# Physiologic parameters of faba bean grown under saline condition, deficit irrigation and biochar 

M. R. Bahadori-Ghasroldashti ${ }^{1}$, F. Razzaghi ${ }^{1,2^{*}}$, A. R. Sepaskhah ${ }^{1,2}$<br>${ }^{1}$ Department of Water Engineering, School of Agriculture, Shiraz University, Shiraz, I. R. Iran<br>${ }^{2}$ Drought Research Center, Shiraz University, Shiraz, I. R. Iran

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#### Abstract

Salinity and water stresses and also low fertile soils are the main constraints in the agricultural production of Iran. The purposes of this study were to examine whether the application of wheat straw biochar (with EC of $9.3 \mathrm{dS} / \mathrm{m}$ ) can enhance faba bean's physiological characteristics and growth under greenhouse conditions. Hence, three levels of biochar ( $0,1.25$ and $2.5 \% \mathrm{w} / \mathrm{w}$ ), irrigation regimes ( 50,75 and $100 \%$ of crop water requirement) and irrigation water salinities $(0.6,4$ and 8 $\mathrm{dS} / \mathrm{m}$ ) were applied using the factorial arrangement in a complete randomized design with four replications. The application of $2.5 \%$ biochar under $0.6 \mathrm{dS} / \mathrm{m}$ saline water and $50 \%$ deficit irrigation significantly increased crop height, leaf area index, stomatal conductance by 12,14 and $11 \%$, respectively, and declined leaf temperature by $3 \%$ in comparison with that obtained at no biochar application. The addition of $2.5 \% \mathrm{w} / \mathrm{w}$ biochar under $8 \mathrm{dS} / \mathrm{m}$ and $50 \%$ deficit irrigation significantly decreased crop height and stomatal conductance by 21 and $29 \%$, respectively, in comparison with that obtained at no biochar application, $8 \mathrm{dS} / \mathrm{m}$ and $50 \%$ deficit irrigation. Moreover, the application of $2.5 \% \mathrm{w} / \mathrm{w}$ biochar together with $0.6 \mathrm{dS} / \mathrm{m}$ saline water and $100 \%$ irrigation water led to having maximum fresh seed yield and 100 -seed dry weight. In conclusion, the application of $2.5 \% \mathrm{w} / \mathrm{w}$ biochar with high electrical conductivity was not appropriate for faba bean under saline conditions at any water regimes.


## INTRODUCTION

Legumes such as faba bean, along with cereals, play an essential role in agricultural production due to their high nutritional value and biological nitrogen fixation (Duc et al., 2010). The faba bean seeds are very nutritious for humans and animals due to their high protein concentration (more than $30 \%$ on a dry weight basis) and energy supply (more than $40 \%$ starch content) (Ruisi et al., 2017). Faba bean is produced in different parts of the world, such as the Middle East, the Mediterranean region, China and Ethiopia (Rahate et al., 2021). From 1961 to 2013, due to faba bean high sensitivity to different kinds of abiotic and biotic stresses (Khan et al., 2010), a $15 \%$ reduction in its production was observed (Ruisi et al., 2017). Faba bean is very sensitive to water deficit during anthesis and pod filling due to its taproot system (Loss and Siddique, 1997).

According to FAOSTAT (2017), in Iran, 28375 and 17882 tons of faba bean seeds were harvested from 12225 and 8217 ha in 2010 and 2017, respectively. Lower faba bean production in Iran apart from lower cultivation area might be due to drought occurrence, low rainfall, low quality of irrigation water resources and low soil organic matter (Kazemi et al., 2016; Mesgaran et al., 2017). Sixty
seven percent of the land area in Iran has organic carbon less than $1 \%$ and around $25 \%$ are recognized saline soil ( $\mathrm{ECe}>4 \mathrm{dS} / \mathrm{m}$ ) (Mesgaran et al., 2017). Low rainfall, high evaporation, low quality of water and soil are the main constraints for low agricultural production in arid and semi-arid regions (Shrivastava and Kumar, 2015) including Iran (Alizadeh-Choobari and Najafi, 2018). Crops frequently face soil water deficit and salinity simultaneously, in arid and semi-arid regions. Many researchers showed that these factors reduce soil water potential, lower soil water availability (Glenn and Brown, 1998), diminish leaf water potential and water content, close stomata and decline photosynthesis, decrease cell enlargement and consequently reduce crop growth and yield (Farooq et al., 2009; Jaleel et al., 2009). Further, the ability of roots to uptake water and nutrients from the soil and transport them to the shoot (Navarro et al., 2008) and also the amount of water moving from the root to the shoot (Navarro et al., 2007) is limited under water stress conditions (Luo et al., 2015).

Biochar is produced through pyrolysis of biomass subjected to high temperatures under limited oxygen
supply (Lehmann, 2007). Previous research has shown that biochar application improves soil fertility, enhances plant growth, increases biomass and facilitates nutrient uptake under water and salt stress conditions (Haider et al., 2015). It has been reported that the application of biochar increased the leaf area and plant height of maize (Haider et al., 2015) and okra (Batool et al., 2015) under water stress conditions. Also, it has been shown that the application of biochar increased the biomass of wheat under field conditions (Olmo et al., 2014). Moreover, the application of biochar significantly improved the stomatal conductance and photosynthetic rate of tomato (Akhtar et al., 2014) and grape (Baronti et al., 2014) under water stress conditions. The application of $30 \mathrm{~g} / \mathrm{m}^{2}$ of biochar in salt-stressed soil did not affect the soil pH , but increased the soil saturated electrical conductivity as compared with that obtained in the control treatment (Thomas et al., 2013). Similar to the results obtained by Lashari et al. (2015) for maize, Akhtar et al, (2015) showed that wood-derived biochar at the rates of $0 \%$ and $5 \%$ W/W increased root length and volume, shoot biomass and stomatal conductance of salt-stressed potato.

So far, to our knowledge, no studies have considered the interaction effect of irrigation regimes, salinity of water and biochar on physiological parameters and shoot growth and development of faba bean. Therefore, the purposes of this study were to examine whether biochar can alleviate the negative effects of deficit water regimes and salinity of irrigation water on faba bean growth and physiological parameters.

## MATERIALS AND METHODS

The experiment was carried out in a greenhouse of Drought Research Center, Shiraz University, Shiraz, I.R. Iran during the 2017 growing season. The longitude, latitude and altitude of the research station are $52^{\circ} 32^{\prime} \mathrm{E}$, $29^{\circ} 36^{\prime} \mathrm{N}$ and 1810 m above mean sea level, respectively.

Three levels of irrigation regimes, irrigation water salinity and biochar were imposed using the factorial arrangement in a complete randomized design with four replications. The irrigation water salinity levels were well water with an EC (electrical conductivity) of $0.6 \mathrm{dS} / \mathrm{m}$ (as control treatment) 4 and $8 \mathrm{dS} / \mathrm{m}$ (named as $\mathrm{S}_{0.6}, \mathrm{~S}_{4}$ and $\mathrm{S}_{8}$, respectively). Fifty $\% \mathrm{NaCl}$ and $50 \% \mathrm{CaCl}_{2}$ (by weight) was added to the well water to prepare saline water. The amount of applied saline water was equal to the amount of irrigation water requirement that was calculated for each
treatment. The three irrigation levels were 50, 75, and 100 $\%$ of the crop water requirement (named as $\mathrm{I}_{50 \%}, \mathrm{I}_{75 \%}$, and $\mathrm{I}_{100 \%}$, respectively). The biochar levels were $0,1.25$ and 2.5 weight percentage (named as $B_{0}, B_{1.25}$ and $B_{2.5}$, respectively). The biochar was produced by pyrolyzing the wheat straw at $550^{\circ} \mathrm{C}$ for 24 hr under low oxygen conditions. The sandy loam soil and produced biochar were analyzed separately prior faba bean cultivation (Table 1).

Prior to filling the pots ( 108 pots, each with 20 cm height and 20 cm diameter), three holes were created at the bottom of the pots and a 1.0 cm gravel layer was put on. The sieved soil and biochar ( 2 mm ) were mixed with the experimental ratios. Thereafter, the pots were filled with the mixture to reach a final pot weight of 6 kg . Triple super-phosphate ( $43.2 \mathrm{mg} \mathrm{P} / \mathrm{kg}$ soil) was added to each pot based on soil analysis prior to cultivation. Urea fertilizer was added at faba bean vegetative and flowering stages (81 $\mathrm{mg} \mathrm{N} / \mathrm{kg}$ soil at each stage). Faba bean seeds (Five seeds) of the Barkat cultivar were sown in each pot on first October 2017 and thinned to three seedlings. Initially, all pots were irrigated to pot water-holding capacity to ensure germination and full plant establishment. The gravimetric soil water content at pot water-holding capacity was 19.0 , 20.9 and $23.0 \% \mathrm{w} / \mathrm{w}$ for $0,1.25$ and $2.5 \% \mathrm{w} / \mathrm{w}$ biochar, respectively (determined prior to cultivation).

The application of irrigation water salinity treatments and irrigation regimes was initiated 36 days after sowing (DAS) (coincided with the vegetative growth (6-7 leaves) of faba bean). The amount of irrigation water for $\mathrm{I}_{100 \%}$ was determined using the amount of water that was needed to increase the soil water content to $100 \%$ of pot waterholding capacity plus $15 \%$ as leaching fraction. Prior to the initiation of the experiment, different levels of leaching fraction were applied to $\mathrm{B}_{0} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%}$ treatment (three replications were considered) and $15 \%$ and higher leaching fraction caused water to drain. Also, the amount of irrigation water for $\mathrm{I}_{50 \%}$, and $\mathrm{I}_{75 \%}$ was calculated based on the amount of irrigation water for $\mathrm{I}_{100 \%}$ at each biochar level. In order to prevent the plant disturbance due to an increase in soil salinity, the irrigation requirement for $\mathrm{B}_{0} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%}$ treatments plus $30 \%$ extra water (leaching) was applied to all pots at 100 DAS (initiation of pod formation). The average temperature and relative humidity of $19^{\circ} \mathrm{C}$ and $57 \%$ were recorded during the crop growing season in the greenhouse, respectively. Natural light was received by plants throughout the experiment and the daylight period was approximately 10 hr .

Table 1. Chemical and physical and properties of biochar and soil

| Properties | Unit | Biochar | Soil |
| :--- | :--- | :--- | :--- |
| Electrical conductivity | $\mathrm{dS} / \mathrm{m}$ | $9.30^{*}$ | $0.66^{* *}$ |
| Cation exchange capacity | $\mathrm{meq} / 100 \mathrm{~g}$ soil | 25.76 | 13.59 |
| Nitrogen | $\%$ | 0.25 | 0.02 |
| Calcium | $\mathrm{meq} / \mathrm{l}$ | 2.3 | 2 |
| Potassium | $\mathrm{meq} / \mathrm{l}$ | 67.52 | 0.63 |
| Magnesium | $\mathrm{meq} / \mathrm{l}$ | 5.80 | 5.1 |
| Bulk density | $\mathrm{g} / \mathrm{cm}^{3}$ | 0.25 | 1.53 |
| pH |  | $8.19^{*}$ | 7.52 |

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## Measured parameters

## Plant Height

Plant height was measured seven times (50, 62, 74, 86, 112,128 and 157 DAS) during the growing season at one represented plant in each pot.

## Leaf area index

Leaf area (A, $\mathrm{cm}^{2}$ ) was measured seven times ( $50,62,74$, $86,112,128$ and 157 DAS) during the growing season by the following equation, which was developed using data of the present study.
$\mathrm{A}=0.6271$ ( $\mathrm{L} \times \mathrm{W}$ ) -0.0109

$$
\begin{equation*}
\mathrm{R}^{2}=0.92 \tag{1}
\end{equation*}
$$

where W is the width $(\mathrm{cm})$ and L is the leaf length ( cm ). Then, the leaf area index $\left(\mathrm{cm}^{2} / \mathrm{cm}^{2}\right)$ was calculated by dividing the leaf area to ground surface area.

## Stomatal conductance

Stomatal conductance ( $\mathrm{mmol} / \mathrm{m}^{2}$ per second) ) was measured 5 times during the growing season ( $55,77,89$, 128 and 148 DAS) using a portable porometer (Leaf Porometer, Decagon Devices, Pullman, Washington, USA). Measurements were performed between 11 am and 2 pm for each treatment in two fully expanded leaves from the top of the represented plant.

## Leaf temperature

Leaf temperature ( ${ }^{\circ} \mathrm{C}$ ) was measured four times during the crop growing season ( $55,77,128$ and 155 DAS) using a portable infrared thermometer (Kyorisu Model 5500). The measurement was conducted at a 0.5 m distance and a $45^{\circ}$ inclination angle to the leaf surface between 11 am to 2 pm.

## Crop yield, 100 -seed dry weight and seed protein

Faba bean fresh seeds were harvested gradually and weighted when the seeds were fully ripened. The fresh seeds were dried at $70^{\circ} \mathrm{C}$ for 48 h to calculate 100 -seed dry weight. The nitrogen concentration of dried seed (two grams) was determined using the Kjeldahl method. To determine the protein concentration by the nitrogen concentration, a conversion factor of 5.75 was considered in this study, because not all of the nitrogen in the seed is converted to protein (Mariotti et al. 2008).

The PROC GLM of SAS was used to perform the statistical analyses (SAS Institute Inc., 2007). All the data satisfied the normality and homogeneity of variance tests. Interaction effects between different levels of biochar, irrigation water salinity and irrigation regimes on the measured parameters were evaluated by analysis of variance (ANOVA). The Duncan's Multiple Range Test (DMRT) at the $5 \%$ level of probability was used to compare means.

## RESULTS AND DISCUSSION

## Plant height

The main effects of irrigation water salinities, biochar levels and irrigation water regimes on plant height during the growing season are shown in Fig. 1. Results showed that increasing salinity levels (Fig. 1a) and decreasing the irrigation water amount (Fig. 1c) decreased the plant height at different days of measurement during the growing season. Moreover, the application of $2.5 \% \mathrm{w} / \mathrm{w}$ biochar increased the plant height in comparison with that obtained in $\mathrm{B}_{0}$ at each day of measurement except in 112 DAS and 128 DAS (Fig. 1b). Considering the interaction effects of the treatments at 128 DAS, the application of deficit irrigation decreased plant height in both biochar and salinity levels (Table 2). Moreover, increasing the salinity levels at each biochar level and irrigation water level declined plant height, while, increasing the biochar from zero to $2.5 \% \mathrm{w} / \mathrm{w}$ significantly increased plant height at salinity level of $0.6 \mathrm{dS} / \mathrm{m}$ (control) and in three levels of irrigation water regimes. On the contrary, plant height was reduced by increasing biochar from zero to $2.5 \% \mathrm{w} / \mathrm{w}$ and at irrigation water salinity levels of $8 \mathrm{dS} / \mathrm{m}$ and three levels of irrigation water regimes (Table 2). It was reported by Akhtar et al. (2015) that wheat height was declined 31.9 \% by the application of 25 mmol of NaCl . Mojid et al. (2013) observed that wheat height was decreased by $9.3 \%$ and $10.3 \%$ at salinity levels of 10 and $13 \mathrm{dS} / \mathrm{m}$, respectively. In another study, increasing the biochar application rate from zero to $2.5 \% \mathrm{w} / \mathrm{w}$ significantly declined the faba bean height, as the produced biochar had an EC of $7.5 \mathrm{dS} / \mathrm{m}$, and a higher level of biochar application increased the soil salinity (Rezaie et al., 2019).

## Statistical analysis



Fig. 1. The main effect of irrigation water salinities (a), biochar levels (b) and irrigation water regimes (c) on plant height (cm) during crop growing season. Error bars are drawn but they are not visible as they are very small. Arrow bars indicate the application of mid-season leaching fraction

## Leaf area index

Figure 2 shows the main effects of biochar levels, irrigation water salinities and irrigation water regimes on leaf area index (LAI) during the growing season. Leaf area index had an increasing trend to 74 DAS and it declined under irrigation water salinities, biochar levels and irrigation water regimes before the application of $30 \%$ leaching fraction except in $\mathrm{S}_{0.6}$ and $\mathrm{S}_{4}$ (Fig. 2a) and $\mathrm{B}_{0}$ (Fig. 2b). Thereafter, LAI increased to 128 DAS and then decreased due to leaf senescence. Considering the interaction effects at 128 DAS, maximum and minimum LAI was observed in $\mathrm{B}_{2.5} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%} \quad\left(2.29 \mathrm{~cm}^{2} / \mathrm{cm}^{2}\right) \quad$ and $\quad \mathrm{B}_{2.5} \mathrm{~S}_{8} \mathrm{I}_{50 \%} \quad(0.62$ $\mathrm{cm}^{2} / \mathrm{cm}^{2}$ ) treatments, respectively (Table 2).

Decreasing irrigation level and saline irrigation water had a negative effect on LAI of faba bean (Mojid et al., 2013). Biochar addition of $2.5 \% \mathrm{w} / \mathrm{w}$ at salinity levels of $0.6 \mathrm{dS} / \mathrm{m}$ increased LAI in comparison with that obtained in $\mathrm{B}_{0}$ (Uzoma et al., 2011), while the
application of simultaneously saline water and biochar in this study declined LAI, as the applied biochar had $\mathrm{EC}_{\mathrm{e}}$ of $9.3 \mathrm{dS} / \mathrm{m}$. However, Huang et al. (2019) indicated that the application of biochar significantly reduced the negative effects of saline irrigation water through lowering leaf $\mathrm{Na}^{+} / \mathrm{K}^{+}$ratio and maintaining higher leaf relative water content, and thereby increased the leaf area index.

## Stomatal conductance

Variation of stomatal conductance $\left(\mathrm{g}_{\mathrm{s}}\right)$ during the growing season (Fig. 3) was almost similar to LAI (Fig. 2 ), as $g_{s}$ had an increasing trend by crop growth; however, the $g_{s}$ of all levels of treatments were decreased due to an increase in soil salinity at 89 DAS (Fig. 3a, b and c). After the application of a higher leaching fraction ( $30 \%$ ) at 100 DAS , the $\mathrm{g}_{\mathrm{s}}$ again increased to 128 DAS and after that dropped towards the end of the growing season due to leaf senescence.


Fig. 2. The main effect of irrigation water salinities (a), biochar levels (b) and irrigation water regimes (c) on leaf area index $\left(\mathrm{cm}^{2} / \mathrm{cm}^{2}\right)$ during crop growing season. Error bars are drawn but they are not visible as they are very small. Arrow bars indicate the application of mid-season leaching fraction


Fig. 3. The main effect of irrigation water salinities (a), biochar levels (b) and irrigation water regimes (c) on stomatal conductance ( $\mathrm{mmol} / \mathrm{m}^{2}$ per second) during crop growing season. Arrow bars indicate the application of mid-season leaching fraction

Considering the interaction effects of treatments at 128 DAS, decreasing irrigation level and increasing salinity significantly declined the stomatal conductance at three biochar levels (Table 2). Besides under full irrigation conditions, the addition of biochar to soil increased $\mathrm{g}_{\mathrm{s}}$ for each salinity level; however, under 50 $\%$ and $75 \%$ irrigation water levels, increasing the biochar declined $g_{s}$ in salinity levels of 8 and $4 \mathrm{dS} / \mathrm{m}$. It has been shown that water and salinity stresses decline water use, leaf growth, and yield through restriction on stomatal apertures to mediate carbon metabolism and leaf photochemistry (Negrão et al., 2017). The application of biochar in saline soils enhanced the stomatal conductance of herbaceous plants, wheat and potato (Akhtar et al., 2015; Thomas et al., 2013).

The positive effect of biochar on the physiological responses of plants such as green canopy temperature and stomatal conductance and (Thomas et al., 2013) may be explained by biochar's capability to increase soil porosity and hence soil water availability, and to decrease soil bulk density. Under water stress conditions, stomata are closed in response to the loss of turgor pressure, causing a lower transpiration rate and hence, the higher canopy temperature (Kramer, 1983). Ali et al. (2017) reviewed the effect of biochar on alleviation of salt and drought stress in plants and showed that the application of biochar to soil increased plant growth and also improved gas exchange characteristics under either salt and/or drought stress.

Table 2. Faba bean height, leaf area index, stomatal conductance and leaf temperature at 128 days after sowing as influenced by different levels of biochar, irrigation water regimes and irrigation water salinity and their analysis of variance

| Characteristic | $\mathrm{B}_{0}$ |  |  | $\mathbf{B}_{1.25}$ |  |  | $\mathbf{B}_{2.5}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{S}_{0.6}$ | $\mathrm{S}_{4}$ | $\mathbf{S}_{8}$ | $\mathrm{S}_{0.6}$ | $\mathrm{S}_{4}$ | $\mathrm{S}_{8}$ | $\mathrm{S}_{0.6}$ | $\mathrm{S}_{4}$ | $\mathbf{S}_{8}$ |
| Plant height (cm) |  |  |  |  |  |  |  |  |  |
| $\mathrm{I}_{100 \%}$ | $95.0^{\text {c }}$ | $87.7{ }^{\text {de }}$ | $72.0{ }^{\text {h }}$ | $101.7^{\text {b }}$ | $75.72^{\text {h }}$ | $62.0{ }^{\text {jk }}$ | $107.3^{\text {a }}$ | $80.0^{\text {g }}$ | $64.3{ }^{\text {ij }}$ |
| $\mathbf{I}_{75 \%}$ |  |  | $58.0{ }^{\mathrm{klmn}}$ | $88.3{ }^{\text {de }}$ | $60.3{ }^{\text {jkl }}$ | $59.0{ }^{\text {klm }}$ | $91.7{ }^{\text {cd }}$ | $60.0{ }^{\text {jkl }}$ | $56.7{ }^{\text {mno }}$ |
| 150\% | 75.3 ${ }^{\text {h }}$ | $60.3{ }^{\text {jkl }}$ | $53.7^{\text {no }}$ | $73.3{ }^{\text {h }}$ | $52.3{ }^{\circ}$ | $46.3{ }^{\text {p }}$ | $84.7{ }^{\text {ef }}$ | $55.3{ }^{\text {mno }}$ | $42.3{ }^{\text {p }}$ |

Leaf area index ( $\mathrm{cm}^{2} / \mathrm{cm}^{2}$ )

| $\mathbf{I}_{\mathbf{1 0 0 \%}}$ | $1.66^{\mathrm{d}}$ | $1.32^{\mathrm{e}}$ | $1.21^{\mathrm{f}}$ | $1.78^{\mathrm{c}}$ | $1.60^{\mathrm{d}}$ | $0.99^{\mathrm{ij}}$ | $2.29^{\mathrm{a}}$ | $1.11^{\mathrm{h}}$ | $0.77^{\mathrm{op}}$ |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
|  |  |  |  |  |  |  |  |  |  |
| $\mathbf{I}_{75 \%}$ | $1.36^{\mathrm{e}}$ | $1.14^{\mathrm{gh}}$ | $0.97^{\mathrm{ijk}}$ | $1.35^{\mathrm{e}}$ | $0.80^{\circ}$ | $0.89^{\mathrm{km}}$ | $2.03^{\mathrm{b}}$ | $0.95^{\mathrm{jkl}}$ | $0.83^{\mathrm{no}}$ |
| $\mathbf{1 5 0 \%}$ | $1.18^{\mathrm{gg}}$ | $0.78^{\circ}$ | $0.62^{\mathrm{q}}$ | $0.87^{\mathrm{mn}}$ | $0.92^{\mathrm{km}}$ | $0.72^{\mathrm{p}}$ | $1.35^{\mathrm{e}}$ | $1.02^{\mathrm{i}}$ | $0.62^{\mathrm{q}}$ |

## Stomatal conductance ( $\mathbf{m m o l} / \mathrm{m}^{2}$ per second)

| $\mathrm{I}_{100 \%}$ | $162.8{ }^{\text {d }}$ | $131.4{ }^{\text {ghi }}$ | $90.1^{\circ}$ | $170.8^{\text {c }}$ | $136.8{ }^{\text {fg }}$ | $109.9{ }^{1}$ | $194.1^{\text {a }}$ | $148.5^{\text {e }}$ | $121.0{ }^{\text {jk }}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathrm{I}_{75 \%}$ | $138.0{ }^{\text {f }}$ | $133.2{ }^{\text {fgh }}$ | $105.1{ }^{\text {lm }}$ | $151.5^{\text {e }}$ | $125.7{ }^{\text {ij }}$ | $107.2^{\text {lm }}$ | $180.7{ }^{\text {b }}$ | $116.5^{\text {k }}$ | $88.0^{\circ}$ |
| 150\% | $150.0^{\text {e }}$ | $128.3{ }^{\text {hi }}$ | $98.9^{\text {n }}$ | $132.7^{\text {fgh }}$ | $109.2^{1}$ | $87.7^{\circ}$ | $166.3{ }^{\text {cd }}$ | $101.6^{\mathrm{mn}}$ | $69.9^{\text {p }}$ |
| Leaf temperature ( ${ }^{\circ} \mathrm{C}$ ) |  |  |  |  |  |  |  |  |  |
| $\mathrm{I}_{100 \%}$ | $26.9{ }^{\text {kl }}$ | $27.1{ }^{\text {jk }}$ | $28.5{ }^{\text {fg }}$ | $26.6{ }^{\text {lm }}$ | $27.0^{\mathrm{jkl}}$ | $28.0{ }^{\text {h }}$ | $23.6{ }^{\circ}$ | $26.5^{\text {m }}$ | $29.1{ }^{\text {de }}$ |
| $\mathbf{I}_{75 \%}$ | $27.5{ }^{\text {i }}$ | $27.1{ }^{\text {jk }}$ | $29.3{ }^{\text {d }}$ | $\begin{gathered} 26.9^{\mathrm{kl}} \\ 27.5^{\mathrm{i}} \\ \hline \end{gathered}$ | $\begin{aligned} & 28.3^{\mathrm{g}} \\ & 29.3^{\mathrm{d}} \end{aligned}$ | $\begin{gathered} 29.7^{\mathrm{c}} \\ 29.83^{\mathrm{b}} \\ \hline \end{gathered}$ | $\begin{aligned} & 25.9^{\mathrm{n}} \\ & 27 . \mathrm{i}^{\mathrm{jk}} \\ & \hline \end{aligned}$ | $\begin{gathered} 27.3^{\mathrm{ij}} \\ 28.80^{\mathrm{ef}} \end{gathered}$ | $\begin{gathered} 29.1^{\mathrm{de}} \\ 31.3^{\mathrm{a}} \\ \hline \end{gathered}$ |
| 150\% | $27.9^{\text {h }}$ | $29.3{ }^{\text {d }}$ | $30.3{ }^{\text {b }}$ |  |  |  |  |  |  |
| Analysis of variance |  |  |  |  |  |  |  |  |  |
| Source of variation |  | Degree of freedom | Plant height | Leaf area index |  | Stomatal conductance |  | Leaftemperature |  |
|  |  | Mean Square |  |  |  |  |  |  |  |
| Biochar (B) Irrigation water salinity (S) |  |  | 2 | $942.27^{*}$ | 0.09 * |  | 602.67 |  | 17.73 * |  |
|  |  | 2 | $29.21{ }^{\text {ns }}$ | 0.07 * |  | 606.95 * |  | $0.00{ }^{\text {ns }}$ |  |
| Irrigation regimes (I) |  | 2 | $56.6{ }^{\text {ns }}$ | 1.19 * |  | $221.20{ }^{\text {ns }}$ |  | $0.28{ }^{\text {ns }}$ |  |
| Interaction (BxS $\times$ ( $)$Error |  | 8 | $176.0{ }^{\text {ns }}$ | 0.15 * |  | $39.27{ }^{\text {ns }}$ |  | 4.32 ** |  |
|  |  | 54 | 100.00 | 0.00 |  | 80.17 |  |  |  |  |

In each trait, means followed by the common letters are not significantly different at $5 \%$ level of probability; **; * significant at $0.05,0.01$ levels, respectively; ns, non-significant

## Leaf temperature

Considering the main effects of irrigation water salinities, biochar levels and irrigation water regimes on leaf temperature during the growing season (Fig. 4), increasing salinity of irrigation water from 0.6 to $8 \mathrm{dS} / \mathrm{m}$ (Fig. 4a) and decreasing irrigation water level from 100 $\%$ to 50 \% irrigation water requirement (Fig. 4c), increased the leaf temperature due to imposed water stress. In addition, the application of $2.5 \% \mathrm{w} / \mathrm{w}$ biochar reduced the leaf temperature all over the growing season in comparison with that in $\mathrm{B}_{0}$ and $\mathrm{B}_{1.25}$ and no significant difference was seen between leaf temperature of $\mathrm{B}_{0}$ and $\mathrm{B}_{1.25}$ during the growing season (Fig. 4b).

The interaction effect of different treatments on leaf temperature at 128 DAS showed that increasing salinity from 0.6 to $8 \mathrm{dS} / \mathrm{m}$ and declining irrigation water level from 100 to $50 \%$ of full irrigation water increased the leaf temperature (Table 2). The most and least stressed plants were those with leaf temperature of $31.3{ }^{\circ} \mathrm{C}$ $\left(\mathrm{B}_{2.5} \mathrm{~S}_{8} \mathrm{I}_{50 \%}\right)$ and $23.6{ }^{\circ} \mathrm{C}\left(\mathrm{B}_{2.5} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%}\right)$, respectively, indicating that the application of $2.5 \% \mathrm{w} / \mathrm{w}$ biochar caused the leaf temperature to rise by $32.6 \%$ under $50 \%$ deficit irrigation and salinity level of $8 \mathrm{dS} / \mathrm{m}$ in comparison with that obtained at full irrigation water and no saline water. Comparing the leaf temperature in treatments of $\mathrm{B}_{0} \mathrm{~S}_{8} \mathrm{I}_{50 \%}\left(30.3{ }^{\circ} \mathrm{C}\right)$ and $\mathrm{B}_{2.5} \mathrm{~S}_{8} \mathrm{I}_{50 \%}\left(31.3{ }^{\circ} \mathrm{C}\right)$ showed that the addition of biochar has not minimized the negative effect of water stresses on the plant (Table 2 ), which could be due to the fact that the plants significantly affected by both salinity ( $8 \mathrm{dS} / \mathrm{m}$ ) and water deficit ( $50 \%$ ) and also the salinity of biochar itself. Rezaie et al. (2019) showed that under full irrigation, the application of biochar significantly increased stomatal conductance and decreased leaf
temperature of faba bean under all salinity levels, as biochar increases the soil water holding capacity and enhanced uptake of water and minerals.

Linear regression analysis was used to fit the relationship between the $\mathrm{g}_{\mathrm{s}}$ and leaf temperature in different levels of biochar (Fig. 5). Results showed that by increasing water stresses (increase in leaf temperature) the stomatal conductance declined and vice versa. The minimum and maximum stressed plants were those treated with $\mathrm{B}_{2.5} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%}$ and $\mathrm{B}_{2.5} \mathrm{~S}_{8} \mathrm{I}_{50 \%}$, respectively (Fig. 5), indicating that application of biochar under both low irrigation levels and saline water cannot diminish the effect of water and salinity stresses on the stomatal conductance.

## Seed weight and protein concentration

Fresh seed yield (Table 2) significantly declined by increasing irrigation water salinity level and at each irrigation and biochar level, while increasing biochar to $2.5 \% \mathrm{w} / \mathrm{w}$ significantly increased seed yield at each irrigation water and salinity level, except for $4 \mathrm{dS} / \mathrm{m}$ salinity at all irrigation water levels. Moreover, no significant difference was observed in fresh seed yield between $\mathrm{B}_{0} \mathrm{~S}_{8} \mathrm{I}_{50 \%}$ and $\mathrm{B}_{2.5} \mathrm{~S}_{8} \mathrm{I}_{50 \%}$ treatments. The maximum fresh seed yield was $37.2 \mathrm{~g} /$ pot obtained in $\mathrm{B}_{2.5} \mathrm{~S}_{0} \mathrm{I}_{100 \%}$ treatment. Maximum and minimum 100seed dry weight was obtained in $\mathrm{B}_{2.5} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%}$ and $\mathrm{B}_{0} \mathrm{~S}_{8} \mathrm{I}_{75 \%}$ treatments, respectively. However, no significant difference was observed between the 100seed dry weight of $\mathrm{B}_{2.5} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%}$ and $\mathrm{B}_{1.25} \mathrm{~S}_{0.6} \mathrm{I}_{100 \%}$ treatments. Similar to Suppadit et al. (2012), the application of saline irrigation water and deficit irrigation declined the 100 -seed dry weight of faba bean, while, the application of biochar increased the 100 -seed dry weight.


Fig. 4. The main effect of irrigation water salinities (a), biochar levels (b) and irrigation water regimes (c) on leaf temperature $\left({ }^{\circ} \mathrm{C}\right)$ during crop growing season. Arrow bars indicate the application of mid-season leaching fraction


Fig. 5. Relationship between the stomatal conductance and leaf temperature at 128 days after sowing for different levels of biochar

The application of saline irrigation water at each irrigation water and biochar level reduced the concentration of protein in faba bean seed (Table 3). The addition of biochar increased the concentration of protein in seed in all salinity and irrigation water levels, except in salinity level of $8 \mathrm{dS} / \mathrm{m}$ and irrigation water of $50 \%$, which might be due to a decrease in soil water potential and therefore, a decline in water and nutrient uptake. The addition of biochar application on the growth of soybean under water stress was investigated by Hafeez et al. (2017). They indicated that protein concentration in soybean leaves was not significantly affected under water stress or biochar application; however, a slight increase in protein concentration was observed by the application of 10 ton ( t ) $\mathrm{ha}^{-1}$ biochar in
comparison with control (no water stress and no biochar). In another study, Ali et al. (2017) indicated that $27 \%$ increase in maize seed protein concentration was obtained by the addition of up to 50 tha - rice husk biochar. A two-year field experiment was carried out by Wacal et al. (2019) to investigate the effect of biochar (rice husk) on sesame (Sesamum indicum L.) seed yield and quality. The results showed that the application of biochar did not significantly affect seed protein concentration in the first year, while higher application of biochar (more than $20 \mathrm{t} \mathrm{ha}^{-1}$ ) significantly increased protein concentration in the second year. Maximum protein concentration in sesame seed was observed by $50 \mathrm{t} \mathrm{ha}^{-1}$ biochar in both years.

Table 3. Faba bean fresh seed yield, 100-seed dry weight and seed's protein concentration for different treatments and their analysis of variance

| Characteristics | $\mathbf{B}_{0}$ |  | $\mathrm{B}_{1.25}$ |  |  | $\mathbf{B}_{2.5}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\mathrm{S}_{4}$ | $\mathrm{S}_{8}$ | $\mathrm{S}_{0.6}$ | $\mathrm{S}_{4}$ | $\mathrm{S}_{8}$ | $\mathrm{S}_{0.6}$ | $\mathrm{S}_{4}$ | $\mathrm{S}_{8}$ |
| Fresh seed yield (g pot ${ }^{-1}$ ) |  |  |  |  |  |  |  |  |
| $\mathbf{I}_{\mathbf{1 0 0 \%}} \quad 32.4{ }^{\text {b }}$ | $24.5{ }^{\text {de }}$ | $17.0{ }^{\text {ijk }}$ | $29.8{ }^{\text {c }}$ | $22.4{ }^{\text {defg }}$ | $16.2{ }^{\mathrm{kl}}$ | $37.2^{\text {a }}$ | $24.0{ }^{\text {d }}$ | $21.0{ }^{\text {fg }}$ |
| $\mathbf{I}_{75 \%} \quad 24.6{ }^{\text {d }}$ | $18.5{ }^{\text {efg }}$ | $13.8{ }^{1}$ | $23.7{ }^{\text {d }}$ | $18.7{ }^{\text {hi }}$ | $15.3{ }^{\text {kl }}$ | $32.4{ }^{\text {b }}$ | $20.5{ }^{\text {gh }}$ | $21.3{ }^{\text {efg }}$ |
| $\mathbf{I}_{\mathbf{5 0 \%}} \quad 23.3{ }^{\text {def }}$ | $21.3{ }^{\text {hij }}$ | $14.8{ }^{\text {kl }}$ | $23.0{ }^{\text {def }}$ | $17.0{ }^{\text {ijk }}$ | $14.3{ }^{1}$ | $28.0^{\text {c }}$ | $16.0{ }^{\text {kl }}$ | $14.9{ }^{\text {kl }}$ |
| 100-seed dry weight (g) |  |  |  |  |  |  |  |  |
| $\mathbf{I}_{\mathbf{1 0 0 \%}}$ | $84.5{ }^{\text {c }}$ | $51.4{ }^{\text {kl }}$ | $91.9^{\text {a }}$ | $78.9{ }^{\text {d }}$ | $58.9{ }^{\text {j }}$ | $96.0^{\text {a }}$ | $75.8{ }^{\text {def }}$ | $71.9^{\text {g }}$ |
| $\mathbf{I}_{75 \%} \quad 78.4{ }^{\text {de }}$ | $73.6{ }^{\text {fg }}$ | $47.9{ }^{\mathrm{m}}$ | $87.0{ }^{\text {c }}$ | $63.7{ }^{\text {i }}$ | $52.3{ }^{\mathrm{kl}}$ | $86.8^{\text {c }}$ | $67.2{ }^{\text {h }}$ | $77.1{ }^{\text {de }}$ |
| 150\% $53.9^{\mathrm{k}}$ | $62.7{ }^{\text {i }}$ | $49.6{ }^{\text {lm }}$ | $75.3{ }^{\text {ef }}$ | $52.3{ }^{\text {kl }}$ | $51.1{ }^{\mathrm{klm}}$ | $66.7^{\text {h }}$ | $63.3{ }^{\text {i }}$ | $49.7{ }^{\text {lm }}$ |
| Protein concentration (\%) |  |  |  |  |  |  |  |  |
| $\mathbf{I}_{\mathbf{1 0 0 \%}} \quad 24.5^{\mathrm{gh}}$ | $22.0{ }^{\text {kl }}$ | $21.6{ }^{\text {lm }}$ | $26.0{ }^{\text {d }}$ | $24.1{ }^{\text {hi }}$ | $23.8{ }^{\text {i }}$ | $27.7{ }^{\text {b }}$ | $25.4{ }^{\text {e }}$ | $23.8{ }^{\text {i }}$ |
| $\mathbf{I}_{\mathbf{7 5 \%}} \quad 24.8^{\mathrm{fg}}$ | $21.3{ }^{\text {m }}$ | $19.7{ }^{\text {op }}$ | $26.7^{\text {c }}$ | $24.5{ }^{\text {gh }}$ | $22.5{ }^{\text {k }}$ | $27.9{ }^{\text {b }}$ | $25.3{ }^{\text {ef }}$ | $23.9{ }^{\text {i }}$ |
| $\mathbf{I}_{50 \%} \quad 25.2{ }^{\text {ef }}$ | $20.4{ }^{\text {n }}$ | $20.1{ }^{\text {no }}$ | $26.9{ }^{\text {c }}$ | $23.2^{\text {j }}$ | $19.0{ }^{\text {q }}$ | $28.8{ }^{\text {a }}$ | $24.6{ }^{\text {gh }}$ | $19.5{ }^{\text {pq }}$ |
| Analysis of variance |  |  |  |  |  |  |  |  |
| Source of variation | Degree of freedom |  | Fresh seed yield 100-seed dry |  |  | Protein concentration |  |  |
|  |  |  | Mean Square |  |  |  |  |  |
| Biochar (B) | 2 |  | $107.51{ }^{\text {* }}$ |  | 392.95* |  | 64.01** |  |
| Irrigation water salinity (S) | 2 |  | 970.47* |  | 3699** |  | 169.46* |  |
| Irrigation regimes (I) | 2 |  | 234.08* |  | 2455* |  | 11.25* |  |
| Interaction ( $\mathrm{B} \times \mathrm{S} \times \mathrm{I}$ ) | 8 |  | $2.34{ }^{\text {ns }}$ |  | 124.1 * |  | 2.09 * |  |
| Error | 54 |  | 1.76 |  | 3.21 |  | 0.095 |  |

In each trait, means followed by the common letters are not significantly different at $5 \%$ level of probability; $* *$; significant at $0.05,0.01$ levels, respectively; ns, non-significant

## CONCLUSIONS

Addition of $2.5 \%$ w/w biochar enhanced physiological parameters of faba bean under $50 \%$ deficit irrigation with no saline water in comparison with that obtained under no biochar application. However, the application of the irrigation water salinity of $8 \mathrm{dS} / \mathrm{m}$ caused the biochar application (especially the high application rate of $2.5 \% \mathrm{w} / \mathrm{w}$ ) to negatively influence the physiological parameters such as stomatal conductance. Moreover, the application of biochar alleviated the negative effects of deficit water regimes on faba bean production under no saline conditions. In conclusion, the application of a high rate of biochar ( $2.5 \% \mathrm{w} / \mathrm{w}$ ) with high electrical conductivity of $9.3 \mathrm{dS} / \mathrm{m}$ is not appropriate for faba

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bean under saline water use at any irrigation water levels. It is recommended to evaluate the result of this study using biochar with lower electrical conductivity.

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پارامترهاى فيزيولوزيكى باقلا كشت شده در شرايط شورى، كم آبيارى و


## بيوحار



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چحكيده－تنشهاى شورى و آبى و همچنين خاكیهاى با حاصلخيزى كم از محدوديتهاى اصلى در توليد محصولات كشاورزى ايران هستند．هدف از اين مطالعه بررسى كاربرد بيوچار كاه و كلش كا گـندم




 شاخص سطح برگ و هدايت روزنهاى به مقدار K مقايسه با عدم كاربرد بيوپار شد．افزودن Y／Q درصد وزنى بيوچار همراه با آب شور＾دسى زيمنس بر
 درصد در مقايسه با بدون استفاده از بيوچار و با آب شور

 بيوچار پ／Q درصد وزنى با هدايت الكتريكى بالا برای باقلا در شرايط شور در هيج سطح آب آبيارى مناسب نبود．

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> وازههاى كليدى:
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> غلظت پروتئين
> محصول دانه
> هدايت روزنهاى


[^0]:    * Corresponding Author: razzaghi@shirazu.ac.ir DOI: 10.22099/IAR.2022.42360.1470

[^1]:    ${ }^{*} \mathrm{pH}$ and EC of biochar was measured in 1:10 biochar: water
    ${ }^{* *}$ EC of soil was measured in saturated extract

