



Sulfur-Enriched Biochar Soil Amendment Enhances Tolerance to Drought Stress in Faba Bean (*Vicia faba* L.) Under Saline Soil Conditions



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Saad M. Howladar¹, Wael M. Semida², Taia A. Abd El-Mageed³, Abeer M. Kutby¹ and Majed M. Howladar⁴

¹ Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, Saudi Arabia

² Horticulture Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt

³ Soil and Water Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt

⁴ Biological Sciences Department, Faculty of Science, King Abdulaziz University, Saudi Arabia

AGRICULTURAL sustainability and crop yield are seriously threatened by drought stress, especially in areas with limited water resources. In this study, we investigated the potential of sulfur-enriched biochar (S-ACB) as a soil amendment to mitigate the negative impacts of drought stress on plants (*Vicia faba*). Experiments were conducted in a randomized split plot design. Treatments were divided into three levels of irrigation water applied (100, 80 and 60% of crop evapotranspiration) and three S-ACB treatments (0, 5 and 10 t ha⁻¹). Irrigation treatments were assisted in the main plots, while S-ACB treatments were allocated in the sub-plots. Our results revealed that deficit irrigation substantially impaired plant water status, photosynthetic efficiency, and nutrient acquisition, ultimately resulting in a decrease in the growth and yield components. However, the incorporation of S-ACB into the soil emerged as a powerful strategy to counteract these detrimental effects. S-ACB played a pivotal role in enhancing plant water status, improving photosynthetic efficiency, and facilitating nutrient uptake. Soil incorporation of S-ACB₅ and S-ACB₁₀ to faba bean plants significantly ($P < 0.05$) enhanced different growth, physiological responses and yield of faba bean under different irrigation regimes in both seasons relative to the controls. Notably, the positive effects of S-ACB extended beyond mere stress mitigation, as it enabled *Vicia faba* plants to achieve growth and yield comparable to or even surpassing those of well-irrigated plants. Furthermore, S-ACB application-maintained leaf nutrient status and reduced the Na⁺/K⁺ ratio, highlighting its potential to sustain nutrient balance under drought stress conditions. This study highlights the potential use of sulfur-enriched biochar as an efficient and long-lasting soil supplement to increase *Vicia faba* plants' resistance to drought. The significant improvements in plant water status, photosynthetic efficiency, growth, yield, and nutrient uptake indicate the potential of S-ACB to address the challenges posed by drought stress in agriculture. These findings offer valuable insights into the development of strategies for sustainable crop production in water-scarce environments, emphasizing the importance of biochar as a tool to enhance resilience and productivity.

Keywords: water stress, yield, soil properties, saline soil, N₂ fixer, Water use efficiency.

1. Introduction

Faba bean (*Vicia faba* L.) is one of the greatest popular legume yields, and it is widely cultivated all over the world with 5.43 million tons produced from 2.57 million ha (FAOSTAT, 2019). Fresh pods and dry seeds are great sources of protein for humans due to their nutritious richness (Khazaei and Vandenberg, 2020; Rady et al., 2021). Faba bean is commonly cultivated in salted affected soil that is characterized by poor structure and low-fertile soil (Abd El-Mageed et al., 2021). These adverse conditions severely obstruct faba bean growth and yield.

Abiotic stressors are thought to be the primary cause of a 70% decline in worldwide crop yields, and are thus a major limit to crop production (Semida et al., 2020; Singh et al., 2016). Due to the freshwater shortage, farmers in some areas are forcing to use partially blending sewage water with freshwater to irrigate their crops (Abd El-Mageed et al., 2020; Abid et al., 2016; Khalifa, 2019). Long-term salts accumulation alters the composition and

*Corresponding author e-mail: smhowladar@uj.edu.sa

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microbial activities of the soil (Xie et al., 2016). Plants grown on salt affected soil or received saline irrigation water take up excessive amounts of them, which accumulate in plant tissues. Direct consequences are oxidative stress by hyper production of (ROS) that provokes damage to the organelle's structures and several alterations in physio-biochemical and morphological attributes, and inhibition of cytoplasmic enzymes (Mishra et al., 2017; Semida et al., 2018). Furthermore, salt stress inhibits root growth, reduces the biomass and chlorophyll biosynthesis, and declines the photosynthetic capacity as well as causes chlorosis and alters water status, membrane stability and nutrient assimilation (Irshad et al., 2020; Singh et al., 2016; Sallam et al., 2014). Therefore, there is a growing focus on sustainable strategies to lower the soil's salt bioavailability, reclaim the saline soil, and preserve soil fertility (Mishra et al., 2017; Yan et al., 2020). As a sustainable organic-based material with superior sorption capacity, in situ immobilisation, and cost efficiency (Inyang et al., 2016; Semida et al., 2019; Zhang et al., 2019; El-Zemrany et al., 2016), biochar (BC) is an ecofriendly organic product that is used for remediation and also for ameliorating salinity stress on plants (Zhang et al., 2019).

Biochar is almost produced as a by-product of biomass pyrolysis for energy recovery, and characterized by high specific surface area, cation exchange capacity (CEC), microporosity, and active surface functional groups as well as reduces the soil bulk density and hydraulic conductivity, which improves the soil physiochemical properties and the soil biota (Abd El-Mageed et al., 2021; Godlewska et al., 2017; Semida et al., 2019). Therefore, these properties make the biochar an effective green sorbent of pollutants, the metal immobilization mechanism, and attenuate the heavy metals uptake in plants by retaining them on the biochar particle surfaces (Irshad et al., 2020; Xu et al., 2014; Zhang et al., 2016), thus it used to alleviate the heavy metal stress on plants (Jing et al., 2020; Nzediegwu et al., 2019). Although biochar may be used to stabilise a variety of soil salts, its effectiveness in saline soils can be inconsistent (Zhao et al., 2020; Zubairu et al., 2023). Furthermore, in semi-arid and arid regions, soil is mostly slight alkaline to highly alkaline (7.5-8.7) (Abd-Alla et al., 2014; Hammam and Mohamed, 2020), which reduces the macro and micronutrients availability, in addition to alkali stress causes ion-induced damage, osmotic and oxidative stresses (Chen et al., 2017; Guo et al., 2017). However, the high pH of CB is a significant impediment to optimizing nutrient use efficiency, particularly in alkaline soils (Rehman et al., 2020; Sultan et al., 2020). Increasing the soil pH has also been observed under soil supplemented biochar, which could be not appropriate for alkaline soil (Bass et al., 2016; Ding et al., 2016). Recently, various CB modifications/enrichments have been proposed, including chemical changes to biochar structure to develop a novel remediation biochar with superior features for the stabilization of contaminants, as well as improving soil quality and microbial activity in the soil (O'Connor et al., 2018; Rehman et al., 2020; Sultan et al., 2020; Zhao et al., 2020). Therefore, we incorporated sulfur or citric acid with biochar in our previous studies to produce acidified biochar for decreasing the biochar pH (Abd El-Mageed et al., 2020; Khalil et al., 2023). Furthermore, applied sulfur could increase soil sulfur content, which can regulate metals mobilization as a result of sulfide/sulfate precipitation of metals in accordance to reducing/oxidizing conditions in the soil (Shaheen et al., 2017). An important macronutrient for plants, sulphur (S) controls abiotic stress reactions, growth, and development (Liang et al., 2016). Acidification of biochar produced significant improvements in soil health and nutrient status (Sultan et al., 2020), enhanced growth of plants (Rehman et al., 2020).

We hypothesized that the incorporation of sulfur-enriched biochar (S-ACB) into the soil would alleviate the damaging impact on *Vicia faba* plants under drought stress. Specifically, we anticipated that S-ACB would enhance plant water status, improve photosynthetic efficiency, facilitate nutrient uptake, and subsequently lead to increased growth, yield, and overall resilience of *Vicia faba* plants exposed to deficit irrigation (DI). We further hypothesized that the beneficial impact of S-ACB would be most pronounced when combined with optimal irrigation levels, potentially surpassing the performance of well-irrigated plants.

2. Materials and methods

2.1. Experimental set-up

In 2020/21 and 2021/22 successive growing seasons, at a private farm in the Al Bahah region (19.9930 N, 41.5183 E), Saudi Arabia. 2 field trails were carried out. The local climatic conditions is classified as arid/hyper-arid (Ponce et al., 2000). The physical - chemical characteristics of the tested soil were assessed according to (Klute and Dirksen, 1986; Page et al., 1982) techniques and displayed in Table 1. The experiments were conducted in Randomized block design with three replications.

Treatments were divided into three irrigation water requirements (IWR) and three S-ACB rates. One of the following three treatments; $I_{100\%} = 100\%$, $I_{80\%} = 80\%$, and $I_{60\%} = 60\%$ of ET_c -was applied as a percentage of ET_c through irrigation process. The main plots received irrigation treatments, and the sub-plots received the S-ABC rates, which were $S-ACB_0 = 0 \text{ t ha}^{-1}$ as a control, $S-ACB_5 = 5 \text{ t ha}^{-1}$, and $S-ACB_{10} = 10 \text{ t ha}^{-1}$. S-ACB [(3:100 (w/w) sulphur: citrus wood biochar)] was added to the soil three weeks prior to faba bean seeding. Properties of the S-ACB are presented in Table 2. Each plot comprised three planting rows, with a 15 cm between each plant within the same row. The experimental unit area was 14 m length \times 0.8 m row width (11.2 m²), individually plot elaborate 2 planting rows and the 40 cm space was between plants in the rows.

2.2. Agronomic management

On October 8 and 10 of 2020/21 and 2021/22, healthy faba bean (*Vicia faba* L, cv. Sakha 1) seeds were planted and harvested on 15 April 2020/21 and 20 April 2021/22. Drip irrigation was the method used, with each elementary test plot having two drip lines spaced 0.5 m apart. Following complete germination, irrigation treatments were initiated. In order to supply phosphorus (370 kg ha⁻¹) to the soil, calcium superphosphate (15.5% P₂O₅) was added during seedbed preparation. Nitrogen was supplied during the growing seasons (15 and 30 days after planting) at two dosages with a total amount of 150 kg ha⁻¹ in the form of ammonium nitrate (33.5% N). Furthermore, after thirty days from the planting date, 125 kg ha⁻¹ of 50% K₂O potassium sulphate was applied according to the recommendation of the Ministry of Agriculture in Saudia Arabia.

2.3. Irrigation water requirements (IWR)

The following formula was used to calculate the irrigation water requirements (Allen et al., 1998):

$$IWR = [(A * ETc * Ii) / Ea * (1 - LR)]$$

IWR (m³) = The water used for irrigation, A (m²) = The unit area, ETc (mm d⁻¹) = Crop evapotranspiration, Ii (day) = The period between irrigations, Ea (%) = The efficiency of irrigation and LR (%) = The requirements for leaching. Allen et al. (1998) approach was used to estimate the ETc;

$$ETc = K_{pan} \times E_{pan} \times K_c$$

K_{pan} = Coefficient of the pan, E_{pan} = Class A pan evaporation (mm d⁻¹), and K_c = Crop coefficient

2.4. Measurements of water status, physiological traits, and growth

The percentages of the membrane stability index (MSI %) and relative water content (RWC %) were calculated using the methods detailed in Rady (2011) and Abd El-Mageed et al. (2016), respectively. A chlorophyll fluorometer (Handy PEA, Hansatech Instruments Ltd., Kings Lynn, UK) was used to measure the three types of chlorophyll fluorescence (F_v/F_m , F_v/F_0 , and PI), which are useful indicators of photosynthetic efficiency and were determined in accordance with (Clark et al., 2000; Maxwell and Johnson, 2000). For determining the relative amounts of chlorophyll, the SPAD chlorophyll meter (SPAD-502; Minolta, Osaka, Japan) was used. To measure the number of leaves per plant, plant height, number of branches per plant, leaf area, and dry weight per plant, samples of faba bean plants were taken from plots 90 days after sowing (DAS).

2.5. Nutrients status determinations

Faba bean leaves were oven-dried and then wet digested with a mixture of HClO₄ and H₂SO₄ (at 1: 3, v/v, respectively) to determine the concentrations of N, P, K, Ca, and Na. The blue color method of Jackson, (1973) was used to analyses the samples for P spectrophotometrically. As shown by Jackson (1973), the K⁺, Ca²⁺, and Na⁺ contents were assessed via flame photometry (Gallenkamp Co., London, UK.). Kjeldahl digestion (Ningbo Medical Instruments Co., Ningbo, China) was used to determine the N content using the techniques outlined in Jackson (2005) study.

2.6. Yield, and water -use efficiency

Ten sample plants from each plot were selected at the harvesting stage, and these plants were used to calculate the yield components: number of pods per plant, weight of pods per plant (g), and weight of 100 seeds (g). Seeds collected from plants in each plot were used to calculate the seed yield (t ha⁻¹). The following formulas were used to compute water use effceincy (WUE).

$$WUE = (\text{Seeds yield, kg ha}^{-1} / \text{Evapotranspiration, mm})$$

2.7. Soil microbial activity

At 90 DAS, 10 plants were removed randomly from each plot and used to count of nodules number plant⁻¹. Counts of microorganisms and free N₂-fixing bacteria were determined according to (Shoukry, 2016).

2.8. Data analyses

Using ANOVA procedures in the GenStat statistical programme (version 11) (VSN International Ltd, Oxford, UK), the findings were statistically analyzed in accordance with Gomez and Gomez (Gomez and Gomez, 1984)

Table 1. Some initial soil hydro-physical and chemical properties.

Particle size distribution				Soil moisture content at							
Sand	Silt	Clay	Texture class	BD gcm ⁻³	θ_{Fc}	AW	WHP	ECe dS m ⁻¹	pH	OM %	CaCO ₃ %
%					%						
75.75	12.65	11.60	LS	1.58	15.41	9.72	10.52	8.42	7.89	0.88	4.37

LS= loamy sand, BD= bulk density, FC=field capacity, AW= available water, WHP= water holding pores ECe = the average of electrical conductivity, and OM =organic matter.

Table 2. Properties of sulfur-enriched biochar (S-ACB) amendment.

Attribute	Value	Unit
EC	3.62	dS m ⁻¹
BD	0.83	g cm ⁻³
pH	7.10	
MC	20.21	
C	40.62	%
CEC	43.20	cmol+/kg
Macronutrients		
N	1.31	%
P	0.074	
K	2879	
Ca	3425	mg kg ⁻¹
Na	15	
Micronutrients		
Fe	71.00	
Zn	55.00	
Mn	132.00	mg kg ⁻¹
Cu	19.00	

BD is the bulk density, MC is moisture content and CEC is the cation exchange capacity

3. Results

3.1. Soil chemical hydro-physical properties and microbial activity in response to sulfur-enriched biochar soil amendment

The addition of S-ACB₅ or S-ACB₁₀ significantly ($p < 0.05$) changed the primary chemical- hydro-physical characteristics of the soil (Table 3). As compared to S-ACB, adding S-ACB₅ and /or S-ACB₁₀ led to a decline in pH, ECe, BD values by 3.8-5.5%, 14.8-20.5%, and 2.8-6.9%, respectively. In contrast, S-ACB₅ and S-ACB₁₀ -amended soil demonstrated a progressive enhancement in OM by 27.5 and 51.1%, WHP by 27.5 and 67.5%, and θ_{Fc} by 40.0 and 59.4% and AW by 33.6 and 51.6%, respectively, compared to un- amended soil. Furthermore, microbial activity of tested soil was markedly ($p \leq 0.05$) increased due to the application of S-ACB₅ or S-ACB₁₀ (Table 4).

The no. of nodules plant-1, no. of bacteria cell g-1 soil, and N₂ fixer cell g -1 soil for the S-ACB₅ and/or S-ACB₁₀ -amended soil were increased by 91.58 and 136.57%, 1.84×10^6 and 3.43×10^6 , 360 and 44% respectively, as compared to untreated soil (S-ACB).

Table 3. Effect of sulfur-enriched biochar (S-ACB) on soil chemical, and hydro-physical properties during 2020/21 (Season I) and 2021/22 (Season II).

S-ACB (t h ⁻¹)	Soil chemical properties			Soil hydro-physical properties			
	pH	ECe, dS m ⁻¹	OM, %	BD, g cm ⁻³	WHP %	θ _{Fc}	AW
Season I							
S-ACB ₀	7.86a	8.47a	0.87c	1.58a	10.54c	15.41c	9.74c
S-ACB ₅	7.57b	7.01b	1.11b	1.54b	13.25b	20.47b	12.34b
S-ACB ₁₀	7.41c	6.64c	1.33a	1.49c	17.23a	24.12a	14.57a
Season II							
S-ACB ₀	7.77a	8.12a	0.95c	1.60a	10.63c	14.76c	9.41c
S-ACB ₅	7.46b	7.13b	1.21b	1.55b	13.75b	19.35b	13.24b
S-ACB ₁₀	7.36c	6.55c	1.42a	1.47c	18.24a	23.98a	14.46a

ECe=electrical conductivity of soil extract, OM= organic matter, BD= bulk density, WHP= water holding pores, θ_{Fc} = field capacity, and AW= available water.

S-ACB₀= 0 t per hectare of sulfur-enriched biochar, S-ACB₅= 5 t per hectare of sulfur-enriched biochar, and S-ACB₁₀= 10 t per hectare of sulfur-enriched biochar

Table 4. Effect of sulfur-enriched biochar (S-ACB) on soil chemical, hydro-physical properties, and microbial activity during 2020/21 (Season I) and 2021/22 (Season II).

S-ACB (t h ⁻¹)	Microbial activity		
	≠Nodules plant ⁻¹	Number of bacteria cell g ⁻¹ soil	N ₂ fixer cell g ⁻¹ soil
Season I			
S-ACB ₀	7.86a	8.47a	0.87c
S-ACB ₅	7.57b	7.01b	1.11b
S-ACB ₁₀	7.41c	6.64c	1.33a
Season II			
S-ACB ₀	7.77a	8.12a	0.95c
S-ACB ₅	7.46b	7.13b	1.21b
S-ACB ₁₀	7.36c	6.55c	1.42a

S-ACB₀= 0 t per hectare of sulfur-enriched biochar, S-ACB₅= 5 t per hectare of sulfur-enriched biochar, and S-ACB₁₀= 10 t per hectare of sulfur-enriched biochar.

3.2. Effect of sulfur-enriched biochar physio-biochemical attributes under drought stress.

Drought stress (DS) significantly decreased physio-biochemical attributes (i.e., MSI, RWC, SPAD, and F_v/F_m , F_v/F_0 , and PI (Tables 5 and 6). Sulfur-enriched biochar (S-ACB) appeared to reduce irrigation stress's detrimental effects on physio-biochemical characteristics of faba bean plants, particularly when plants received full irrigation requirements. The positive effect of S-ACB on physio-biochemical attributes was more prominent when combined with full irrigation level (I_{100%}). Although maximum values of physio-biochemical attributes were obtained with I_{100%} + S-ACB₅ and/or I_{100%} + S-ACB₁₀ treatments, soil incorporation of S-ACB₅ and/or S-ACB₁₀ to faba bean plants grown under 20% DI (I_{80%} + S-ACB₅ and I_{80%} + S-ACB₁₀) enabled faba bean plants to enhance physio-biochemical attributes greater than or comparable to the plants grown with full watering (I_{100%}) as shown in Tables 5 and 6.

Table 5. Effect of sulfur-enriched biochar on membrane stability index (MSI %), relative water content (RWC %) of faba bean grown under water stress during 2020/21 (Season I) and 2021/22 (Season II).

Treatments	MSI (%)		RWC (%)	
	Season _I	Season _{II}	Season _I	Season _{II}
I _{100%}	51.1b [#]	58.7ab	66.5ab	69.1a
I _{80%}	51.5b	53.7c	63.7bc	62.9c
I _{60%}	29.1c	35.5d	61.5c	61.4c
I _{100%} + S-ACB ₅	60.1a	59.2ab	66.5ab	69.1a
I _{100%} + S-ACB ₁₀	60.4a	61.3a	69.1a	68.3ab
I _{80%} + S-ACB ₅	58.3a	55.8bc	67.0ab	66.3b
I _{80%} + S-ACB ₁₀	57.5a	56.1bc	67.0ab	67.0ab
I _{60%} + S-ACB ₅	53.5b	55.1bc	63.8bc	63.1c
I _{60%} + S-ACB ₁₀	57.4a	57.8abc	66.7ab	66.3b

#Values are means of (n = 9). Mean values in each column followed by a different lower-case-letter are significantly different by Student Newman Keuls test at $P \leq 0.05$. I_{100%} = irrigation with 100% of ETc, I_{80%} = irrigation with 80% of ETc and I_{60%} = irrigation with 60% of ETc. S-ACB₀ = 0 t per hectare of sulfur-enriched biochar, S-ACB₅ = 5 t per hectare of sulfur-enriched biochar, and S-ACB₁₀ = 10 t per hectare of sulfur-enriched biochar.

Table 6. Effect of sulfur-enriched biochar on relative chlorophyll content (SPAD value), chlorophyll *a* fluorescence (*Fv/Fm* and *Fv/F0*), and performance index (PI) of faba bean grown under water stress during 2020/21 (Season I) and 2021/22 (Season II).

Treatments	SPAD		<i>Fv/Fm</i>		<i>Fv/F0</i>		PI	
	Season _I	Season _{II}	Season _I	Season _{II}	Season _I	Season _{II}	Season _I	Season _{II}
I _{100%}	45.8d [#]	43.3b	0.81ad	0.80b	5.40a	5.20a	5.50bcd	5.90b
I _{80%}	41.8e	40.8b	0.79d	0.80b	4.00c	4.20b	3.60e	3.40d
I _{60%}	27.4f	30.0c	0.75e	0.75c	3.10d	3.00c	1.50f	1.80e
I _{100%} + S-ACB ₅	51.7ab	49.3a	0.84abc	0.85a	5.20a	5.50a	6.00abc	7.80a
I _{100%} + S-ACB ₁₀	49.3bc	49.2a	0.85ab	0.85a	5.50a	5.60a	6.60ab	6.50b
I _{80%} + S-ACB ₅	47.8cd	46.5a	0.84abc	0.84a	5.30a	5.20a	5.10cd	3.60d
I _{80%} + S-ACB ₁₀	53.6a	49.3a	0.85a	0.85a	5.50a	5.50a	6.90a	6.80b
I _{60%} + S-ACB ₅	41.4e	40.3b	0.84d	0.80b	4.40ac	4.30b	2.40f	3.10d
I _{60%} + S-ACB ₁₀	45.1d	41.5b	0.80cd	0.84a	5.20ab	5.10a	4.40de	4.90c

#Values are means of (n = 9). Mean values in each column followed by a different lower-case-letter are significantly different by Student Newman Keuls test at $P \leq 0.05$. I_{100%} = irrigation with 100% of ETc, I_{80%} = irrigation with 80% of ETc and I_{60%} = irrigation with 60% of ETc. S-ACB₀ = 0 t per hectare of sulfur-enriched biochar, S-ACB₅ = 5 t per hectare of sulfur-enriched biochar, and S-ACB₁₀ = 10 t per hectare of sulfur-enriched biochar.

3.3. Effect of sulfur-enriched biochar on growth characteristics under drought stress Growth characteristics (i.e., plant height, leaves and branches number, dry matter, and leaves area) as influenced by soil incorporation of sulfur-enriched biochar (S-ACB) under different irrigation regimes are shown in (Tables 7 and 8). Water stress markedly inhibited the growth of faba bean plants as estimated by plant height, leaves number, branches number, dry matter, and leaves area by raising water stress from 20% (I_{80%}) to 40% (I_{60%}). Data

noticeably presented that soil incorporation of S-ACB₅ and/or S-ACB₁₀ to faba bean plants significantly ($P < 0.05$) enhanced different growth characteristics under different irrigation regimes in both seasons relative to the controls. While the highest growth characteristics were noted with I_{100%} + S-ACB₅ or S-ACB₁₀ treatments. Adding 5 and 10 t ha⁻¹ of S-ACB for plants grown under (I_{80%} + S-ACB₅ and/or I_{80%} + S-ACB₁₀) treatments enhanced growth characteristics to a level equal to or even surpassing those of plants cultivated in full irrigation (I_{100%} treatment), as showed in (Tables 7 and 8).

Table 7. Effect of sulfur-enriched biochar on plant height, leaves number, and branches number of faba bean grown under water stress during 2020/21 (Season I) and 2021/22 (Season II).

Treatments	Plant height (cm)		Leaves No.		Branches No.	
	Season _I	Season _{II}	Season _I	Season _{II}	Season _I	Season _{II}
I _{100%}	106.7bc [#]	101.7cd	20.0b	22.0c	4.8bc	5.3ab
I _{80%}	100.3cd	97.3cd	18.0bc	19.7d	4.3bc	5.8ab
I _{60%}	79.0e	88.7e	14.3d	15.7f	3.0d	2.8c
I _{100%} + S-ACB ₅	111.7ab	114.7a	24.0a	24.7ab	5.7ab	6.0ab
I _{100%} + S-ACB ₁₀	116.7a	119.7a	24.7a	25.7a	6.3a	6.7a
I _{80%} + S-ACB ₅	101.3cd	103.6c	19.3bc	20.3d	4.7bc	6.0ab
I _{80%} + S-ACB ₁₀	107.0bc	109.3b	22.7a	23.7b	5.3ab	6.3ab
I _{60%} + S-ACB ₅	94.0d	96.0d	18.3bc	18.0e	3.7cd	5.0b
I _{60%} + S-ACB ₁₀	99.3cd	101.3cd	17.0c	19.3d	4.3bc	3.7c

[#]Values are means of (n = 9). Mean values in each column followed by a different lower-case-letter are significantly different by Student Newman Keuls test at $P \leq 0.05$. I_{100%} = irrigation with 100% of ETc, I_{80%} = irrigation with 80% of ETc and I_{60%} = irrigation with 60% of ETc. S-ACB0= 0 t per hectare of sulfur-enriched biochar, S-ACB5= 5 t per hectare of sulfur-enriched biochar, and S-ACB10= 10 t per hectare of sulfur-enriched biochar.

Table 8. Effect of sulfur-enriched biochar on dry matter, and leaves area of faba bean grown under water stress during 2020/21 (Season I) and 2021/22 (Season II).

Treatments	Dry matter (g)		Leaves area (dm ²)	
	Season _I	Season _{II}	Season _I	Season _{II}
I _{100%}	87.2c [#]	88.4c	246.0c	249.4c
I _{80%}	61.4d	58.4d	173.2d	164.7d
I _{60%}	56.1d	51.9d	158.3d	146.5d
I _{100%} + S-ACB ₅	114.0b	156.4a	437.7a	324.9b
I _{100%} + S-ACB ₁₀	155.2a	115.2b	321.5b	441.1a
I _{80%} + S-ACB ₅	85.1c	82.1c	329.0b	231.5c
I _{80%} + S-ACB ₁₀	116.7b	113.7b	239.9c	320.6b
I _{60%} + S-ACB ₅	82.9c	78.7c	264.4c	221.9c
I _{60%} + S-ACB ₁₀	93.8c	89.6c	233.8c	252.6c

[#]Values are means of (n = 9). Mean values in each column followed by a different lower-case-letter are significantly different by Student Newman Keuls test at $P \leq 0.05$. I_{100%} = irrigation with 100% of ETc, I_{80%} = irrigation with 80% of ETc and I_{60%} = irrigation with 60% of ETc. S-ACB0= 0 t per hectare of sulfur-enriched biochar, S-ACB5= 5 t per hectare of sulfur-enriched biochar, and S-ACB10= 10 t per hectare of sulfur-enriched biochar.

3.4. Effect of sulfur-enriched biochar on yield components, and water use efficiency (WUE) under drought stress.

Results presented in (Table 9) showed that, yield components such as; pods number, 100 -seed weight, and seed yield of plants grown under water stress ($I_{80\%}$ and $I_{60\%}$) were decreased significantly in both seasons compared to those grown under $I_{100\%}$ treatment. As average for both seasons, pods number, 100 seed weight and seed yield were decreased by 22.3, 16.2 and 23.3% when plants were subjected to severe water stress 40% ($I_{60\%}$) as compared by 20% ($I_{80\%}$). Nevertheless, soil incorporation of S-ACB significantly enhanced yield components. The data presented clear evidence that soil incorporation of S-ACB₅ or S-ACB₁₀ significantly ($P < 0.05$) enhanced yield and its various components in faba bean plants across different irrigation regimes in both seasons in contrast to the controls. The effect of S-ACB₁₀ addition was more pronounced under different irrigation regimes when compared with S-ACB₀. The addition of S-ACB at 10t ha⁻¹ increased pods number, 100 seed weight and seed yield by 50.7, 19.2 and 36.8%, respectively, as compared S-ACB₀ as average for two seasons. While the highest yield was achieved with the $I_{100\%} + S-ACB_5$ and/or $I_{100\%} + S-ACB_{10}$ treatments, soil incorporation of S-ACB₅ or S-ACB₁₀ into faba bean plants grown under 20% DI ($I_{80\%} + S-ACB_5$ and/or $I_{80\%} + S-ACB_{10}$) allowed these plants to significantly enhance their yield, either matching or surpassing the yield of plants cultivated under optimal irrigation ($I_{100\%}$ treatment), as illustrated in Table 9. Water use efficiency (WUE) was significantly impacted by water stress. Compared to $I_{60\%}$ and $I_{100\%}$, the average value of WUE under $I_{80\%}$ was greater. However, soil integration of S-ACB₅ had a significant ($p < 0.05$) impact on WUE. The superior WUE values was obtained under $I_{60\%} + S-ACB_5$ and/or $I_{60\%} + S-ACB_{10}$ (Table 9 and Figure 1). Figure 1 show the curvilinear polynomials of second order were used to characterise the relationship between WUE and IWR, and seed yield during the two sowing seasons under study. The following formulas can be used to illustrate these relationships:

$$Y = 1E-07 \times IWR^2 + 0.0035 \times IWR + 0.32 \quad R^2 = 0.73 \quad (2020/21)$$

$$Y = -4E-07 \times IWR^2 + 0.0077 \times IWR - 7.29 \quad R^2 = 0.72 \quad (2021/22)$$

$$WUE = 7E-08 \times IWR^2 - 0.0006 \times IWR + 5.34 \quad R^2 = 0.58 \quad (2020/21)$$

$$WUE = -1E-07 \times IWR^2 + 0.0009 \times IWR + 2.69 \quad R^2 = 0.51 \quad (2021/22).$$

Table 9. Effect of sulfur-enriched biochar on pods no, 100 seed weight, seed yield, and water use efficiency (WUE) of faba bean grown under water stress during 2020/21 (Season_I) and 2021/22 (Season_{II}).

Treatments	Pods No.		100 seed weight (g)		Seed yield (t ha ⁻¹)		WUE (m ³ ha ⁻¹)	
	Season _I	Season _{II}	Season _I	Season _{II}	Season _I	Season _{II}	Season _I	Season _{II}
I_{100%}	29.0d [#]	26.0d	78.3bc	78.1d	4.0c	3.9c	0.96c	0.88c
I_{80%}	24.7e	22.3e	72.0c	74.2d	3.8c	3.5c	1.1b	0.99b
I_{60%}	22.0e	19.0f	58.2e	59.4f	2.3d	2.6d	0.92c	1.0b
I_{100%} + S-ACB₅	29.0d	30.3c	87.0a	85.8ab	5.1a	4.6b	1.2b	1.1b
I_{100%} + S-ACB₁₀	39.0abc	36.0b	91.7a	90.5a	4.9ab	5.8a	1.2b	1.3a
I_{80%} + S-ACB₅	43.0a	40.0a	77.3bc	79.4cd	3.9c	3.9c	1.2b	1.1b
I_{80%} + S-ACB₁₀	39.0b	36.0b	82.0b	84.1bc	4.7b	4.6b	1.4a	1.3a
I_{60%} + S-ACB₅	31.3d	28.3cd	66.1d	67.3e	3.6c	3.8c	1.4a	1.4a
I_{60%} + S-ACB₁₀	35.0b	32.0c	75.7bc	77.0d	3.7c	3.8c	1.5a	1.4a

#Values are means. Mean values in each column followed by a different lower-case-letter are significantly different by Student Newman Keuls test at $P \leq 0.05$. $I_{100\%}$ = irrigation with 1100% of ETc, $I_{80\%}$ = irrigation with 80% of ETc and $I_{60\%}$ = irrigation with 60% of ETc. S-ACB₀ = 0 t per hectare of sulfur-enriched biochar, S-ACB₅ = 5 t per hectare of sulfur-enriched biochar, and S-ACB₁₀ = 10 t per hectare of sulfur-enriched biochar.

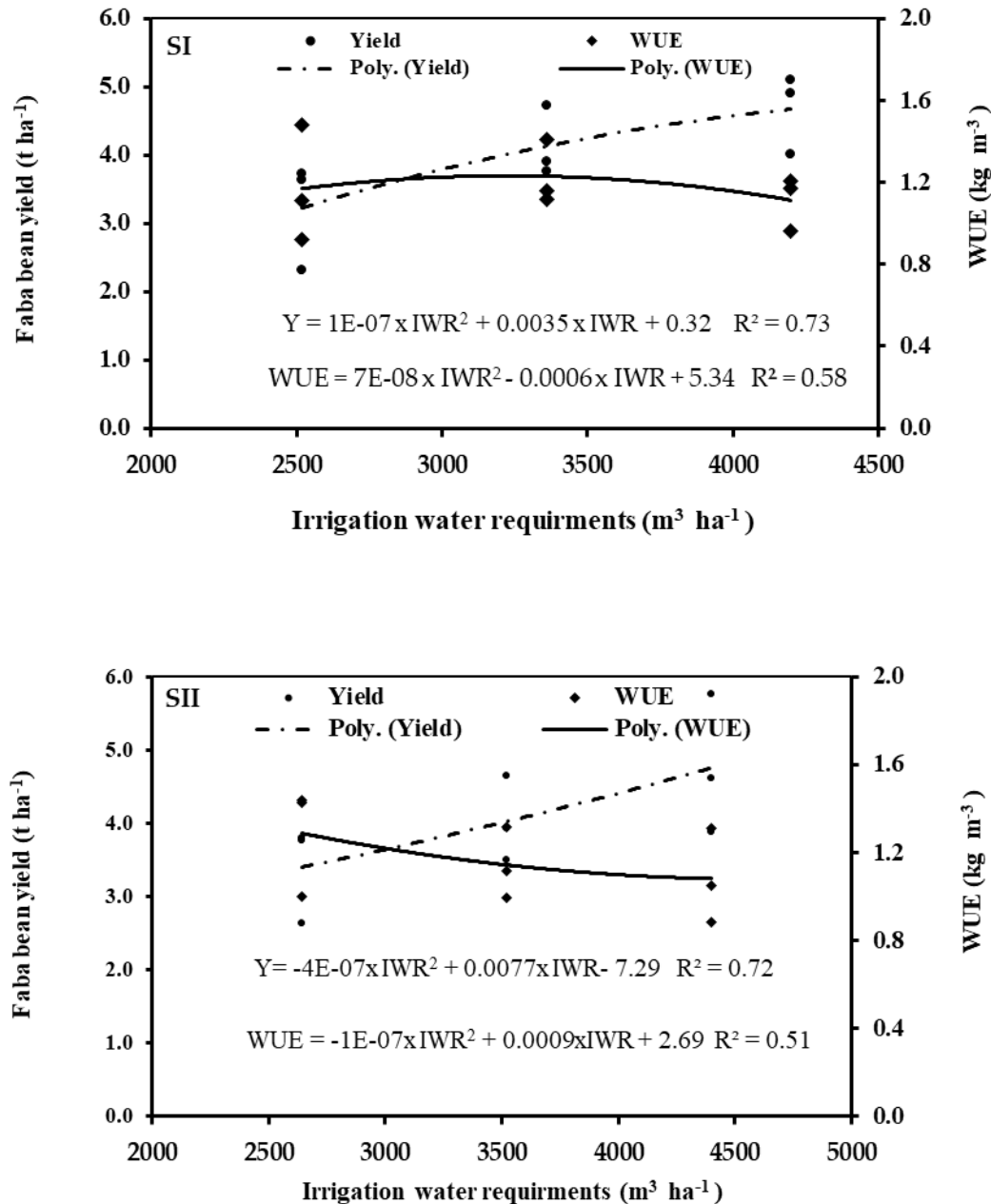


Fig. 1. Regression analysis between irrigation requirements (m³ ha⁻¹) seed yield (t ha⁻¹) and irrigation use efficiency (kg m⁻³) of sugar beet for SI (2020/21) and SII 2021/22.

3.5. Impact of sulfur-enriched biochar under water stress on the nutrients (N, P, K Ca, and Na⁺/K⁺ ratio) in faba bean leaves

Nutrients acquisition (i.e., N, P, K, Ca, Na, and Na⁺/K⁺ ratio) in response to soil incorporation of sulfur-enriched biochar (S-ACB) under different irrigation regimes are shown in Tables 10 and 11. Under water stress, the nutritional value of faba bean leaves has declined, with the exception of Na in both seasons compared to those grown under full irrigation conditions (I_{100%} treatment). However, faba bean plants grown under water stress (I_{80%} and I_{60%} treatment) remarkably ($p \leq 0.05$) exhibited higher Na⁺ in both seasons compared to those grown under full irrigation conditions (I_{100%} treatment). Soil incorporation of S-ACB₅ and/or S-ACB₁₀ to faba bean plants significantly ($P < 0.05$) showed a raise in N, P, K, Ca, and Na⁺/K⁺ ratio; however, simultaneously lowered Na⁺ content compared to the controls. The influence of adding S-ACB₁₀ was more prominent in both season across various irrigation regimes compared to the effect of S-ACB₅.

Table 10. Effect of sulfur-enriched biochar on nutrients status (N, P, K) of faba bean grown under water stress during 2020/21 (Season_I) and 2021/22 (Season_{II}).

Treatments	N		P		K	
	(g kg ⁻¹ DW)					
	Season _I	Season _{II}	Season _I	Season _{II}	Season _I	Season _{II}
I _{100%}	28.0e [#]	28.3e	3.2d	3.4c	27.9b	28.0b
I _{80%}	26.3f	27.0e	3.4cd	3.5c	23.4c	25.8c
I _{60%}	23.7g	23.7f	2.1e	2.3d	22.7c	21.9d
I _{100%} + S-ACB ₅	35.6ab	35.6ab	4.0b	4.1b	31.2a	26.9bc
I _{100%} + S-ACB ₁₀	36.8a	37.0a	4.6a	4.9a	27.6b	32.6a
I _{80%} + S-ACB ₅	31.1d	30.6d	3.9b	4.0b	27.9b	26.1bc
I _{80%} + S-ACB ₁₀	32.9c	33.3c	4.7a	4.2b	28.2b	28.7b
I _{60%} + S-ACB ₅	34.6bc	36.4a	3.1d	3.4c	25.8bc	25.4c
I _{60%} + S-ACB ₁₀	33.0c	34.2bc	3.8bc	3.7bc	25.3bc	25.7c

#Values are means. Mean values in each column followed by a different lower-case-letter are significantly different by Student Newman Keuls test at $P \leq 0.05$. I_{100%} = irrigation with 1100% of ETc, I_{80%} = irrigation with 80% of ETc and I_{60%} = irrigation with 60% of ETc. S-ACB₀ = 0 t per hectare of sulfur-enriched biochar, S-ACB₅ = 5 t per hectare of sulfur-enriched biochar, and S-ACB₁₀ = 10 t per hectare of sulfur-enriched biochar.

Table 11. Effect of sulfur-enriched biochar on nutrients status (Ca, Na, and Na⁺/K⁺ ratio) of faba bean grown under water stress during 2020/21 (Season_I) and 2021/22 (Season_{II}).

Treatments	Ca		Na		Na ⁺ /K ⁺	
	(g kg ⁻¹ DW)					
	Season _I	Season _{II}	Season _I	Season _{II}	Season _I	Season _{II}
I _{100%}	5.6d [#]	5.2d	9.3d	10.3e	0.36cd	0.39c
I _{80%}	5.2d	5.3d	12.1c	13.8c	0.52b	0.59b
I _{60%}	4.3e	4.3e	17.2a	17.9a	0.76a	0.79a
I _{100%} + S-ACB ₅	8.1a	7.6a	8.5d	8.3g	0.31d	0.29d
I _{100%} + S-ACB ₁₀	7.9a	8.2a	6.9e	7.0h	0.22e	0.22e
I _{80%} + S-ACB ₅	7.9a	7.9a	11.6c	11.9d	0.42c	0.43c
I _{80%} + S-ACB ₁₀	6.8b	7.0b	9.7d	9.3f	0.35cd	0.33d
I _{60%} + S-ACB ₅	5.9bd	5.9c	14.5b	14.9b	0.56b	0.58b
I _{60%} + S-ACB ₁₀	6.8bc	7.6a	12.9c	13.1c	0.51b	0.52b

#Values are means. Mean values in each column followed by a different lower-case-letter are significantly different by Student Newman Keuls test at $P \leq 0.05$. I_{100%} = irrigation with 1100% of ETc, I_{80%} = irrigation with 80% of ETc and I_{60%} = irrigation with 60% of ETc. S-ACB₀ = 0 t per hectare of sulfur-enriched biochar, S-ACB₅ = 5 t per hectare of sulfur-enriched biochar, and S-ACB₁₀ = 10 t per hectare of sulfur-enriched biochar.

4. Discussion

Drought stress stands as a pivotal abiotic stress that exerts a significant effect on plant growth, productivity, and various physiological processes (Loutfy et al., 2012; Rady et al., 2020; Semida et al., 2017; Xiao et al., 2009). Here, we demonstrated how applying S-ACB improved the soil's chemical, hydrological, and physical characteristics. Further research has shown that the addition of S-ACB to soils has improved their physical,

chemical, and biological functionalities (Abd El-Mageed et al., 2021; Mon et al., 2024; Zhang et al., 2019). Consequently, adding S-ACB to the soil improves its physical and chemical qualities (BD, WHP, UP, TP, FC, and AW) as well as its pH, OM, and NPK levels.

The results of our study indicate that S-ACB₅ can play a crucial role in water management strategies to improve the growth, development, and production of faba bean plants cultivated in saline soil during periods of water scarcity. When faba bean plants received 100% of ET_c, the addition of S-ACB₅ led to an increase in seed yield by 35.2% as compared with S-ACB₀. These results are consistent with those of (El-Ramady et al., 2024; Asai et al., 2009). Their findings indicated that adding biochar could also enhance a number of physical - chemical characteristics of the soil, including bulk density, OM%, TP, WHP, and NPK content. Results show that adding S-ACB to saline soil enhanced faba bean productivity through improving biological N₂ fixation (Table 3 and 4). Mia et al. (2014) reported similar results. The increments of soil microbial activity may be ascribed to the affirmative influence of S-ACB as an OM on microbial counts by supplying soil microbes with valuable substrates. Biochar addition to soil improved microbial activity and noticeable upgrading in plant growth (Rondon et al., 2007). In this concern, Ding et al. (2016) obviously that the application of biochar clearly affected microbial soil populations, which may have worthwhile capacities in enhancing soil fertility. Moreover, according to their findings, the microbial growth rates were positively improved by the addition of biochar. Plant production and growth are decreased by deficit irrigation (DI). However, if DI stress persists for an extended period or intensifies, it can lead to irreversible deterioration and eventual plant demise. This stress induced by DI in our study negatively affected plant water status (as seen in RWC and MSI), leading to desiccation, which had detrimental effects on osmotic conditions and overall plant performance (Abd El-Mageed et al., 2021). The adverse impact observed on leaf tissue under deficit irrigation stress may be ascribed to the elevated transpiration rate surpassing the rate of water uptake. DI not only diminishes plant's water balance (as demonstrated in Tables 5, but also disturbs photosynthetic efficiency and nutrients acquisition (as observed in Tables 6, 10 and 11). These adverse effects consequently lead to a decrease in yield components, as evidenced by a reduction in pod numbers, 100-seed weight, and overall seed yield (as indicated in Table 9).

Mixing of biochar into agricultural soils has received escalating interest over the past decade, and the enhancement of biochar through chemical modifications presents a promising approach to mitigate the quantity of biochar needed for enhancing soil productivity (Abd El-Mageed et al., 2021; Semida et al., 2020, 2019). The application of sulfur-enriched biochar (S-ACB) as a cost-effective fertilizer holds significant promise for potentially improving plant growth and morpho-physiological characteristics and consequently enhancing yield of faba bean plants under water scarcity. The response patterns of the faba bean plants, as well as the increased growth (i.e., plant height, leaves no., branches no., dry matter and leaves area) and productivity (pods no., 100 seed weight and seed yield) that were found in this study with the addition of sulfur-enriched biochar (S-ACB) to the soil, were consistent with our hypothesis. They stated that higher amounts of acidified biochar treatment were associated with an increase in F_v/F_m , F_v/F_0 , and PI by 7.3, 29.4 and 66.3%, respectively. Relative water content (RWC) is widely regarded as a significant indicator of dehydration tolerance, while the Membrane Stability Index (MSI) assesses the integrity of cell membranes (Semida et al., 2014). In the presence of deficit irrigation (DI), a decrease in both RWC and MSI was evident, signifying the detrimental consequences of water limitation on *Vicia faba* plants. The adverse impact of I₆₀ on both RWC and MSI implies that at the highest level of deficit irrigation tested, *Vicia faba* plants experienced disintegration of the cell membrane. In alignment with this observation, a reduction in photosynthetic activity was observed due to the decreased leaf RWC induced by water stress (Abdou et al., 2021).

The outcomes of the current study underscore that soil incorporation of S-ACB significantly enhanced plant water status as exemplified by the notable improvements in RWC, MSI, and WUE (Tables 5 and 9 and Figure 1). Improving plant water status led to increased production components as shown by number of pods, weight of 100 seeds and seed yield in plants treated with S-ACB under different irrigation levels (Table 9). Consistent with this (Abd El-Mageed et al., 2021), further noted improved plant water status on drought-stressed *Vicia faba* plants when using acidified biochar. A dependable method for determining how differently photosystem II (PSII) functions in response to different abiotic stresses is chlorophyll-a fluorescence (Belal et al., 2023; Habibi, 2012; Melo et al., 2017; Semida et al., 2017). The results of studies conducted by (Abdelkhalik et al., 2023; Pieters and El Souki, 2005; Semida et al., 2023) suggest that a decrease in PSII's photochemical efficiency could be the cause of reduced photosynthetic activity.

Our findings indicated declines in photosynthetic efficiency (as seen in F_v/F_m , F_v/F_0 , and PI) alongside a decrease in SPAD value under the conditions of deficit irrigation stress (Table 6). These observations are most likely contributed to the decrease in MSI and RWC, both of them are essential for sustaining photosynthesis. Numerous studies have concluded that disruptions in water supply result in a reduction of water content in the absorptive tissue, consequently causing a decline in photosynthetic activity (Abd El-Mageed, et al., 2017; Habibi,

2012; Semida et al., 2020). On the contrary, soil incorporation of sulfur-enriched biochar (S-ACB) was found to enhance chlorophyll *a* fluorescence and increase SPAD value of *Vicia faba* plants subjected to deficit irrigation stress (Table 6). These observed responses align with prior research conducted by (Abd El-Mageed et al., 2021). They reported that higher amounts of acidified biochar treatment led to an increase in chlorophyll *a* fluorescence (i.e., F_v/F_m , F_v/F_0 and PI). In contrast, (Akhtar and Liu, 2015) found that applying biochar to the soil had no appreciable effect on the photochemical efficiency of photosystem II (F_v/F_m) in wheat and maize crops.

Both the total amount of dry matter accumulated and the absorption of nutrients are impacted by irrigation stress. According to Liang et al. (2018), the final concentrations of nutrients in plant tissues during periods of water deficit will depend on how much nutrient absorption declines in comparison to the decline in dry matter accumulation. Water availability is a critical component that regulates nutrient absorption; drought conditions can impede plant growth by reducing the uptake, transportation, and distribution of vital nutrients (Sardans and Peñuelas, 2021; El-Sherpiny and Kany, 2023). In addition to lowering the nutrient supply through mineralization, this kind of stress also lowers the diffusion and mass movement of nutrients within the soil, which prevents the absorption of nutrients (Sanaullah et al., 2012). In line with findings from other (Abd El-Mageed et al., 2020; Zhao et al., 2015) the obtained data demonstrated a significant reduction in the uptake of needed nutrients (i.e., N, P, K, Ca, and Na^+/K^+ ratio) by *Vicia faba* plants under drought stress conditions (Table 10 and 11). This could elucidate their lower growth rate, resulting in decreased biomass accumulation (Table 8) and yield components as shown by pods number, 100 seed weight, seed yield (Table 9) owing to the adverse effects of scarcity on their ability to assimilate.

He and Dijkstra (2014) have suggested that drought stress has an adverse impact on plant nutrition since the decline in nutrient uptake surpasses the decline in plant development. Plant tolerance to water shortage is mostly dependent on nutrient absorption. According to Liang et al. (2018), drought stress prevents nutrients from moving freely in the soil and from being absorbed by plants. In our research, the addition of sulfur-enriched biochar (S-ACB) into the soil under DI was observed to enhance leaf content of (N, P, K, Ca, and Na^+/K^+ ratio) when compared to the corresponding control group. Conversely, the presence of S-ACB resulted in a significant reduction in leaf Na^+ concentration and the Na^+/K^+ ratio. Our findings align with those reported by Major et al. (2010), who observed that the addition of biochar led to an increase in the uptake of potassium (K), magnesium (Mg), and calcium (Ca) in maize. Similarly, Abd El-Mageed et al., (2020) observed that residual acidified biochar utilization enhanced the leaf content of N, P, K, Ca, and Na^+/K^+ ratio and reduced Na^+ uptake under heavy metal stress in relation to the relevant control. The decline in soil pH and electrical conductivity (ECe), as indicated in Table 3, likely contributed to the improved nutrient uptake associated with the incorporation of sulfur-enriched biochar (S-ACB). Particularly in the root zone, the lower pH of the soil facilitated the solubilization of nutrients and the increased mineralization of organic matter. Thus, more vital elements may have been bioavailable in the soil, which would have made it easier for *Vicia faba* plants to absorb and assimilate those nutrients. The increased uptake of nitrogen (N) and phosphorus (P) is most likely the consequence of the improvements in the chemical qualities of the soil, such as the pH and total nitrogen, available P_2O_5 content, CEC, and base saturation. Reliable with this, Abd El-Mageed et al. (2020); Elbasiouny and Kelly (2023) reported that the application of residual acidified biochar resulted in increased leaf levels of (N, P, K, Ca, and the Na^+/K^+ ratio), while simultaneously reducing sodium (Na^+) uptake when subjected to drought stress.

5. Conclusions

Drought stress imposed significant challenges to plant growth and physiological performance, leading to reduced water status, impaired photosynthesis, and compromised nutrient acquisition. However, the incorporation of S-ACB into the soil emerged as a potent mitigating factor against the negative consequences of drought stress. S-ACB played a pivotal role in improving plant water status, enhancing photosynthetic efficiency, and facilitating nutrient uptake and then increased yield components, and water use efficiency. Moreover, the positive effects of S-ACB on leaf nutrient content and Na^+/K^+ ratios underscore its potential in maintaining nutrient status under drought stress conditions. Soil pH reduction, coupled with enhanced nutrient availability due to S-ACB incorporation, likely contributed to these positive outcomes. Overall, the findings of this study emphasize the valuable role of sulfur-enriched biochar as a soil amendment in enhancing the resilience of *Vicia faba* plants to drought stress. These results provide important insights into the development of strategies aimed at mitigating the adverse effects of drought and improving crop productivity while conserving water resources. Further research is warranted to explore the long-term impacts and mechanisms underlying S-ACB -mediated drought tolerance in various crops and environmental conditions.

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