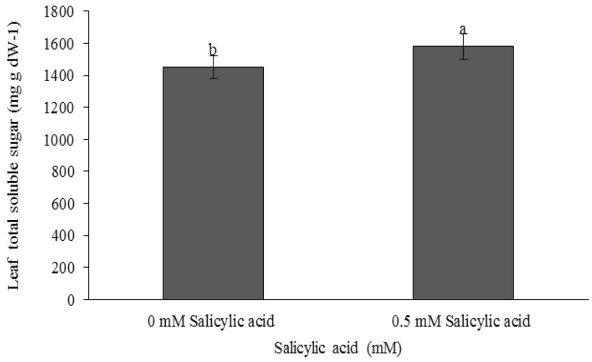

Fig. 11. Effects of salicylic acid on Leaf total soluble sugar in *Borago officinalis* L. in 2018.

Catalase, peroxidase, and superoxide dismutase are important enzymes in plants that are directly involved in the plant scavenging system that degrades H_2O_2 to H_2O at various cellular sites, thereby mitigating the negative effects of ROS. Although several studies have shown that all of these enzymes are increased under drought stress, the overall trend of these enzymes in different plants under stress varies, and in some cases, they are even contradictory (Gao et al., 2020). Drought stress in *Pyrus ussuriensis* initially increased the levels of SOD and POD enzymes but sharply decreased them as drought stress increased. Nevertheless, CAT was continuously decreased (Lyu et al., 2016), while in Chinese cabbage exposed to drought, the opposite trend was observed, such that catalase activity was three times higher than that in control treatment after one week (Shawon et al., 2020). The application of salicylic acid reduced the negative effects of drought stress by increasing these antioxidant enzymes in *Ctenanthe setosa* (Kadioglu et al., 2011), *Brassica rapa* L.(La et al., 2019) and *Portulaca oleracea* L. (Saheri et al., 2020). Several studies have also mentioned that antioxidant enzymes are also affected by the addition of biochar (Abideen et al., 2020) (Farhangi-Abriz & Torabian, 2017). Abideen et al. (2020) reported that biochar reduced the soil evaporation rate and improved the water holding capacity through some physicochemical properties such as higher hydrophilic inner surface area; it means plants maintained a balanced water ratio under water deficit and caused less oxidative stress in biochar treated plants due to higher photosystem II efficiency and stimulated antioxidant defense system activity due to higher water availability. The absence of biochar reduced the demand for ATP and increased the risk of excessive reduction of the physicochemical electron transport chain, increasing the probability of ROS by damaging the photochemical activity of PSII (Abideen et al., 2020; Farhangi-Abriz & Torabian, 2017).

4.2. Leaf proline

In the present study, leaf proline concentration was increased with an increase in 75% WR regime (Fig. 1). The proline accumulation under drought stress conditions is well documented (Farhangi-Abriz & Torabian, 2017; La et al., 2019). Proline is a multifunctional amino acid capable of protecting protein integrity and enhancing the activities of various enzymes. Proline accumulation under drought stress conditions may be related to the decrease in proline oxidase enzyme activity with a concomitant increase in γ-glutamylkinase enzymes (Idrees et al., 2010). Proline is not only an excellent osmolyte but also an osmoprotectant that has been shown to play an important role in improving resistance to drought by stabilizing the protein structure, inhibiting lipid peroxidation, and scavenging the production of ROS by maintaining the $NADPH/NADP^+$ ratio involved in cellular homeostasis (Farhangi-Abriz & Torabian, 2017; Hussain et al., 2018; La et al., 2019). Application of salicylic acid increased the proline concentration in plants subjected to mild water deficit stress. Similarly, numerous studies have reported an increase in the proline concentration by salicylic acid during drought stress (Kordi et al., 2013; Farhangi-Abriz & Torabian, 2017; La et al., 2018). This result agreed with Estaji and Niknam (2020) views, who reported that irrigation intervals increased the leaf proline in *Silybum marianum* L. by 1.5 and 2.5 times compared to well-watered plants.

In addition, our results suggest that biochar can alleviate the water deficit condition by proline reduction through the mechanisms of osmoregulation. Wang et al. (2014) reported a similar decrease in the leaf proline content of *Malus hupehensis* Rehd. seedlings by 3-7% compared to treatments without biochar addition. This decrease could be related to the reduction of proline accumulation in the biochar additive treatments. It could also be attributed to the improvement in stress tolerance due to the increase in soil water retention capacity and water availability for protein degradation (Adejumo et al., 2020).

4.3. Leaf RWC

Leaf RWC is an important indicator of water status in plants; it reflects the balance between leaf tissue water supply and transpiration rate (Lugojan & Ciulca, 2011). It has been observed that water deficit decreases RWC. A possible explanation could be that the plant increases the accumulation of compatible solutes (such as amino acids, sugars, or sugar alcohols) and reduces the osmotic potential in the protoplasm for water absorption under drought conditions compared to well-watered conditions; this allows water to enter the cell and stabilize the turgor potential within the cells, counteractively modulating RWC (Loutfy et al., 2012).

High correlations between plant water potential and carbohydrates have been reported in different plant species (Askari & Ehsanzadeh, 2015). This study also found a significant positive relationship between leaf soluble sugars and the leaf relative water content in the first ($r = 0.44^{**}$) and second ($r = 0.43^{**}$) years (results not shown). In the present study, salicylic acid increased RWC in treated plants under water deficit conditions, which is consistent with observations reported in sunflowers (Chavoushi et al., 2020). This may be due to the role of salicylic acid in activating defense mechanisms and increasing the biosynthesis of secondary metabolites such as flavonoids, anthocyanins, and phenolics, which protect cells from oxidative stress and increase sugar concentration to reduce the deleterious effects of drought (Chavoushi et al., 2020; Saheri et al., 2020). Biochar also caused an increase in RWC in borage leaves. The reduction of RWC due to drought stress positively correlates with the soil moisture content (Dapanage & Bhat, 2018).

Biochar, as a soil conditioner, plays a crucial role in increasing soil and plant water availability (Baronti et al., 2014), so its effect may result from increased cell water content. Biochar application increased membrane stability and the relative leaf water content in tomatoes under drought stress. Biochar application has also been reported to increase the available soil water by 3.2% to 45% and leaf water potential by 24% to 37% in grapes (Baronti et al., 2014).

4.4. Total soluble sugars

The total soluble sugars were decreased under water deficit conditions, but salicylic acid treatments mitigated the negative effects. Accumulation of sugars under drought stress may be due to reduced consumption of these compounds, or hydrolysis of starch. Accumulation of soluble sugars under stress conditions plays a critical role in reducing the osmotic potential of leaves. In this way, they maintain their internal osmotic pressure and thus resist against water loss. It has been reported that SA and soluble sugars increase by 25% in wheat under drought stress (Ilyas et al., 2017). Another study showed that water deficit increased total soluble carbohydrates in all fennel genotypes, and SA affected positively the total soluble carbohydrates (Askari & Ehsanzadeh, 2015).

4. Conclusion

It was found that the application of biochar as a soil amendment and salicylic acid as a regulatory hormone could substantially mitigate the negative effects of water deficit in *Borago officinalis* via increasing plant defense mechanisms, such as the leaf proline, and scavengers such as antioxidant enzymes, and using osmotic adjustment through accumulation of total soluble sugars. Biochar decreased the harmful effects of water stress by the formation of stable aggregates that increases the water holding capacity and RWC which helps the plant better cope with water deficits. Salicylic acid treatment enhances the sugar accumulation, which may cause the more remarkable changes in the key compound that participates in glycolysis, i.e., acetyl-CoA, which starts the biosynthesis of long-chain fatty acids. Furthermore, salicylic acid increases sugar accumulation as well. The results may be applicable in medicinal plant production cropping systems in arid regions.

References

Aebi, H. (1984). Catalase in Vitro. *Methods in Enzymology*. 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Abdelli, M., Moghrani, H., Aboun, A. & Maachi, R. (2016). Algerian *Mentha pulegium* L. leaves essential oil: Chemical composition, antimicrobial, insecticidal and antioxidant activities. *Industrial Crops and Products*. 94, 197–205. https://doi.org/10.1016/j.indcrop.2016.08.042

Abideen, Z., Koyro, H.W., Huchzermeyer, B., Ansari, R., Zulfiqar, F. & Gul, B. (2020). Ameliorating effects of biochar on photosynthetic efficiency and antioxidant defence of Phragmites karka under drought stress. *Plant Biology*. 22, 259–266. https://doi.org/10.1111/plb.13054

Adejumo, S.A., Arowo, D.O., Ogundiran, M.B. & Srivastava, P. (2020). Biochar in combination with compost reduced Pb uptake and enhanced the growth of maize in lead (Pb)-contaminated soil exposed to drought stress. *Journal of Crop Science and Biotechnology.* 23, 273–288. https://doi.org/10.1007/s12892-020-00035-8

Aebi, H. (1984). Catalase in Vitro. *Methods in Enzymology*. 105, 121–126. https://doi.org/10.1016/S0076-6879(84)05016-3

Akhtar, S.S., Li, G., Andersen, M.N. & Liu, F. (2014). Biochar enhances yield and quality of tomato under reduced irrigation. *Agriculture and Water Management*. 138, 37–44. https://doi.org/10.1016/j.agwat.2014.02.016

Askari, E. & Ehsanzadeh, P. (2015). Drought stress mitigation by foliar application of salicylic acid and their interactive effects on physiological characteristics of fennel (*Foeniculum vulgare* Mill.) genotypes. *Acta Physiologiae Plantarum*. 37. https://doi.org/10.1007/s11738-014-1762-y

Bahadori, M. & Tofighi, H. (2016). A Modified Walkley-Black Method Based on Spectrophotometric Procedure. *Communication in Soil Science and Plant Analysis.* 47, 213–220. https://doi.org/10.1080/00103624.2015.1118118

Baronti, S., Vaccari, F.P., Miglietta, F., Calzolari, C., Lugato, E., Orlandini, S., Pini, R., Zulian, C. & Genesio, L. (2014). Impact of biochar application on plant water relations in *Vitis vinifera* (L.). *European Journal of Agronomy*. 53, 38–44. https://doi.org/10.1016/j.eja.2013.11.003

Barrs, H.D. & Weatherley, P.E. (1962) A Re-Examination of the Relative Turgidity Techniques for Estimating Water Deficits in Leaves. *Australian Journal of Biological Sciences.* 15, 413 - 428. http://dx.doi.org/10.1071/BI9620413.

Bates, L.S., Waldren, R.P. & Teare, I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*. 39**,** 205–207. https://doi.org/10.1007/BF00018060

Batool, A., Taj, S., Rashid, A., Khalid, A., Qadeer, S., Saleem, A.R. & Ghufran, M.A. (2015). Potential of soil amendments (Biochar and gypsum) in increasing water use efficiency of *abelmoschus esculentus* L. Moench. *Frontiers in Plant Science*. 6, 1–13. https://doi.org/10.3389/fpls.2015.00733

Beauchamp, C. & Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analals of Biochemistry*. 44, 276–287. https://doi.org/10.1016/0003-2697(71)90370-8

Borchard, N., Schirrmann, M., Cayuela, M.L., Kammann, C., Wrage-Mönnig, N., Estavillo, J.M., Fuertes-Mendizábal, T., Sigua, G., Spokas, K., Ippolito, J.A. & Novak, J. (2019). Biochar, soil and land-use interactions that reduce nitrate leaching and N2O emissions: A meta-analysis. *Science of The Total Environment.* 651, 2354–2364. https://doi.org/10.1016/j.scitotenv.2018.10.060

Cao, Y., Luo, Q., Tian, Y. & Meng, F. (2017). Physiological and proteomic analyses of the drought stress response in Amygdalus Mira (Koehne) roots. *Plant Biolology*. 17, 1–16. https://doi.org/10.1186/s12870-017-1000-z

Chance, B. & Maehly, A.C. (1955). [136] Assay of catalases and peroxidases. {black small square}. *Methods in Enzymology*. 2, 764–775. https://doi.org/10.1016/S0076-6879(55)02300-8

Chavoushi, M., Najafi, F., Salimi, A. & Angaji, S.A. (2020). Effect of salicylic acid and sodium nitroprusside on growth parameters, photosynthetic pigments and secondary metabolites of safflower under drought stress. *Scientia Horticulturae*.

(Amsterdam). 259. https://doi.org/10.1016/j.scienta.2019.108823

Chen, L., Liu, M., Ali, A., Zhou, Q., Zhan, S., Chen, Y., Pan, X. & Zeng, Y. (2020). Effects of Biochar on Paddy Soil Fertility Under Different Water Management Modes. *Journal of Soil Science and Plant Nutrition*. 20, 1810–1818. https://doi.org/10.1007/s42729-020-00252-8

Cheng, W. & Kuzyakov, Y. (2015). Root effects on soil organic matter decomposition. Roots Soil Manag. Interact. between Roots *Soil*. 48, 119–143. https://doi.org/10.2134/agronmonogr48.c7

Chow, P.S. & Landhäusser, S.M. (2004). A method for routine measurements of total sugar and starch content in woody plant tissues. *Tree Physiology*. 24, 1129–1136. https://doi.org/10.1093/treephys/24.10.1129

Dapanage, M. & Bhat, S. (2018). Physiological responses of commercial sugarcane (Saccharum spp. hybrids) varieties to moisture deficit stress tolerance. *Indian Journal of Plant Physiology*. 23, 40–47. https://doi.org/10.1007/s40502-017-0328-6

Dong, C.J., Liu, X.Y., Xie, L.L., Wang, L.L. & Shang, Q.M. (2020). Salicylic acid regulates adventitious root formation via competitive inhibition of the auxin conjugation enzyme CsGH3.5 in cucumber hypocotyls. *Planta*. 252, 1–15. https://doi.org/10.1007/s00425-020-03467-2

Darvizheh, H., Zahedi, M., Abbaszadeh, B. & Razmjoo, J. (2019). Changes in some antioxidant enzymes and physiological indices of purple coneflower (*Echinacea purpurea* L.) in response to water deficit and foliar application of salicylic acid and spermine under field condition. *Scientia Horticulturae*. (Amsterdam). 247, 390–399. https://doi.org/10.1016/j.scienta.2018.12.037

Diatta, A.A., Fike, J.H., Battaglia, M.L., Galbraith, J.M. & Baig, M.B. (2020). Effects of biochar on soil fertility and crop productivity in arid regions: a review. *Arabian Journal of Geosciences.* 13, 595. https://doi.org/10.1007/s12517-020-05586-2

Du, C., Shen, F., Li, Y., Zhao, Z., Xu, X., Jiang, J. & Li, J. (2021). Effects of salicylic acid, jasmonic acid and reactive oxygen species on the resistance of Solanum peruvianum to Meloidogyne incognita. *Scientia Horticulturae*. (Amsterdam). 275, 109649. https://doi.org/10.1016/j.scienta.2020.109649

Estaji, A. & Niknam, F. (2020). Foliar salicylic acid spraying effect' on growth, seed oil content, and physiology of drought-stressed *Silybum marianum* L. plant. *Agriculture and Water Management*. 234, 106116. https://doi.org/10.1016/j.agwat.2020.106116

Farhangi-Abriz, S. & Torabian, S. (2017). Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. *Ecotoxicology and Environmental Safety*. 137, 64–70. https://doi.org/10.1016/j.ecoenv.2016.11.029

Farhadi, N. & Ghassemi-Golezani, K. (2020). Physiological changes of Mentha pulegium in response to exogenous salicylic acid under salinity. *Scientia Horticulturae*. (Amsterdam). 267, 109325. https://doi.org/10.1016/j.scienta.2020.109325

Friedman, S.P. (2005). Soil properties influencing apparent electrical conductivity: A review. *Computer and Electronic in Agriculture*. 46, 45–70. https://doi.org/10.1016/j.compag.2004.11.001

Gacnik, S., Veberic, R., Marinovic, S., Halbwirth, H. & Mikulic-Petkovsek, M. (2021). Effect of pre-harvest treatments with salicylic and methyl salicylic acid on the chemical profile and activity of some phenylpropanoid pathway related enzymes in apple leaves. *Scientia Horticulturae*. (Amsterdam). 277, 109794. https://doi.org/10.1016/j.scienta.2020.109794

Gao, S., Wang, Y., Yu, S., Huang, Y., Liu, H., Chen, W. & He, X. (2020). Effects of drought stress on growth, physiology and secondary metabolites of Two Adonis species in Northeast China. *Scientia Horticulturae*. (Amsterdam). 259, 108795. https://doi.org/10.1016/j.scienta.2019.108795

Ghaffari, H., Tadayon, M.R., Nadeem, M., Cheema, M. & Razmjoo, J. (2019). Proline-mediated changes in antioxidant enzymatic activities and the physiology of sugar beet under drought stress. *Acta Physiologiae Plantarum*. 41, 23. https://doi.org/10.1007/s11738-019-2815-z

Ghassemi-Golezani, K. & Farhangi-Abriz, S. (2018). Changes in Oil Accumulation and Fatty Acid Composition of Soybean Seeds under Salt Stress in Response to Salicylic Acid and Jasmonic Acid. *Russian Journal of Plant Physiology*. 65, 229–236. https://doi.org/10.1134/S1021443718020115

Ghassemi, S., Ghassemi-Golezani, K. & Salmasi, S.Z. (2019). Changes in antioxidant enzymes activities and physiological traits of ajowan in response to water stress and hormonal application. *Scientia Horticulturae*. (Amsterdam). 246, 957–964. https://doi.org/10.1016/j.scienta.2018.11.086

Gorni, P.H., Pacheco, A.C., Moro, A.L., Silva, J.F.A., Moreli, R.R., de Miranda, G.R., Pelegrini, J.M., Spera, K.D., Bronzel, J.L. & da Silva, R.M.G. (2020). Salicylic acid foliar application increases biomass, nutrient assimilation, primary metabolites and essential oil content in *Achillea millefolium* L. *Scientia Horticulturae.* (Amsterdam). 270, 109436. https://doi.org/10.1016/j.scienta.2020.109436

Gong Z, Li P, Guo S, Jing X, Wang X, Zhang H. (2001).Bioslurry remediation of soil contaminated with polycyclic aromatic hydrocarbons. Sep;22(5):112-6. Chinese. PMID: 11769215.

Guo, L., Bornø, M.L., Niu, W. & Liu, F. (2021). Biochar amendment improves shoot biomass of tomato seedlings and sustains water relations and leaf gas exchange rates under different irrigation and nitrogen regimes. *Agriculture and Water Management.* 245. https://doi.org/10.1016/j.agwat.2020.106580

Han, M., Sun, L., Gan, D., Fu, L. & Zhu, B. (2020). Root functional traits are key determinants of the rhizosphere effect on soil organic matter decomposition across 14 temperate hardwood species. *Soil Biology and Biochemistry*. 151, 108019. https://doi.org/10.1016/j.soilbio.2020.108019

Hossain, M.Z., Bahar, M.M., Sarkar, B., Donne, S.W., Ok, Y.S., Palansooriya, K.N., Kirkham, M.B., Chowdhury, S. & Bolan, N. (2020). Biochar and its importance on nutrient dynamics in soil and plant, *Biochar*. 2, 379-420. https://doi.org/10.1007/s42773-020-00065-z

Huang, L., Li, M., Zhou, K., Sun, T., Hu, L., Li, C. & Ma, F. (2018). Uptake and metabolism of ammonium and nitrate in response to drought stress in Malus prunifolia. *Plant Physiology and Biochemistry*. 127, 185–193. https://doi.org/10.1016/j.plaphy.2018.03.031

Huang, Y.T., Cai, S.Y., Ruan, X.L., Chen, S.Y., Mei, G.F., Ruan, G.H. & Cao, D.D. (2021). Salicylic acid enhances sunflower seed germination under Zn2+ stress via involvement in Zn2+ metabolic balance and phytohormone interactions. *Scientia Horticultura.* (Amsterdam). 275, 109702. https://doi.org/10.1016/j.scienta.2020.109702

Hussain, M., Farooq, S., Hasan, W., Ul-Allah, S., Tanveer, M., Farooq, M. & Nawaz, A. (2018). Drought stress in sunflower: Physiological effects and its management through breeding and agronomic alternatives. *Agriculture and Water Management*. 201, 152–166. https://doi.org/10.1016/j.agwat.2018.01.028

Idrees, M., Khan, M.M.A., Aftab, T., Naeem, M. & Hashmi, N. (2010). Salicylic acid-induced physiological and biochemical changes in lemongrass varieties under water stress. *Journal of Plant Interaction*. 5, 293–303. https://doi.org/10.1080/17429145.2010.508566

Ilyas, N., Gull, R., Mazhar, R., Saeed, M., Kanwal, S., Shabir, S. & Bibi, F. (2017). Influence of Salicylic Acid and Jasmonic Acid on Wheat Under Drought Stress. *Communication in Soil Science and Plant Analysis*. 48, 2715–2723. https://doi.org/10.1080/00103624.2017.1418370

Jovanovic, N., Pereira, L.S., Paredes, P., Pôças, I., Cantore, V. &Todorovic, M. (2020). A review of strategies, methods and technologies to reduce non-beneficial consumptive water use on farms considering the FAO56 methods. *Agriculture and Water Management.* 239, 106267. https://doi.org/10.1016/j.agwat.2020.106267

Kadioglu, A., Saruhan, N., Sağlam, A., Terzi, R., Acet, T., 2011. Exogenous Salicylic acid alleviates effects of long term drought stress and delays leaf rolling by inducing antioxidant system. *Plant Growth Regulation*. 64, 27–37. https://doi.org/10.1007/s10725-010-9532-3v

Karimi, A., Moezzi, A., Chorom, M. & Enayatizamir, N. (2020). Application of Biochar Changed the Status of Nutrients and Biological Activity in a Calcareous Soil. *Journal of Soil Science and Plant Nutrition*. 20, 450–459. https://doi.org/10.1007/s42729-019-00129-5

Karimi, E., Oskoueian, E., Karimi, A., Noura, R. & Ebrahimi, M. (2018). *Borago officinalis* L. flower: a comprehensive study on bioactive compounds and its health-promoting properties. *Journal of Food Measurement and Characterization*. 12, 826–838. https://doi.org/10.1007/s11694-017-9697-9

Kambo, H.S. & Dutta, A. (2015). A comparative review of biochar and hydrochar in terms of production, physico-chemical properties and applications. *Renewable and Sustainable Energy Reviews*. 45, 359–378. https://doi.org/10.1016/j.rser.2015.01.050

Kordi, S., Saidi, M. & Ghanbari, F. (2013). Induction of Drought Tolerance in Sweet Basil (*Ocimum basilicum* L) by Salicylic Acid. *International Journal of Agricultural Food Research.* 2, 18–26. https://doi.org/10.24102/ijafr.v2i2.149

La, V.H., Lee, B.R., Zhang, Q., Park, S.H., Islam, M.T. & Kim, T.H. (2019). Salicylic acid improves drought-stress tolerance by regulating the redox status and proline metabolism in *Brassica rapa*. *Horticulture, Environment and Biotechnology*. 60, 31–40. https://doi.org/10.1007/s13580-018-0099-7

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C. & Crowley, D. (2011). Biochar effects on soil biota - A review. *Soil Biology and Biochemistry*. 43, 1812–1836. https://doi.org/10.1016/j.soilbio.2011.04.022

Lorenz, K. & Lal, R. (2014). Biochar application to soil for climate change mitigation by soil organic carbon sequestration. *Journal of Plant Nutrition and Soil Science*. 177, 651–670. https://doi.org/10.1002/jpln.201400058

Loutfy, N., El-Tayeb, M.A., Hassanen, A.M., Moustafa, M.F.M., Sakuma, Y. & Inouhe, M. (2012). Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum*).

Journal of Plant Research. 125, 173–184. https://doi.org/10.1007/s10265-011-0419-9

Lugojan, C. & Ciulca, S. (2011). Evaluation of relative water content in winter wheat. *Journal of Horticulture, Forestry and Biotechnol.* 15(2), 173–177.

Lyu, S., Du, G., Liu, Z., Zhao, L. & Lyu, D. (2016). Effects of biochar on photosystem function and activities of protective enzymes in Pyrus ussuriensis Maxim. under drought stress. *Acta Physiologiae Plantarum*. 38. https://doi.org/10.1007/s11738-016-2236-1.

Malamos, N., Barouchas, P.E., Tsirogiannis, I.L., Liopa-Tsakalidi, A. & Koromilas, T.H. (2015). Estimation of monthly FAO Penman-Monteith evapotranspiration in GIS environment, through a geometry independent algorithm. *Agriculture and Agricultural Science Procedia*. 4, 290-299.

Rayment, G.E. & Higginson, F.R. (1992). Australian Laboratory Handbook of Soil and Water Chemical Methods. Melbourne, Inkafa Press. Australian Soil and Lab Survey Handbooks, 3.

Safahani Langeroodi, A.R., Campiglia, E., Mancinelli, R. & Radicetti, E. (2019). Can biochar improve pumpkin productivity and its physiological characteristics under reduced irrigation regimes? *Scientia Horticulturae*. (Amsterdam). 247, 195–204. https://doi.org/10.1016/j.scienta.2018.11.059

Shah Jahan, M., Wang, Y., Shu, S., Zhong, M., Chen, Z., Wu, J., Sun, J. & Guo, S. (2019). Exogenous salicylic acid increases the heat tolerance in Tomato (*Solanum lycopersicum* L) by enhancing photosynthesis efficiency and improving antioxidant defense system through scavenging of reactive oxygen species. *Scientia. Horticulturae*. (Amsterdam). 247, 421–429. https://doi.org/10.1016/j.scienta.2018.12.047

Shahbazi, Z., Salehi, A., Hazrati, S. & Dehnavi, M.M. (2019). Enhancing the Quality and Yield of European Borage (Borago officinalis) by Simultaneous Application of Granulated Compost, Vermicompost and Mycorrhiza. *International Journal of Horticultural Science and Technology*. 6, 283–298. https://doi.org/10.22059/ijhst.2019.274604.281

Shawon, R.A., Kang, B.S., Lee, S.G., Kim, S.K., Ju Lee, H., Katrich, E., Gorinstein, S. & Ku, Y.G. (2020). Influence of drought stress on bioactive compounds, antioxidant enzymes and glucosinolate contents of Chinese cabbage (*Brassica rapa*). *Food Chemistry*. 308, 125657. https://doi.org/10.1016/j.foodchem.2019.125657

Verheijen, F., Jeffery, S., Bastos, A.C., Van Der Velde, M. & Diafas, I. (2010). Biochar Application to Soils: A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. European Communities Publication, pp.164.

Wang, Yanfang, Pan, F., Wang, G., Zhang, G., Wang, Yanling, Chen, X. & Mao, Z. (2014). Effects of biochar on photosynthesis and antioxidative system of *Malus hupehensis Rehd*. seedlings under replant conditions. *Scientia Horticulturae*. (Amsterdam). 175, 9–15. https://doi.org/10.1016/j.scienta.2014.05.029

Yin, H., Wheeler, E. & Phillips, R.P. (2014). Root-induced changes in nutrient cycling in forests depend on exudation rates. *Soil Biology and Biochemistry*. 78, 213–221. https://doi.org/10.1016/j.soilbio.2014.07.022

Zamani, S., Naderi, M.R., Soleymani, A., Nasiri, B.M., Miransari, M., 2020. Sunflower (*Helianthus annuus* L.) biochemical properties and seed components affected by potassium fertilization under drought conditions. *Ecotoxicology and Environmental Safety*. 190, 110017. https://doi.org/10.1016/j.ecoenv.2019.110017

Zhang, C., Li, X., Yan, H., Ullah, I., Zuo, Z., Li, L. & Yu, J. (2020). Effects of irrigation quantity and biochar on soil physical properties, growth characteristics, yield and quality of greenhouse tomato. *Agriculture and Water Management*. 241, 106263. https://doi.org/10.1016/j.agwat.2020.106263

Zhang, X., Qu, J., Li, H., La, S., Tian, Y. & Gao, L. (2020). Biochar addition combined with daily fertigation improves overall soil quality and enhances water-fertilizer productivity of cucumber in alkaline soils of a semi-arid region. *Geoderma*. 363, 114170.