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ORIGINAL PAPER

Jasmonates Improve Drought Tolerance of *Hordeum vulgare* L. After Biochar Treatment

Sajjad Nasiri[1] · **Babak Andalibi**[1] · **Afshin Tavakoli**[1] · **Mohammad Amir Delavar**[2] · **Ali El-Keblawy**[3] · **Andrea Mastinu**[4]

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Abstract

To investigate how and in what amounts biochar and methyl jasmonate can improve drought tolerance of barley. A two-year experimental study was conducted in a factorial randomized complete block design ($n = 5$) in the research greenhouse of Zanjan University, Iran, to investigate the possible effects of biochar and methyl jasmonate on some traits of winter barley under drought conditions. Two irrigation regimes, D_0 (full irrigation in soil field capacity as control) and D_1 (withholding irrigation immediately after flowering stage), three methyl jasmonate spray densities [0 (M_0), 50 (M_{50}), and 100 (M_{100}) µM] and four levels of biochar in soil [0% (B_0), 0.25% ($B_{0.25}$), 0.5% ($B_{0.5}$), 1% (B_1) per soil weight] were used in this experiment. In this study, drought reduced two-year average leaf area (LA) by 96%, stomatal conductance (gs) by 84%, and photosynthetic water use efficiency (PWUE) by 64%. In addition, drought reduced chlorophyll-b by 1.5% and 81% and transpiration rate (Tr) by 2.5% and 78% in the first and second years, respectively. However, the application of biochar and methyl jasmonate improved all the traits studied in both D0 and D1 drought-treated plants. For most of the parameters studied, the optimal combination of biochar and methyl jasmonate that optimized water use efficiency and alleviated drought was 0.25% and 50 µM, respectively. The synergistic action of biochar and jasmonates improved the tolerance of barley to water stress.

Keywords Gas exchange · Photosynthetic pigments · Leaf area · Soil amendment · Water use efficiency · Yield

1 Introduction

Barley (*Hordeum vulgare* L.) is among the most strategic crop plants, providing food for loads of population of the world. However, the productivity of the barley is considerably restricted under the influence of climate change catastrophic environmental circumstances (Nasiri et al. 2023a). It is reported that the world barley production was decreased in 2021–2022 cultivation season compared with 2020–2021 mainly due to the adverse effects of drought stress (Shahbandeh 2022). It is a multidimensional kind of stress which takes place largely for the sake of restricted rainfall and a consequent dry spell. Over the past decades, the expansion in areas of dry-land agriculture has increased, and drought has caused approximately 30 billion dollars in damage to the agriculture sector (Gupta et al. 2020). Numerous studies worldwide have assessed the effects of abiotic stresses on crops and how to alleviate their effects (El-Seyed et al. 2014). Although, plants have specific adaptation mechanisms to cope with water deficits, there is an urgent need to maintain productivity at a high level under drought conditions (Dey et al. 2021; Sirhindi et al. 2020). Drought can

✉ Sajjad Nasiri
sajjadnasiri1989@yahoo.com

✉ Andrea Mastinu
andrea.mastinu@unibs.it

Babak Andalibi
andalibi@znu.ac.ir

Afshin Tavakoli
tavakoli@znu.ac.ir

Mohammad Amir Delavar
adelavar443@gmail.com

Ali El-Keblawy
akeblawy@sharjah.ac.ae

[1] Department of Genetic and Plant Production, University of Zanjan, Zanjan, Iran

[2] Department of Soil Science, University of Zanjan, Zanjan, Iran

[3] Department of Applied Biology, University of Sharjah, Sharjah, United Arab Emirates

[4] Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy

damage plant growth and development, reducing economic yield due to adverse effects on photosynthesis, respiration, nitrogen metabolism, and absorption of other essential elements (Liu et al. 2022). Drought leads to negative effects on photosynthesis and gas exchange parameters (Bryant et al. 2021), cell wall composition Tenhaken 2014) nutrient translocation (Demidchik 2015), the transcriptional activity of genes, transposable elements (Mamnoei and Seyed Sharifi 2010), the lipid signaling (Hou et al. 2021), metabolites, proteins (Nohong and Nompo 2015), and antioxidant profile (Dey et al. 2021). Drought also results in cell dehydration by reducing relative water content, chloroplast degradation, and a severe reduction in photosynthetic pigments content, decreasing photosynthesis and final productivity (Dey et al. 2021). In addition, researchers reported that drought decreased some physiological and biochemical traits of wheat, such as photosynthetic pigments content, relative water content (RWC), net photosynthesis (An), stomatal conductance (gs), and grain yield; Furthermore, under water deficit conditions, electron transport through PSII is reduced, and the oxygen-evolving complex of photosystem II (PSII) is damaged (Gupta et al. 2020). However, there are many ways to alleviate the detrimental impacts of water deficit on soil water content, plant growth, and yield.

Several reports have documented the alleviation of water deficit stress by applying soil amendments such as biochar. Biochar has recently received attention because it improves soil fertility, carbon content, water use efficiency, and crop production (Khan et al. 2021). Biochar practically sequester carbon, enhances soil organic carbon and microbial activity and improves the whole soil quality and function which affect positively the attributes of plants in water shortage conditions (Cantrell et al. 2012). Mixing biochar with soil can diminish irrigation frequency by holding water from percolation to a deeper layer away from the root system. In addition, mixing biochar with soil increases nutrient retention (Thi Pham et al. 2021). For example, cations retention was higher in soil mixed with biochar than in other forms of organic matter (Sombroek et al. 2004). Therefore, the increase in water retention and soil fertility of soil amended with biochar mitigates the sensitivity of crops to soil water deficit (Abideen et al. 2020; Katterer et al. 2019). It is reported that, under drought circumstances, application of biochar can be effective approach in alleviating the adverse effects of it on the enzymatic activity of plant and the concentration of non-enzymatic substances. For instance, in several researches that, although the activity of plant defense system including reactive oxygen species (ROS) scavengers such as catalase, peroxidase, ascorbate peroxidase and superoxide dismutase as well as proline concentration is increased in stressful circum-stances, adding biochar, due to alleviation of drought effects, results in reduced enzymatic activity and non-enzymatic components' accumulation (Nasiri et al. 2023b). In addition, biochar application significantly enhanced plant normal functions, such as chlorophyll-a, and chlorophyll-b contents, RWC, and decreased the concentration of proline and the percentage of electrolyte leakage resulting in higher economic yield (Abd El-Mageed et al. 2021). Similarly, other researchers (Iqbal et al. 2022) reported that the biochar amendment significantly alleviated the effect of drought in broad beans (*Vicia faba* L.) by increasing the plants' height, dry weight, chlorophyll content, the maximum quantum performance of photosystem II, improving the physical and biochemical properties of the soil.

Jasmonates, lipid-based plant hormones, are essential in alleviating several biotic and abiotic stresses and play a crucial role in plant response (Ali and Baek 2020). Besides, jasmonic acid (JA) regulates various plant processes, including growth, photosynthesis, and reproductive functions (Soliman et al. 2018; Munir et al. 2022; Sheteiwy et al. 2022). Furthermore, methyl jasmonate (M) relieves water deficit stress by regulating stomatal closure, ROS production, and profound root growth (Riemann et al. 2015; Iqbal et al. 2022). For example, the foliar application of methyl jasmonate can benefit plants exposed to drought by regulating enzymatic and non-enzymatic antioxidants that scavenge ROS (Fugate et al. 2018). Researchers illustrated that the foliar application of biochar can mitigate the adverse effects of drought on barley enhancing the enzymatic activity of catalase, peroxidase, ascorbate peroxidase and superoxide dismutase, improving the concentration of proline and leaf greenness index (SPAD) in leaves (Nasiri et al. 2023b). Besides, other scientists reported a substantial positive impact of spraying sugar beet subjected to drought with different levels of methyl jasmonate by positively improving RWC, fresh and dry weight, net photosynthesis rate, transpiration rate, stomatal conductance, water use efficiency (WUE), and PSII quantum efficiency (Fugate et al. 2018). Moreover, it is stated that exogenous application of methyl jasmonate relieved salinity stress by improving chlorophyll content, Hill reaction rate, transpiration, and stomatal conductance (Rezaei et al. 2018).

In light of these findings, due to the cumulative influences of climate change, drought stress is getting the most important environmental issue day by day. Furthermore, biochar amends the soil structure, enhances the microbial community and activity of the soil, resulting in the improved water availability and crop productivity under controlled environments. Methyl jasmonate also in specific and in combination with biochar can be utilized as a promising method to alleviate the drastic impacts of water shortage in a wide range of crop plants. To the best of our knowledge, no experiments have been explored to study the combined effects of biochar and methyl jasmonate on drought alleviation. According to what we stated in introduction, we aimed in this study to assess that how the individual and joint application of biochar and foliar application of methyl jasmonate influence the several physiological and biochemical traits of barley plants under drought stress and the normal conditions. We assumed that the application of biochar can ameliorate the repercussions of drought stress by improving soil properties and the external application of methyl jasmonate will also be useful due to its positive impact in elevation of the plant's enzymatic activity. The results of this study will help to determine the optimum combination of biochar amendment and methyl jasmonate for relieving the drought effect on barley.

2 Materials and Methods

2.1 Site Description

To evaluate the effects of the biochar addition to the soil and exogenous application of methyl jasmonate, an experimental glasshouse study was carried out in northwest Iran at the University of Zanjan. The study was conducted over two consecutive years (2021 and 2022) using a randomized complete block design (n = 5).

2.2 Growth Conditions

Barley (*Hordeum vulgare* L. var. Jolgeh) seeds were purchased from a well-known barley seed producer company in Zanjan, Iran (Zanjan Kesht Kheir Abad company) and were soaked with a density of 17 plants per pot in black plastic pots (25 cm top diameter with 30 cm height) containing 10 kg of loamy sand soil (dry weight equivalent). Seeds were sown on the 15th of October in the first year and the 22nd of October in the second year. During the experiment, the temperature and humidity of the glasshouse were set between 16–21 °C (minimum–maximum) and 60%, respectively, with natural light conditions with a day/night cycle of 14/10 h. Tables 1 and 2 show the physicochemical properties of the soil and biochar used in this experiment, which were analyzed and determined at the agricultural research center of Zanjan, Iran. The recommended doses of NPK fertilizers were based on the recommendations of the local agricultural organization for barley (100, 60, and 45 kg ha^{-1}, respectively).

2.3 Experimental Layout

Two levels of drought stress, namely non-stress conditions (100% field capacity as a control, D$_0$) and withholding irrigation for two weeks at the flowering stage (D$_1$), was applied as a main factor. The field capacity (FC) is the amount of

Table 1 Physical and chemical properties of the soil and the biochar

Material													
The soil	Parameter	Fe	Mn	Zn	Cu	EC×10^3	pH	O.C	K	S.P	N	sand	silt	clay
	Unit	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(Ms cm)	-	(%)	(mg kg^{-1})	(mg kg^{-1})	(%)	(%)	(%)	(%)
	Quantity	2.79	4.20	0.38	0.28	1.20	8.17	0.12	181	5.6	0.012	72	16	12
The biochar	Parameter	Fe	Mn	Zn	Cu	EC×10^3	pH	O.C	K	S.P	N	Pb	Cd	-
	Unit	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mg kg^{-1})	(mS cm)	-	(%)	(%)	(%)	(%)	(mg kg^{-1})	(mg kg^{-1})	-
	Quantity	4.65	3.62	1.34	0.46	3.21	7.9	63.85	0.83	0.25	0.28	0.38	0.02	-

In this Table, Fe, Mn, Zn, Cu, EC, pH, O.C, K, S.P, N, Pb and Cd stand for Iron, Magnesium, Zinc, Copper, electrical conductivity, potential hydrogen, organic Carbon, Potassium, Soluble Phosphorus, Nitrogen, Lead and Cadmium respectively

Table 2 Analysis of variance results (F-Values) testing the effects of drought (D), methyl jasmonate (M) and biochar (B) on leaf area, relative water content (RWC), water use efficiency (WUE), chlorophyll (Chl-a,Chl-b) and carotenoids (CAR) content, maximal quantum performance of photosystem II (MQPPSII) of barley

Source of variation	DF	LA	RWC	WUE	Chl-a	Chl-b	Total chlorophyll	CAR	MQPPSII
Year	1	8.63*	144.00 ns	2.748**	1.77 ns	47.17**	29.54*	5.80**	0.061*
Block	8	0.50	29.98	0.142	1.38	0.31	3.42	0.36	0.006
D	1	22.88**	3344.32**	2.522**	235.62**	51.52**	504.02**	20.71**	2.748**
B	3	38.00**	864.70**	0.979**	75.58**	14.03**	155.33**	4.61**	0.503**
M	2	14.31**	844.43**	0.833**	39.18**	4.90**	42.12**	2.49**	0.379**
D×B	3	1.70**	169.94 ns	0.024 ns	2.20 ns	0.60 ns	2.90 ^{ns}	0.26 ns	0.007 ns
D×M	2	0.02 ns	91.88 ns	0.004 ns	1.46 ns	0.30 ns	2.37 ^{ns}	0.09 ns	0.032*
B×M	6	1.41**	194.00*	0.098**	6.36**	1.29**	9.17**	0.33 ns	0.064**
D×B×M	6	0.43*	79.91 ^{ns}	0.016 ns	1.64 ns	0.44 ns	3.11 ns	0.11 ns	0.014 ns
Year×D	1	0.06 ns	28.91 ^{ns}	0.212**	4.70*	2.99**	15.50**	0.02 ns	0.002 ns
Year×B	3	0.74**	9.40 ns	0.089**	1.70 ns	1.26**	4.15 ^{ns}	0.85*	0.079**
Year×M	2	0.84**	28.41 ns	0.205**	0.41 ns	2.73**	5.15 ^{ns}	0.20 ns	0.017 ns
Year×D×B	3	0.93**	57.30 ns	0.013 ns	1.10 ns	0.16 ns	1.41 ^{ns}	0.21 ns	0.007 ns
Year×D×M	2	1.02**	73.26 ns	0.006 ns	1.39 ns	0.68 ns	2.60 ns	0.02 ns	0.003 ns
Year×B×M	6	0.17 ns	51.20 ns	0.010 ns	0.49 ns	1.06**	2.51 ^{ns}	0.09 ns	0.035**
Year×D×B×M	6	0.09 ns	49.37 ns	0.008 ns	0.61 ns	1.05**	0.39 ns	0.15 ns	0.008 ns
C.V	-	18.56	13.28	23.94	18.45	26.56	18.24	18.49	8.89

* and ** = significant at 0.05 and 0.01 levels, respectively; ns = non-significant

water in the soil after a rain or irrigation event when gravity has removed the remaining water from the soil.

The soils in the pots were saturated with water and left until they had constant weight. The soil was dried, and the weight difference between dry and FC soils was calculated and kept constant throughout the experiment in the D_0. The treatment of D_1 was started at the beginning of the flowering stage. In this treatment, the soils were kept without irrigation until the soil water potential reached under 2 mega pascal, indicating that drought stress is applied adequately. To analyze and determine the physical and chemical properties of the soil, Surface soil samples were collected from a 0–30 cm depth of the soil profile. Then the air-dried soil samples were sieved by a 2-mm sieve, homogenized, mixed, and their properties were measured according to the standard methods outlined by Lindsay and Norvell (1978) and Page et al. (1982).

Biochar was obtained from soybean stover subjected to pyrolysis temperature of 700 °C. This biochar showed these characteristics: specific surface area 419.6 m^2 g, volatile matter 15.6%, ash 17.2%, cation exchange capacity 47.5 cmol kg. In terms of particle size distribution, 21.7% were between 5 and 2 mm, 24% were between 2 and 0.5 mm, 48% were between 0 and 0.5 mm. Finally, biochar showed 48% water holding capacity. It was mixed with the soil at the beginning of the experiment at four levels: 0 (B_0), 0.25% ($B_{0.25}$), 0.5% ($B_{0.5}$), 1% (B_1) w/w. To analyze the studied biochar, first the samples were homogenized, then they were divided into portions. The physicochemical properties of the biochar were determined based on method introduced by Cantrell et al. (2012) and Singh et al. (2017).

A foliar spray of methyl jasmonate (was purchased from Sigma-Aldrich company) was applied simultaneously with withholding irrigation during the flowering stage at three levels: 0 as the control (M_0), 50 µM (M_{50}), and 100 µM (M_{100}). In both years, this method was used. The soils were changed in the second year, and biochar treatments were applied as in the first year. Samples were collected from all pots two weeks after starting D_1 treatment.

Physiological and biochemical traits were determined and measured in the plant physiology laboratory at the University of Zanjan, Iran.

2.4 Leaf Area (LA)

Leaf area was measured five weeks after the flowering stage from all replications and plots, using a leaf surface meter device, model delta T, from three sampled plants from each pot selected randomly before applying drought and earmarked using colorful ribbons. Three samples from each plot were mixed and considered as one sample. The final leaf surface of the plants was calculated as the average of the three samples from each pot.

2.5 Determination of Relative Water Content of Leaf (RWC) and Water Use Efficiency (WUE)

The leaf fresh weight was measured from three randomly matured leaves from the top of each plant. Fresh leaves were weighed, placed in distilled water for 24 h, dried with tissue paper, and weighed to calculate turgid weight. The leaves were dried in an oven set (at 70 °C for 48 h) till constant weights. The RWC was calculated using the Eq. 1 (Pieczynski et al. 2013):

$$\mathrm{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100 \tag{1}$$

The water use efficiency (WUE), which is known as the ratio of plant yield to used water during the cultivation season, was calculated according to the Eq. 2 (Smart and Bingham 1974):

$$\mathrm{WUE} = \text{grain yield (g.pot)} / \text{used water} \mathrm{v}(\mathrm{m}^{-3}) \tag{2}$$

2.6 Photosynthetic Pigments

The fresh flagged leaves were separated and snap-frozen immediately in liquid nitrogen. A 0.5 g fresh flag leaf was used to determine the photosynthetic pigments content, according to approach introduced by Arnon (1940). To calculate chlorophyll a, b, and carotenoid contents, leaf extracts were absorbed at wavelengths of 663, 645, and 470 nm, respectively, using a UV/VIS device (PerkinElmer, Lambda 25, USA). The final concentrations of pigments were calculated based on the formulas 3, 4 and 5:

$$\mathrm{Chla}\left(\mathrm{mg}g^{-1}\mathrm{DW}\right) = \frac{[12.7(\mathrm{A}663) - 2.69(\mathrm{A}645)] \times \mathrm{V}}{1000 \times \mathrm{W}} \tag{3}$$

$$\mathrm{Chlb}\left(\mathrm{mg}g^{-1}\mathrm{DW}\right) = \frac{[22.9(\mathrm{A}645) - 4.68(\mathrm{A}663)] \times \mathrm{V}}{1000 \times \mathrm{W}} \tag{4}$$

$$\mathrm{Carotenoid}\ (\mathrm{mg}g^{-1}\mathrm{DW}) = \frac{[(1000 \times \mathrm{A}470) - (1.82 \times \mathrm{Chla}) - (85.02 \times \mathrm{Chlb})] \times \mathrm{V}}{198 \times 1000 \times \mathrm{W}} \tag{5}$$

2.7 Gas Exchange Parameters and the Maximum Quantum Performance of Photosystem II

The net photosynthetic rate (NP, µM CO_2 m^{-2} s^{-1}), intercellular CO_2 concentration (Ci, µM CO_2/M air), stomatal conductance (gs, M H_2O m^{-2} s^{-1}) and transpiration rate (Tr, mM H_2O m^{-2} s^{-1}) were determined from the activity of the flagged leaves of each replicate between 8:00 and 10:00 using a portable gas analyzer device (IRGA, Model LCA4, UK). In addition, mesophyll conductance (Mc) was estimated as the ratio of An to gs (mM CO_2 m^{-2} s^{-1}), and leaf-intrinsic water use efficiency (µM CO_2/mM H_2O) was calculated as an NP to gs ratio (photosynthetic water use efficiency, PWUE). The measurements were performed at CO_2 concentrations of 412 ppm, relative humidity of 60%, natural light conditions, and day/night cycle of 14/10 h in both years.

Fluorescence parameters were measured in the greenhouse using a portable plant fluorescence meter (Opti Sciences, model OS-30), and the maximal quantum performance of photosystem II (MQPPSII) was calculated using Fm-F0 / Fm. In this equation, Fm is the maximum fluorescence and F0 is the minimum fluorescence. The dark adaptation period in measurements of Fm-F0 was 20 min.

2.8 Statistical Analysis

Four-way analyses of variance (ANOVA) were used in factorial based on randomized complete block design to test the effects of the main factors (i.e., year, drought, biochar, and methyl jasmonate) on the studied biochemical and physiological attributes and yield. Duncan's test assessed the significant differences between the means of different treatments. The figures were delineated using Excel software. All statistical analyses were performed using the SAS software version 9.1.

3 Results

The ANOVA test showed significant effects for the main factors, i.e., the study year, drought treatment, methyl jasmonate) on the physiological attributes, e.g., RWC, WUE, and Chl-a, Chl-b, carotenoids contents, and MQPPSII (Table 2), leaf gas exchange parameters, e.g., NP, Tr, Ci, gs, Mc, PWUE, and drain yield of barley ($P < 0.05$, Table 3). However, the interactions between the different combinations of the main factors were not significant in most studied attributes.

Table 3 Analysis of variance results (F-Values) testing the effects of drought (D), methyl jasmonate (M) and biochar (B) on net photosynthesis rate (NP), transpiration rate (Tr) intercellular CO_2 (Ci), stomatal conductance (gs), mesophyll conductance (Mc), Leaf photosynthetic water use efficiency (PWUE) and grain yield barley

Source of variation	DF	NP	Tr	Ci	gs	Mc	PWUE	Grain yield
Year	1	10.66 ns	45.69**	46,259.27*	0.009*	0.0098*	0.0010*	345.82*
Block	8	4.51	0.81	5342.87	0.0007	0.0011	0.0000	22.01
D	1	910.34**	66.35**	519,312.07**	0.060**	0.1088**	0.0002*	612.32*
B	3	205.08**	12.88*	102,823.16**	0.013**	0.0293**	0.0005**	114.07*
M	2	67.05**	6.39*	77,064.70**	0.003**	0.0133**	0.0008**	105.87*
D × B	3	7.71 ns	0.63 ^{ns}	14,178.86*	0.0007 ns	0.0051**	0.0004**	4.01 ns
D × M	2	2.84 ns	3.22 ^{ns}	22,783.23**	0.002**	0.0023 ns	0.0002*	2.28 ns
B × M	6	14.20**	0.86 ^{ns}	14,211.40**	0.0015**	0.0019*	0.0001 ns	12.39*
D × B × M	6	5.24 ns	1.25 ^{ns}	4424.83 ns	0.0019**	0.0013 ^{ns}	0.0002**	1.70 ns
Year × D	1	2.56 ns	51.35**	132.02 ns	0.0016 ns	0.0004 ns	0.0001 ns	8.30 ns
Year × B	3	1.73 ns	5.25**	6691.28 ns	0.0013*	0.0014 ^{ns}	0.0003**	12.66**
Year × M	2	2.91 ns	0.65 ^{ns}	521.05 ns	0.0008*	0.0004 ^{ns}	0.0002*	27.87*
Year × D × B	3	1.76 ns	6.30**	10,762.96*	0.0006 ^{ns}	0.0002 ^{ns}	0.00001 ^{ns}	2.42 ns
Year × D × M	2	0.26 ns	1.76**	5376.38 ns	0.0009 ns	0.0005 ns	0.0002 ns	0.93 ns
Year × B × M	6	1.72 ns	2.79**	2548.15 ns	0.0009*	0.0003 ^{ns}	0.0001 ns	1.42 ns
Year × D × B × M	6	1.65 ns	1.86**	1589.67 ns	0.001 ns	0.0003 ^{ns}	0.0001 ns	0.92 ns
C.V	-	28	28.81	16.85	9.55	13.4	14.21	17.61

* and ** = significant at 0.05 and 0.01 levels, respectively; ns = non-significant

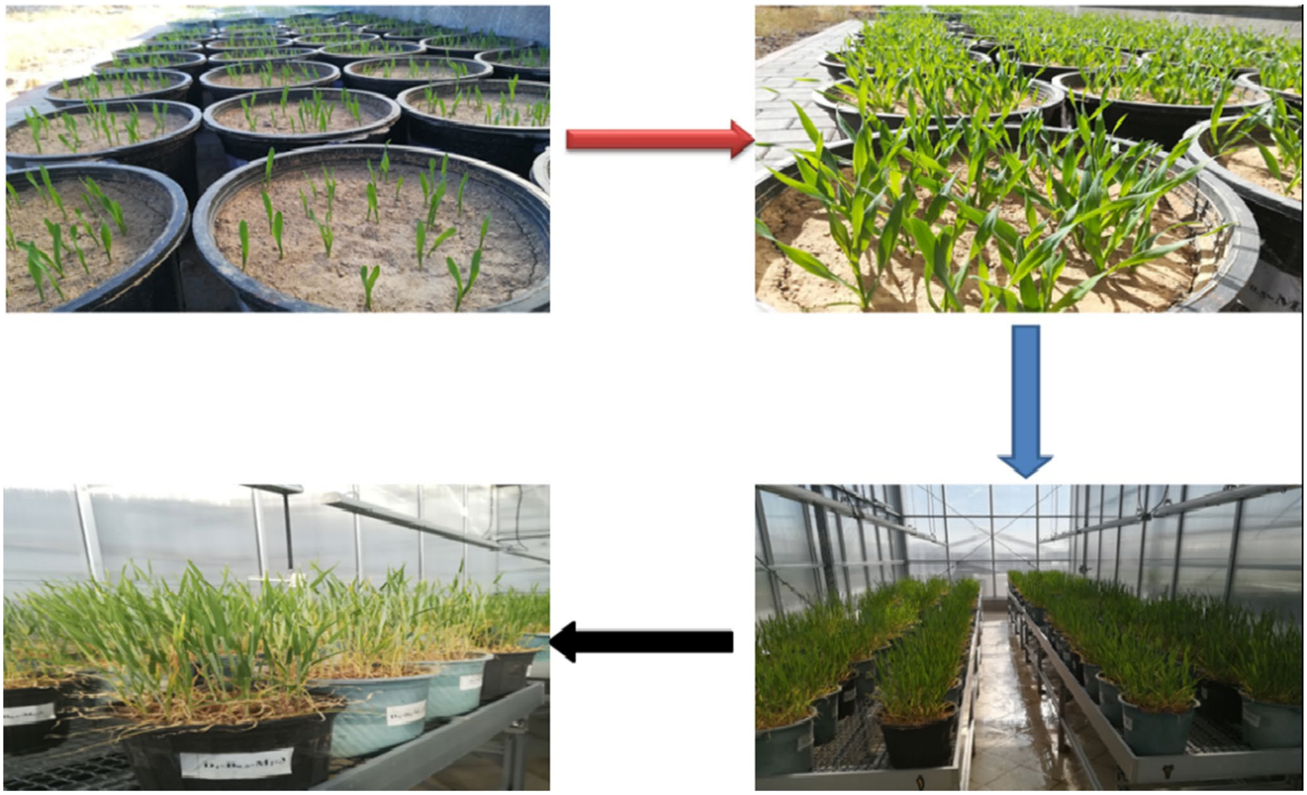


Fig. 1 A summary of the steps for conducting the two-year experiment

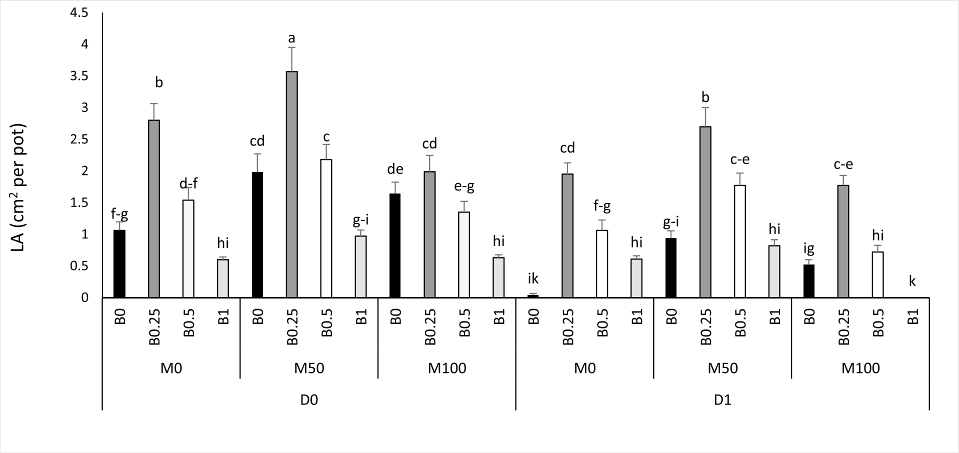


Fig. 2 The effect of triple interaction between drought, biochar and methyl jasmonate on the two-year average of leaf area (LA) of barley plants. In this figure, D0 and D1 refer to normal irrigation and drought stress conditions respectively, B0, B0.25, B0.5 and B1 respectively stand for control treatment without biochar, 0.25, 0.5 and one % of biochar addition into the soil per soil weight, and M0, M50, M100 refer to control treatment without spraying methyl jasmonate, 50 and 100 µM/L of spraying

3.1 Leaf Area (LA)

The main factors had significant effects on the leaf area (LA). Besides, the two-way (B x D, B x M) and three-way interactions (D x B x M) had significant effects on the LA (Table 2). Drought stress significantly reduced the LA, but biochar and methyl jasmonate significantly increased the LA in drought-stressed plants (Figs. 1, 2). In fact, drought decreased the two-year average of LA by 96%, while the application of biochar and methyl jasmonate increased this parameter comparable to the control. Regardless of the effect of methyl jasmonate, the interaction between biochar and methyl jasmonate was significant; the enhancement of biochar application was significantly greater in drought-non-stressed than in drought-stressed plants. Besides, the maximum increase in the LA was recorded in plants treated with $B_{0.25}$ and M_0, with $B_{0.25}$ and M_{50} in drought-non-stressed plants, and in plants treated with $B_{0.25}$ and M_{50} in drought-stressed plants. Such results indicate that the enhancing efficiency of biochar and methyl jasmonate is independent of drought stress. Furthermore, applying 1% biochar (B_1) had a greater inhibitory effect on the LA, than $B_{0.25}$ and $B_{0.5}$, in all methyl jasmonate and drought treatments, indicating 1% biochar might be harmful to soil physicochemical or microbial properties (Fig. 2).

3.2 Relative Water Content and Water Use Efficiency

The relative water content (RWC) and water use efficiency (WUE) were significantly affected by the main factor (D, B and M) and the two-way interaction of biochar and methyl jasmonate (Table 2). Investigating the two-year interaction between biochar and methyl jasmonate in both irrigation systems (DS_0 and DS_1) illustrated that the biochar addition

Table 4 The interactive effect of biochar and methyl jasmonate on average of the two years and drought treatments of some biochemical, and physiological parameters of barley of the average irrigation treatments. In this table methyl jasmonate (M) and biochar (B), relative water content (RWC), water use efficiency (WUE), chlorophyll a (Chl-a) maximal quantum performance of photosystem II (MQPPSII), net photosynthesis rate (NP), intercellular CO_2 (Ci), and mesophyll conductance (Mc) have been illustrated

B (%)	M (µM/L)	RWC	WUE	Chl-a	Total chlorophyll	MQPPSII	NP	Ci	Mc
B_0	M_0	59.33^{e}	0.29^{e}	3.93^{f}	5.41^{f}	0.33^{g}	3.81^{fg}	280.9^{a}	0.02^{f}
	M_{50}	70.26^{b}	0.61^{b}	6.33^{bcd}	8.6^{bc}	0.56^{bc}	6.71^{cd}	168.9^{de}	0.05^{bcd}
	M_{100}	64.57^{cde}	0.43^{d}	5.66^{de}	7.07^{e}	0.53^{cd}	6.18^{d}	181.35^{cde}	0.04^{de}
$B_{0.25}$	M_0	71.01^{b}	0.66^{b}	6.85^{b}	9.4^{b}	0.59^{b}	8.05^{b}	168.55^{de}	0.06^{b}
	M_{50}	77.9^{a}	0.77^{a}	8.34^{a}	11.24^{a}	0.69^{a}	9.78^{a}	126.6^{f}	0.1^{a}
	M_{100}	66.32^{bcd}	0.54^{c}	6.58^{bc}	9.08^{b}	0.61^{b}	7.87^{b}	154.8^{ef}	0.06^{b}
$B_{0.5}$	M_0	66.31^{bcd}	0.47^{d}	6.05^{cde}	8.07^{cd}	0.48^{de}	6.46^{cd}	194.4^{cd}	0.04^{cde}
	M_{50}	69.3^{bc}	0.64^{b}	6.52^{bc}	8.64^{bc}	0.59^{bc}	7.29^{bc}	160.9^{def}	0.06^{bc}
	M_{100}	64.01^{cde}	0.41^{d}	6.18^{bcd}	8.61^{bc}	0.43^{ef}	4.84^{ef}	217.6^{bc}	0.03^{ef}
B_1	M_0	62.25^{de}	0.32^{e}	4.36^{f}	5.65^{f}	0.38^{fg}	3.65^{g}	276.1^{a}	0.03^{ef}
	M_{50}	63.51^{de}	0.41^{d}	5.43^{e}	7.39^{de}	0.49^{d}	4.89^{e}	215.8^{c}	0.03^{def}
	M_{100}	63.16^{de}	0.33^{e}	4.27^{f}	5.77^{f}	0.37^{fg}	3.87^{efg}	256.6^{ab}	0.02^{f}

Means with similar letters in each column are not significantly different at the 5% level based on the Duncan test and methyl jasmonate spraying, individually or in combination, improved RWC and WUE (Table 4). The highest RWC (77.9%) was detected by the combined application of $B_{0.25}$ and M_{50}, and the lowest index (59.3%) was observed in the controls (i.e., B_0 and M_0, Table 4). Similarly, the WUE was significantly greater in the combined application of $B_{0.25}$, and M_{50} (0.77 g/L) than in control treatments (B_0 and M_0, 0.29 g/L) (Table 4).

3.3 Leaf Photosynthetic Pigment Content

The concentrations of Chl-a, Chl-b, total chlorophyll, and carotenoids were significantly affected by the man factors (D, B, and M) (Table 2). The results showed significant interactions between the year and both biochar and methyl jasmonate on Chl-a, Chl-b, and total chlorophyll. The results demonstrated that drought reduced the Chl-b in the first year by 1.5% and in the second year by 81% compared with drought-non-stressed plants (Fig. 3). However, application of biochar and methyl jasmonate mitigated the adverse effects of drought in both years. In the first year, the highest accumulation of Chlorophyll-b (2.76 mg g^{-1} DW) was observed in plants exposed to D_0-$B_{0.25}$-M_{50} treatment, and the lowest value (0.71 mg g^{-1} DW) was in D_1-B_1-M_0 treatment. In the second year, the highest concentration of Chlorophyll-b (4.5 mg g^{-1} DW) was detected in D_0-$B_{0.5}$-$M_{100,}$ and the lowest content (0.47 mg g^{-1}DW) in D_1-B_0-M_0 treatment (Fig. 3) Moreover, there was a significant interaction between biochar and methyl jasmonate on the concentration of Chl-a and total chlorophyll. The positive effect of M_{50} was greater in $B_{0.25}$ than in the other biochar treatment. The highest accumulation of Chl-a (8.34 mg g^{-1} DW) and total chlorophyll (11.24 mg g^{-1} DW) was observed in the $B_{0.25}$-M_{50} treatment. Still, the lowest content of Chl-a (3.93 mg g^{-1} DW) and total chlorophyll (5.41 mg g^{-1} DW) was detected in non-treated plants (B_0-M_0) (Table 4).

Table 5 The interactive effect of drought and methyl jasmonate on the average of the two-year and biochar treatments on the maximal quantum performance of photosystem II (MQYPSII) and intercellular CO_2 (Ci) of barley. In this table drought (D), methyl jasmonate (M), maximal quantum performance of photosystem II (MQPPSII), and intercellular CO_2 (Ci) have been illustrated

D	M (μM/L)	MQYPSII	Ci
D_0	M_0	0.57^b	164.7^d
	M_{50}	0.7^a	135.45^e
	M_{100}	0.57^b	160.93^{de}
D_1	M_0	0.33^e	295.28^a
	M_{50}	0.46^c	200.65^c
	M_{100}	0.4^d	244.25^b

Means with similar letters in each column are not significantly different at the 5% level based on the Duncan test

3.4 Maximum Quantum Performance of Photosystem II (Fv/Fm)

There were interactive effects of biochar and methyl jasmonate (B x M, Tables 2 and 4) and drought and methyl jasmonate (D×M, Tables 2 and 5) on the most important chlorophyll fluorescence parameter (MQPPSII). The investigation on the average of the two-year interaction between biochar and methyl jasmonate in both irrigation systems. The MQPPSII was significantly greater in M_{50} than both M_0 and M_{100} in $B_{0.25}$, $B_{0.5}$, and B_1, but not B_0 (Table 4). In B_0, there was no significant difference between M_{50} (0.56) and M_{100} (0.53), and both had significantly greater values than M_0 (0.33). The highest MQPPSII (0.69) was in the $B_{0.25}$-M_{50} treatment, but the lowest (0.33) was in B_0-M_0 (Table 4). Besides, drought significantly reduced MQPPSII, but methyl jasmonate's foliar application significantly increased it under drought-non-stressed and drought-stressed plants. The highest value (0.7) was observed in M_{50}-DS_0, and the lowest value (0.33) in the M_0-D_1 treatment (Table 5).

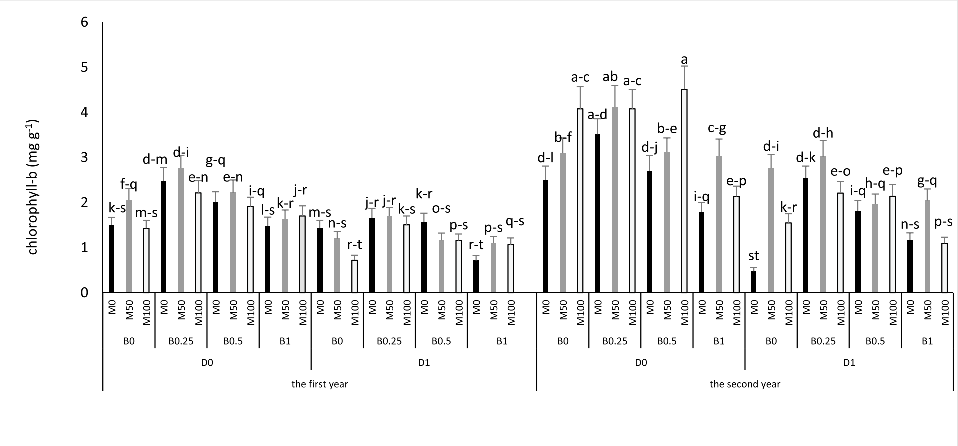


Fig. 3 Four-way interaction between year, drought, biochar and methyl jasmonate on the concentration of chlorophyll-b (Chl-b) in barley plants. In this figure, D0 and D1 refer to normal irrigation and drought stress conditions respectively, B0, B0.25, B0.5 and B1 respectively stand for control treatment without biochar, 0.25, 0.5 and one % of biochar addition into the soil per soil weight, and M0, M50, M100 refer to control treatment without spraying methyl jasmonate, 50 and 100 μM of spraying

3.5 Leaf Gas Exchange Parameters

The individual effects of D, B, and M significantly affected all gas exchange parameters (Table 3). The effect of B × M interaction on NP (net photosynthesis rate) was significant; the average An of the two-year and biochar treatments was greater by 11.3% and 69.5% in $B_{0.25}$ and $B_{0.5}$ compared with control plants (B_0), while the use of B_1 decreased NP by 4.2% (Table 4). Furthermore, spraying methyl jasmonate enhanced NP by 76% and 62% with the application of M_{50} and M_{100} compared with M_0. The highest photosynthesis rate (9.78 μM CO_2 m^{-2} s^{-1}) was obtained in $B_{0.25}$-M_{50} treatment, and the lowest (3.65 μM CO_2 m^{-2} s^{-1}) was in B_1-M_0 (Table 4). Additionally, the application of biochar and methyl jasmonate decreased the accumulation of Ci, which was related to the increase in the net photosynthesis rate (NP) (Table 5). The highest Ci (280.9 μM CO_2/M air) was detected in B_0-M_0, whereas the lowest (126.6 μM CO_2/M air) was in $B_{0.25}$-M_{50} treatment (Table 5). The highest mesophyll conductance (Mc) (0.1 mM CO_2 m^{-2} s^{-1}) was detected in $B_{0.25}$-M_{50} and the lowest (0.02 mM CO_2 0.1 mM CO_2 m^{-2} s^{-1}) was B_0-M_0 treatment (Table 4). The drought significantly elevated the Ci, but the foliar application of methyl jasmonate resulted in a significant reduction in the accumulation of CO_2 (Table 5). The effect of drought and methyl jasmonate interaction on the Ci index was significant; the reduction was greater in J_{50} (32.1% and 17.8% in D_1 and D_0, respectively) than in M_{100} (2.3% and 17.3% in D_1 and D_{S0}, respectively), as compared to in M_0 (Table 5). Similarly, the effect of drought and biochar interaction on the Ci index was significant; in the non-drought-stressed plants (D_0), the Ci was significantly greater in $B_{0.25}$ and $B_{0.5}$ than B_0 by 33.7%, and 3.05%, respectively, in D_0 treatment, and by 44.5% and 15.0% in D_1 treatment. However, Ci was significantly lower in B_1 than B_0 by 16% in D_0 treatment and 20.2 in D_1 treatment (Table 6). Although drought stress caused an increase in the Ci index, it caused a significant reduction in mesophyll conductance (Mc). The highest Mc value was in D_0-$B_{0.25}$ (0.11), and the lowest was in the D_1-B_1 treatment. The results indicate that the best biochar treatment in D_0 and D_1 was $B_{0.25}$, and the worst was B_1 (Tables 5 and 6).

The ANOVA result showed a significant effect for the individual effect of drought, biochar and methyl jasmonate, and year and the interaction of the four factors (i.e., year x D x B x M) on the transpiration rate (Tr) of barley plants (Table 3). The Tr was significantly reduced under drought in both year 1 (2.5%) and year 2 (78%) compared to non-drought-stressed plants (Fig. 3). However, biochar addition increased Tr in Year 2 but not in year 1. The methyl jasmonate had no clear effect on the transpiration rate in both years and drought and biochar treatments (Fig. 4).

There was a significant effect of the three-way interaction of the main factors (D x B x M) on the stomatal conductance (gs) (Fig. 5) and the leaf intrinsic water use efficiency (PWUE) (Fig. 6). Drought stress significantly reduced gs by 84%; however, biochar and methyl jasmonate's individual and combined applications significantly increased it. The

Table 6 Mean comparisons of the two-year average of interaction between drought and biochar on some of leaf gas exchange parameters. In this table drought (D), biochar (B), intercellular CO_2 (Ci), and mesophyll conductance (Mc) have been illustrated

D	B (%)	Ci (μM $CO_2 \times M$ air^{-1})	Mc
D_0	B_0	158.53^{d}	0.06^{bc}
	$B_{0.25}$	118.53^{e}	0.11^{a}
	$B_{0.5}$	153.83^{d}	0.06^{b}
	B_1	183.87^{d}	0.04^{de}
D_1	B_0	262.23^{b}	0.02^{f}
	$B_{0.25}$	181.43^{d}	0.04^{cd}
	$B_{0.5}$	228.1^{c}	0.02^{ef}
	B_1	315.13^{a}	0.01^{f}

Means with similar letters in each column are not significantly different at the 5% level based on the Duncan test

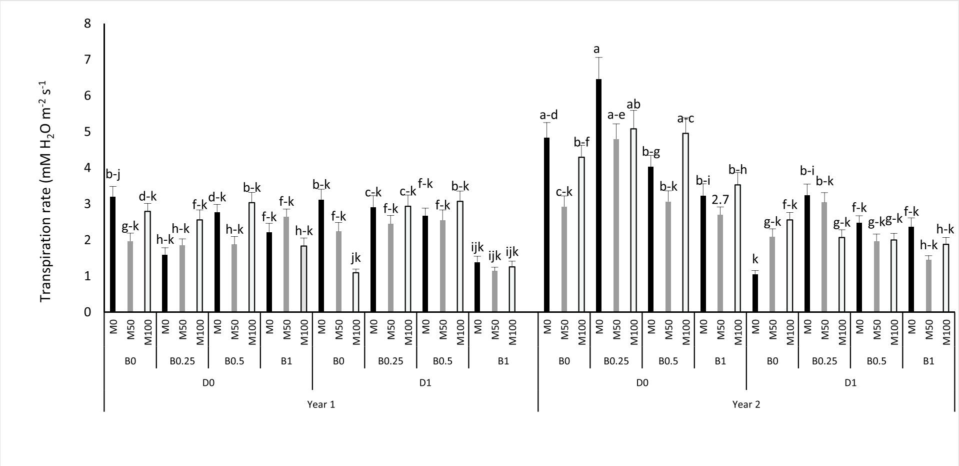
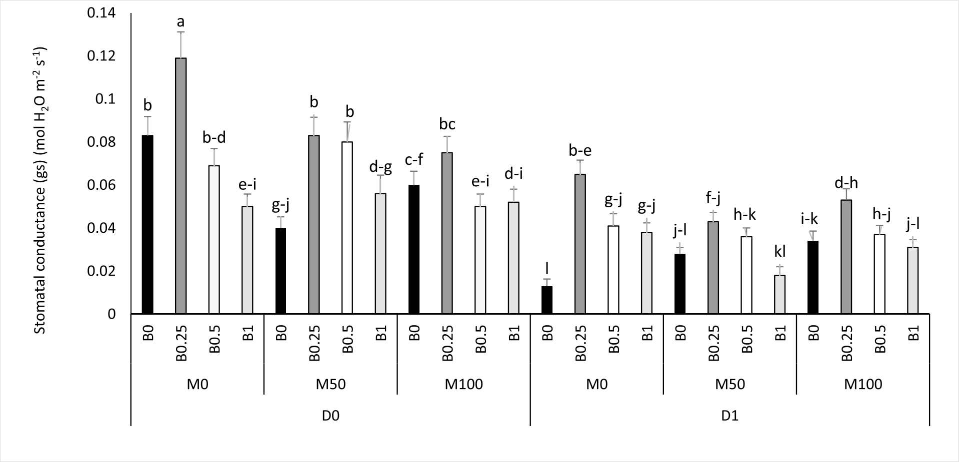


Fig. 4 Four-way interaction between year, drought, biochar and methyl jasmonate on transpiration rate (Tr). In this figure, D0 and D1 refer to normal irrigation and drought stress conditions respectively, B0, B0.25, B0.5 and B1 respectively stand for control treatment without biochar, 0.25, 0.5 and one % of biochar addition into the soil per soil weight, and M0, M50, M100 refer to control treatment without spraying methyl jasmonate, 50 and 100 μM of spraying

Fig. 5 The two-year average of interaction between drought, biochar and methyl jasmonate on stomatal conductance (gs) of barley plants. In this figure, D0 and D1 refer to normal irrigation and drought stress conditions respectively, B0, B0.25, B0.5 and B1 respectively stand for control treatment without biochar, 0.25, 0.5 and one % of biochar addition into the soil per soil weight, and M0, M50, M100 refer to control treatment without spraying methyl jasmonate, 50 and 100 µM of spraying



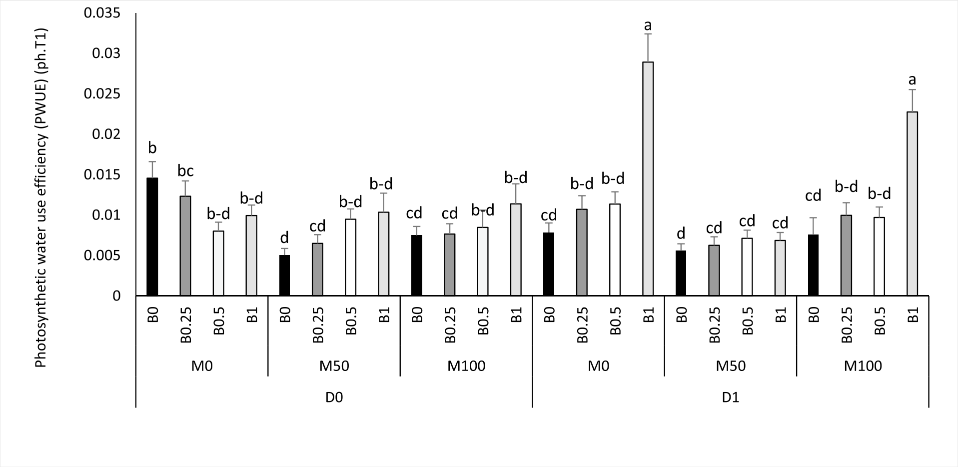


Fig. 6 The two-year average of interaction between drought, biochar and methyl jasmonate on photosynthetic water use efficiency (PWUE) index of barley plants. In this figure, D0 and D1 refer to normal irrigation and drought stress conditions respectively, B0, B0.25, B0.5 and B1 respectively stand for control treatment without biochar, 0.25, 0.5 and one % of biochar addition into the soil per soil weight, and M0, M50, M100 refer to control treatment without spraying methyl jasmonate, 50 and 100 µM of spraying

highest gs was in the $B_{0.25}$-D_0 treatment, and the lowest was B_0—M_0 treatment. Again, the highest dose of biochar (B_1) provided the lowest gs values. Drought declined PWUE by 64% compared to non-drought-stressed plants (D_0). At the same time, the individual and combined applications of biochar and methyl jasmonate significantly enhanced PWUE. The highest PWUE values were in $B_{0.25}$ at all M treatments of both drought-stressed and on-drought-stressed plants (Fig. 5).

4 Discussion

Environmental changes affecting water availability, drought, or flooding, had major impacts on plant growth, and physiological and metabolic processes (Feng et al. 2021). Restricted supply of water can change the physiological, biochemical and photosynthetic attributes of crop plants, leading to the lower production. The present study demonstrated that drought significantly affected some parameters, resulting in substantial alterations in the grain yield of barley. However, our findings revealed that the addition of biochar into the soil and exogenous application of methyl jasmonate as a plant growth regulator, individually and in combination, alleviated the impact of drought in barley plants by maintaining cellular water levels, membrane stability, the accumulation of photosynthetic pigments, and gas exchange attributes, eventually enhancing morpho-physiological traits.

The relative water content is an index, showing the repercussions of drought on plants. Our results further demonstrated that biochar considerably improved RWC and WUE (Table 5). Such a finding also was supported by other studies (Sombroek et al. 2004; Mohi-Ud-Din et al. 2021; Alikhani et al. 2019; Xu et al. 2010). For example, biochar application under drought significantly increased the WUE in Peanut (Xu et al. 2010). Like our results, other researchers concluded that biochar amendment enhanced water use efficiency in sugar beet (*Beta vulgaris* L.), alleviating drought stress under extremely arid or semi-arid climates (Wang et al. 2021). Biochar increases the soil content of carbon and other nutrients, increasing relative water content and enhancing plant height, leaf number, and leaf area (Abd El-Mageed et al. 2021). Additionally, biochar increases water-holding capacity (WHC), mineral nutrients, soil microbial activity, organic carbon, available phosphorous, soil carbohydrate content, and microbial biomass of the soil, and soil aeration, leading to higher water use efficiency and wheat productivity (Cantrell et al. 2012; Wang et al. 2021). It has been reported that biochar amendment improved WUE because it enhances hydrological characteristics, the physical attributes of the soil, and soil water content, resulting in higher grain yield under natural or stress conditions (Todorova et al. 2022). On the other hand, we observed in this study that the exogenous application of methyl jasmonate improved RWC and WUE (Table 5). The same results have been presented by the other (Ahmadian et al. 2021). The methyl jasmonate, at lower densities, has positive impacts on the physiological and biochemical attributes of plants, however, higher doses of this hormone can deteriorate the responses in particular in stress conditions (Feng et al. 2021; Wei et al. 2020). Our study indicated that methyl jasmonate effect was dose-specific; the positive impact of M_{50} was greater than that of M_{100} on most studied attributes. Besides, the study also indicated that the positive effect of methyl jasmonate on different morphological, physicochemical, and grain yields of barley under drought was augmented when applied with other anti-stress elements, i.e., biochar. Water use efficiency is the ratio of plant yield to actual water used during the cultivation season and represents an efficiency indicator (Baiamonte et al. 2020). The positive effect of methyl jasmonate on WUE could be attributed to its role in protecting the photosynthetic apparatus and enhancing its function even under drought-stress conditions, leading to better grain yield in crops (Vatankhah et al. 2016).

In the present experiment, water scarcity significantly reduced the pigment contents (i.e., Chl-a, Chl-b, total chlorophyll, and CAR) in barley leaves. However, adding biochar into the soil and foliar application of methyl jasmonate protected photosynthetic pigments under drought and modulated its destructive effects. Similarly, other researchers have reported a massive reduction in chlorophyll and carotenoid contents in barley under water scarcity (Iqbal et al. 2022; Feng et al. 2021). The negative effect of drought in various crops was attributed to lowering the oxidation that impairs the biosynthesis of photosynthetic pigments, including chlorophyll and carotenoids (Rezaei et al. 2018; Bagheri et al. 2020). It is possible that drought stress negatively impacts photosynthetic enzyme activity, mainly Calvin cycle enzymes and reactive oxygen species, which negatively impacts the chloroplast and reduces carbon assimilation, decreases chlorophyll concentrations and inhibits photosynthetic activity (Shadmand and Afkari 2018). It has also been proved that the reduction in pigment concentration under drought could be due to the prevention of pigment biosynthesis and assembly of the PSI and PSII light-harvesting complexes to stop over-plus absorption and ROS production (Ahmadian et al. 2021). Moreover, drought results in a colossal disorder in absorption of nutrients, inhibiting different stages of chlorophyll production and increasing the activity of cholorophylase which lead in lower pigment production (Hashem et al. 2019). Nevertheless, similar to our results, some other researchers reported that biochar significantly increased pigment accumulation under drought conditions in two seasons (Abd El-Mageed et al. 2021). It has been reported in several studies that adding biochar to the soil increases its water-holding capacity (WHC), cation exchange capacity and physicochemical properties of the soil, as well as modifies the soil pH, improving nutrient retention and availability in the root zone, such as nitrogen, which is necessary for the production of photosynthetic pigments (Abd El-Mageed et al. 2021). It is noticeable that in this study, the application of methyl jasmonate in specific or in combination with biochar inhibited the adverse effects of drought and regulated the concentration of photosynthetic pigments. The positive impact of methyl jasmonate in reducing the detrimental influences of drought stress on photosynthetic pigments in the present study is in agreement with results observed in bean plants (Wei et al. 2020). The elevation in chlorophyll and carotenoid content in drought-stressed plants treated with methyl jasmonate was reported in bean cultivars (Bagheri et al. 2020), Rapeseed (Ahmadi et al. 2018), and wheat (Todorova et al. 2022). Some believe that first of all, methyl jasmonate restricts the stomatal conductance, resulting in lower transpiration rate, then elevates the enzymatic activity in order to detoxification of plants from Malondialdehyde and other toxic components, and eventually protects plants from destructive impacts of drought conditions such as reduction in the concentration of pigments (Nasiri et al. 2023b). Other studies also have reported that methyl jasmonate stimulates the production of antioxidants and supports plants against oxidative stress, which can be beneficial in reducing the devastating effects of drought on pigment syntheses, increasing their concentration (Fugate et al. 2018). Moreover, it has been claimed that methyl jasmonate plays an important role in up-regulating some key genes in the biosynthesis pathway of chlorophyll, which results in the improved biosynthesis of pigments (Razmi et al. 2017). It seems the combined application of biochar and methyl jasmonate, particularly at optimum doses, can be more effective in alleviating drought consequences.

Our results showed that drought in barley plants resulted in a substantial reduction in the transpiration rate (Tr), mesophyll conductance (Mc), stomatal conductance (gs), and intrinsic or photosynthetic water use efficiency (PWUE), which agree with other results in the young sugar beet (*Beta vulgaris* L.) (Fugate et al. 2018) and wheat (Sadaf et al. 2017). In addition, drought resulted in the buildup of intercellular carbon, a clear symptom of decreased photosynthesis rate. The negative effect of drought stress on the gas exchange attributes of plants is a common phenomenon and exacerbation of these characteristics during water scarcity is mainly caused by either stomatal restrictions (stomatal closure due to CO_2 reduction) or non-stomatal limitations, which result in a reduction in the chlorophyll content, inhibition of Rubisco activase, and lower photochemical efficiency of PSII (Sadaf et al. 2017; Liu et al. 2018; Huang et al. 2017). Despite, the withholding irrigation affected some of the gas exchange parameters negatively use of biochar (0.25% soil weight) ameliorated these indicators both in full irrigation and in drought conditions, which is in accordance with findings by (Hafez et al. 2020) in barley. It seems biochar improves soil microbial, biochemical and physical properties of the soil, enhances the soil's water-holding capacity, resulting in a higher RWC, a better stomatal conductance and higher photosynthesis rate and (Demidchik 2015). All these events lead in the consumption of sub-stomatal CO_2 during photosynthesis and reduce the Ci index (Abd El-Mageed et al. 2021). Similarly, spraying methyl jasmonate altered the NP and Ci significantly in peppermint plants under drought stress (Vatankhah et al. 2016). It is explained that methyl jasmonate application could increase photosynthesis by enhancing the relative water content, specific leaf area, relative growth rate, and net assimilation rate (Ahmadi et al. 2018). Our study observed that stomatal conductance was positively affected by methyl jasmonate application compared to control plants (M_0). It is claimed that methyl jasmonate at higher concentrations increases ABA and ethylene concentrations in plants, which causes stomatal closure, resulting in stomatal resistance and reduced transpiration rate (Boutraa et al. 2010) Furthermore, it can lead to a series of enzymes called MAPK, which affect stomata and elevate ABA accumulation under biotic and abiotic stress conditions.

Plants' tolerance against stress function can be due to harming the PII center or lack of water during the plant growth season (Mamnoei and Seyed Sharifi 2010). So the chlorophyll fluorescence mirrors the initial process of photosynthesis, consist of absorbing light energy and transmitting excitation and photochemical energy responses and the degree of damage due to drought stress or other stressful conditions is reverberate measuring this index (Zhang et al. 2022) In our research, drought significantly reduced the maximum quantum performance of photosystem II (MQPPSII). Nonetheless, applying biochar and methyl jasmonate showed a significant positive change in MQPPSII. The reduction in this index under water limitation has been reported in several studies (Iqbal et al. 2022; Fallahi et al. 2013; Pandey et al. 2012; Abbaspour et al. 2011). Chlorophyll fluorescence parameters are in a close relation with the different processes of the photosynthesis (Xia et al. 2019). It is reported that biochar can be beneficial in reduction of the shutdown of active reaction centers which in turn increases the performance of PSII (Zhang et al. 2018). Moreover, it seems that, biochar affects this parameter positively, increasing the accumulation of photosynthetic pigment which eventually results in the higher rates of the photosynthesis (Yang et al. 2021). Furthermore, it has been reported that reducing the amount of chlorophyll-a, a determining indicator of PSII efficiency under stress conditions, is the main reason for the reduction in the MQPPSII (Boutraa et al. 2010). The same as our results, others observed a considerable increase in the function of PSII under drought stress due to using biochar. Besides, it suggested that, biochar, as an organic source of nitrogen, phosphorus, and potassium, significantly improves the efficiency of photosystem II (Iqbal et al. 2022). It has been reported that a high nitrogen concentration increases MQPPSII, an important index of the photosynthetic capability of plants (Klughammer and Schreiber 2008; Kościelniak et al. 2006). Therefore, the improvement in photosynthesis and water use efficiencies could be attributed to the tremendous advantages of biochar, such as improving soil organic carbon, physical soil properties, and soil water holding capacity that inhibits nutrient leaching and increases the availability of essential nutrients (Sombroek et al. 2004) Similarly, methyl jasmonate can protect the photosynthetic system against several stresses by maintaining chloroplast membrane stability and pigment concentration under stress conditions, which leads to decreased Fv, Fm, F0, and increased Fv/Fm (Liu et al. 2018). What is more, Fatma et al. (2021) reported a considerable influence of methyl jasmonate, in protection of the PSII system, maintaining the stability of a series of chloroplast protein named D1 and the acceleration of enzymatic activity under stress-induced conditions. Additionally, it has been reported that applying methyl jasmonate reduces the photo-inhibition in stressed plants compared to control plants, which results in the higher photosynthetic efficiency of PSII (Sirhindi et al. 2020).

5 Conclusions

It can be conclude that, in the present work, drought resulted in destructive repercussions on many parameters such as the net photosynthesis rate, transpiration rate, stomatal conductance, and photosynthetic pigments. However, use of biochar and methyl jasmonate acclimatized barley plants to water shortage, which is associated with enhanced morpho-physiological and biochemical traits. Soil content of biochar at 0.25% w/w and methyl jasmonate leaf applied at 50 µM protected plants against drought stress. Importantly, higher doses of biochar and methyl jasmonate had negative effects, highlighting the need to understand better the optimal doses and mechanisms of action of these amendments. Overuse of biochar and methyl jasmonate can be futile as the biochar increases the soil porosity which causes negative impact on the water availability. Additionally overuse of methyl jasmonate can cause negative effect on the stomatal conductance and molecular attributes of the plant under drought conditions. Last but not least, more experimental studies are needed to figure out more about the influence of different doses of biochar along with different doses of methyl jasmonate on several crop plant to identify the best doses of them for relieving the adverse effects of water shortage.

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Data Availability All data are available by connecting the first author through e-mail address. Furthermore, the extra data and materials used in this study will be made available by the authors upon request as much as possible.

Declarations

Conflicts of Interest The authors declare that they have no conflict of interest.

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