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Biochar alleviates single and combined effects of salinity and drought stress in faba bean plants

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Abstract

This study aimed to evaluate the impact of four biochar concentrations (0, 2, 5, and 8%) on single and interactive effects of salinity and drought stresses on the morphological, physiological, and photosynthetic parameters of faba bean plants. PCA analysis showed that plants displayed different behavior under non-stressed and stressed conditions. The most discriminating quantitative characters were related to plant biomass production and photosynthesis, especially shoot dry mass, root dry mass, plant fresh mass, internal CO_2 concentration, net CO_2 assimilation rate, and relative water content. The obtained results confirm the biochar's important role in promoting plant growth under normal or stressed conditions. Thus, a better understanding of the impact of biochar on plant growth under drought and salinity stresses will be beneficial for sustainable agriculture.

Keywords: biochar; drought; faba bean; gas exchange; growth analysis; salinity.

Introduction

Among various abiotic stresses, soil salinization and drought pose a critical constraint to the future sustainability of global crop production (FAO 2021, Münchinger *et al.* 2023). It has been reported that both stresses could restrain crop yield (Wang *et al.* 2017, Mega *et al.* 2019, Zhang *et al.* 2020, Nefissi Ouertani *et al.* 2022a, Bagues *et al.* 2024). Drought stress may affect the physiological properties of plant leaves, such as reducing transpiration rate and stomatal conductance, thus limiting agricultural productivity (Hashem *et al.* 2019). Water-use efficiency is an important parameter indicating plant resistance under drought conditions (Edwards *et al.* 2012). Plant roots play a crucial role in the shortage of water. Indeed, plants develop deeper roots capable to assimilate more water

Highlights

- Biochar effect on faba beans grown under different stress conditions was assessed
- It improves leaf photosynthetic and biomass parameters under stress
- It has a positive effect on alleviating harmfull effect of salinity and drought

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Abbreviations: B – biochar; C – control; C_i – intercellular CO_2 concentration; D – drought; E – transpiration rate; EL – electrolyte leakage; g_s – stomatal conductance; LN – leaf number; N – normal conditions; PCA – principal component analysis; PDM – plant dry mass; PFM – plant fresh mass; PL – plant length; P_N – net CO_2 assimilation rate; RDM – root dry mass; RFM – root fresh mass; RL – root length; RWC – relative water content; S – salinity; SDM – shoot dry mass; SFM – shoot fresh mass; SL – shoot length; SV – SPAD value; VF – *Vicia faba*; WHC – water-holding capacity; WUE – water-use efficiency.

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and nutrients from deeper soil (Hammer *et al.* 2009). Furthermore, drought might impact plant phenology (*i.e.*, advancing or delaying flowering time) (Farooq *et al.* 2017).

The weathering of saline bedrock and sea level fluctuations along the coast cause primary soil salinization, which is unavoidable. However, secondary salinization, mostly caused by human activities, such as irrigation with salty water, excessive use of mineral fertilizers, and other intense monocultures, can be avoided by implementing sustainable and ecologically friendly farming practices (Tedeschi 2020). It affected around 6% of the total land in the world (Amini *et al.* 2016). Salinity causes decreasing in plant growth and crop yield (Munns and Gilliham 2015, Rajhi *et al.* 2023a) by imparing the opening of stomata, osmotic adjustment, growth rate, root hydraulic conductance, photosynthetic pigments, and nutritional balance (James *et al.* 2011).

Legumes, the second largest plants family, are related to the family of *Fabaceae*, also named *Leguminosae* (Kouris-Blazos and Belski 2016). *Fabaceae* is a big family, containing around 18,000 species, including herbs, trees, climbers, and shrubs. However, a restricted number of species is consumed by humans (Rajhi *et al.* 2022a). Faba beans (*Vicia faba* L.) are considered one of the most important legumes due to their role in soil fertility, human diet, animal nutrition, industry uses, and food chain value (Cazzato *et al.* 2012, Rajhi *et al.* 2022b,c). Faba bean grains contain 28–30% of proteins, and 51–68% of carbohydrates of dry matter (Burbano *et al.* 1995). These consist of vitamins, carotenoids, and essential minerals, such as potassium, magnesium, zinc, iron, selenium, and copper (Labba *et al.* 2021). Additionally, they are a considerable source of antioxidants and have a lipid-lowering effect (Ray and Georges 2010).

Biochar, a stable C-rich byproduct obtained from biomass, is an organic soil amendment applied to low-fertility soils to ameliorate their quality and crop yield (Wei *et al.* 2021). Biochar, a solid residue, is formed *via* a process known as pyrolysis in which different natural biomass (feedstock) including manure, leaves, or wood are thermally treated in the absence of oxygen with oil and gas as co-products (Kameyama *et al.* 2016). Pyrolysis, a thermochemical conversion technology, can be classified into slow and fast pyrolysis (Mohan *et al.* 2006). The first type is distinguished by a slow heating rate under lower temperature conditions (300–400°C). The second is characterized by its high heating rate under high-temperature conditions (500–850°C) (Mohan *et al.* 2006). Biochar's physico-chemical characteristics and structure depend essentially on the type of biomass used and pyrolysis conditions (Gabhi *et al.* 2020). Recently, biochar has attracted the attention of researchers due to its potential to produce farm-based renewable energy in an eco-friendly way with a low-cost process (Hussain *et al.* 2017). Furthermore, biochar can increase the soil pH, improve the ability to absorb moisture, captivate more beneficial microbes, ameliorate the exchange cation ability, maintain the nutrients in the soil, decrease soil density, augment soil aeration, and modify the soil structure *via* the changes in its physico-chemical properties (Lehmann 2007, Jeffery *et al.* 2011, Blanco-Canqui 2017). The impact of biochar on mitigating the harmful effect of salinity and drought on plants was well studied (Hafeez *et al.* 2017, Rezaie *et al.* 2019). However, there is limited information about the role of biochar in alleviating the combined effect of salinity and drought stresses. Therefore, this study aimed to (*1*) evaluate the effect of different concentrations of biochar on physiological, photosynthetic, and biochemical parameters of local faba bean cultivar grown under salinity, drought, and combined salinity and drought stresses and (*2*) to identify the most contributing traits to the variations among investigated parameters.

Materials and methods

Plant materials: Local faba bean seeds (*Vicia faba* L.) were considered in this study. Similar-sized seeds, without any physical damage, were chosen. Legume seeds were stored at 4°C in an opaque aluminum bag until use.

Growth conditions: All experiments were performed in the Experimental Station of the Biotechnology Center of Borj Cedria in Tunisia, under controlled greenhouse conditions; temperature was set at 23°C, photoperiod was 16/8 h day/night, relative humidity was between 55 and 65%, and PAR was 270 μmol(photon) m^{-2} s^{-1}. Seeds were surface disinfected in $HgCl_2$ (0.1%) for 1 min and then rinsed perfectly using sterilized distilled water. These were then sowed in autoclaved perlite moistened with water to germinate at room temperature (20°C) in the dark. Ten days later, germinated seeds were transferred to plastic pots containing soil amended or not with biochar.

Biochar production: Biochar was prepared from forestry wood under aerobic conditions (10 h at 450°C) with the following characteristics (Bagues *et al.* 2024). The biochar was provided by the *Biofire Society* (Tunisia).

Attributes	Units	Contents
Electrical conductivity (EC)	dS cm^{-1}	1.3
pH	-	7.63
Organic matter (OM)	%	81.2
Cation exchangeable capacity (CEC)	meq 100 g^{-1}	54.6
Phosphorus (P)	ppm	325.5
Sodium (Na)	mg kg^{-1}	27.9
Potassium (K)	mg kg^{-1}	58.7
Calcium (Ca)	mg kg^{-1}	1,192.1
Magnesium (Mg)	mg kg^{-1}	9.5
Zinc (Zn)	mg kg^{-1}	0.4
Iron (Fe)	mg kg^{-1}	16.1
Manganese (Mn)	mg kg^{-1}	2.5

Soil preparation and treatments: For the experiment, the soil was composed of 65% sand, 14% silt, and 21% clay. Ten-day-old seedlings were transferred to plastic pots containing different biochar concentrations: 0% (C), 2% (B2), 5% (B5), and 8% (B8). Pots lacking biochar served as controls. Before filling the pots, the soil was well mixed with the corresponding concentration of biochar. Nitrogen fertilizer was also added to the soil at rates of 80 mg kg^{-1} (Yang *et al*. 2020). Then, the water-holding capacity (WHC) was determined for each soil. In pots containing 2 kg of soil mixture, two faba bean seedlings were planted, and then irrigated every other day with tap water. Then, plants were divided into four groups. Within the first group, pots containing different biochar concentrations (0, 2, 5, and 8%) were irrigated with tap water (non-stressed conditions). Salinity treatment was applied to pots containing 0, 2, 5, and 8% of biochar in the second group. Salt stress was gradually applied by increments of 25 mM NaCl a day until it reached 100 mM. To create drought stress, a high level of water shortage (20–25% WHC) was applied to the third group's pots containing 0, 2, 5, and 8% of biochar. Soil moisture was controlled with an electronic balance. Every 1 or 2 d, experiment pots were weighted and distilled water was used to replenish water loss if necessary. For the fourth group, combined stress was applied. Seedlings planted in different concentrations of biochar (0, 2, 5, and 8%) were irrigated with saline water (100 mM NaCl) under high drought conditions (20–25% of pot WHC). Similarly, as above, soil moisture was controlled gravimetrically with an electronic balance, and saline water (100 mM NaCl) was used to replenish water loss if necessary. All treatments were maintained continuously until the final harvest (2 months later). Three independent sets of experiments were performed with three plants for each replication (n = 9 plants for each content of biochar and per treatment).

Morphological measurements: Three morphological parameters were evaluated on faba bean cultivar: root length (RL), shoot length (SL), and leaf number (LN). The SL and RL were determined by measuring the distance between the crown and the leaf tip [cm] and the crown and the root tip [cm], respectively. The number of leaves was counted.

Relative water content: At harvest time, leaves were directly weighted to get the fresh mass designed as FM. To obtain the turgid mass (TM), leaves were weighed after incubation in distilled water for 24 h. Then the saturated leaves were dried for 72 h at 70°C and the dry mass was determined (DM). The RWC was calculated using the following formula (Barrs and Weatherley 1962):

RWC [%] = [(FM/DM)/(TM/DM)] × 100.

Plant biomass: The roots and shoots were collected separately from each plant. All parameters in this study, root fresh mass (RFM), shoot fresh mass (SFM), and plant fresh mass (PFM), were measured on the day of the harvest. The root dry mass (RDM), shoot dry mass (SDM), and plant dry mass (PDM) were assessed after incubation of the samples at 70°C until constant masses.

Photosynthetic gas-exchange parameters: Stomatal conductance to water vapor (g_s), net CO_2 assimilation rate (P_N), transpiration rate (E), and intercellular CO_2 concentration (C_i) were determined using a portable *LCpro T* gas analyzer (*ADC Bioscientific Ltd.,* Hoddesdon, United Kingdom). PAR was about 1,000 μmol(photon) m^{-2} s^{-1} during measurement. The leaf chamber temperature, the leaf surface temperature, and the ambient CO_2 concentration were 31 ± 1°C, 33 ± 1°C, and 517 ± 5 μmol mol^{-1}, respectively. The WUE was measured as the ratio between P_N and E.

Electrolyte leakage: Fragments of 100 mg of the middle part of freshly cut leaves were floated on 10 ml of ultrapure water in assay tubes. First, electrical conductivity (EC1) of the solution was measured after incubation of the tubes in a water bath at 32°C for 2 h using a conductivity meter *Metrohm 712* (*Metrohm*, Herisau, Switzerland). Then the tubes were placed in an oven (90°C). The electrical conductivity (EC2) was measured in the solution after cooling to 25°C. The leakage of electrolyte was measured using the following formula:

EL = EC1/EC2 × 100 (Dionisio-Sese and Tobita 1998).

SPAD index: Leaf SPAD was measured using a standard chlorophyll meter (*Minolta 1500*, Osaka, Japan).

Statistical analysis: Multivariate analysis, analysis of variance (*ANOVA*) (*XLSTAT* software, *version 2014*), and clustering were used to analyze data. The Principal Component Analysis (PCA) was performed with *XLSTAT* software, *version 2014*. For all experiments, all samples were assessed in three replications. *ANOVA* considering the *post hoc* evaluation with Duncan's test was conducted to examine any important variations at $p<0.05$. Data are given as mean ± SD.

Results

Eighteen physiological and morphological parameters were used in this study to characterize the response of faba bean cultivar to different biochar concentrations under stressed and non-stressed conditions (Table 1).

Morphological and physiological parameters under non-stressed conditions: Under normal conditions, control and treated plants [sowed in soil without biochar (0%) or in 2 (B2), 5 (B5), and 8% (B8) of B, respectively] were designed as following: VF-CN, VF-B2N, VF-B5N, and VF-B8N. Plants were watered with tap water for 2 months under identical environmental conditions and harvested at the same time. Fig. 1*A* shows the PCA plot setup for faba bean plants under normal conditions. The first two components counted for 79.2% of the total variation, of which principal components 1 (PC1) and 2 (PC2) defined 46.1 and 33.1% of the variation, respectively. The PC1 was extremely correlated to PFM and PC2 was determined by C_i (Fig. 1*A*). As shown in Table 1, these two parameters were the top contributing variables to the descriptions of PC1 and PC2 with contribution values of 12.0 and 15.3, respectively. Consequently, they were used in the treatment distribution under normal conditions. The PCA plot in

Table 1. Physiological and morphological characteristics of faba bean plants were observed with their contributions to the description of PC1 and PC2 of the statistical analysis of PCA under normal, salinity, drought, and combined stress conditions.

Characteristics	Normal		Salinity		Drought		Combined	
	PC1	PC2	PC1	PC2	PC1	PC2	PC1	PC2
Root length (RL)	9.460	0.245	0.331	1.948	1.945	4.036	6.722	7.259
Shoot length (SL)	1.273	14.992	6.557	5.877	4.660	3.548	11.506	0.199
Plant length (PL)	4.462	9.989	5.006	5.664	6.382	6.309	11.244	0.003
Root fresh mass (RFM)	2.169	8.460	10.011	0.746	7.638	0.846	0.153	9.757
Shoot fresh mass (SFM)	9.713	2.210	0.015	13.413	7.787	0.145	2.119	13.352
Plant fresh mass (PFM)	11.999	0.018	7.447	4.153	8.115	0.187	3.026	9.563
Root dry mass (RDM)	9.941	2.098	9.255	0.112	0.109	21.419	0.030	16.085
Shoot dry mass (SDM)	7.602	6.099	10.493	0.249	3.980	8.420	11.887	0.304
Plant dry mass (PDM)	2.100	0.975	10.341	0.009	3.759	10.346	9.917	2.441
SPAD value (SV)	0.010	1.433	9.184	1.170	4.534	8.259	6.931	0.497
Relative water content (RWC)	8.639	2.377	2.890	10.182	7.639	0.726	0.039	16.418
Leaf number (LN)	4.438	0.950	1.710	13.430	8.141	2.459	9.394	3.557
Intercellular CO_2 concentration (C_i)	0.474	15.308	6.761	4.862	9.133	0.189	1.253	6.292
Transpiration rate (E)	7.822	4.550	0.003	11.863	5.821	8.353	8.739	1.278
Stomatal conductance (g_s)	7.819	5.790	3.500	5.599	6.414	7.617	5.152	0.381
Net CO_2 assimilation rate (P_N)	2.243	12.548	0.993	15.463	5.110	10.959	0.001	11.320
Water-use efficiency (WUE)	3.787	11.472	7.307	5.261	7.294	0.044	5.420	1.204
Electrolytes leakage (EL)	6.048	0.486	8.197	0.001	1.539	6.140	6.467	0.088

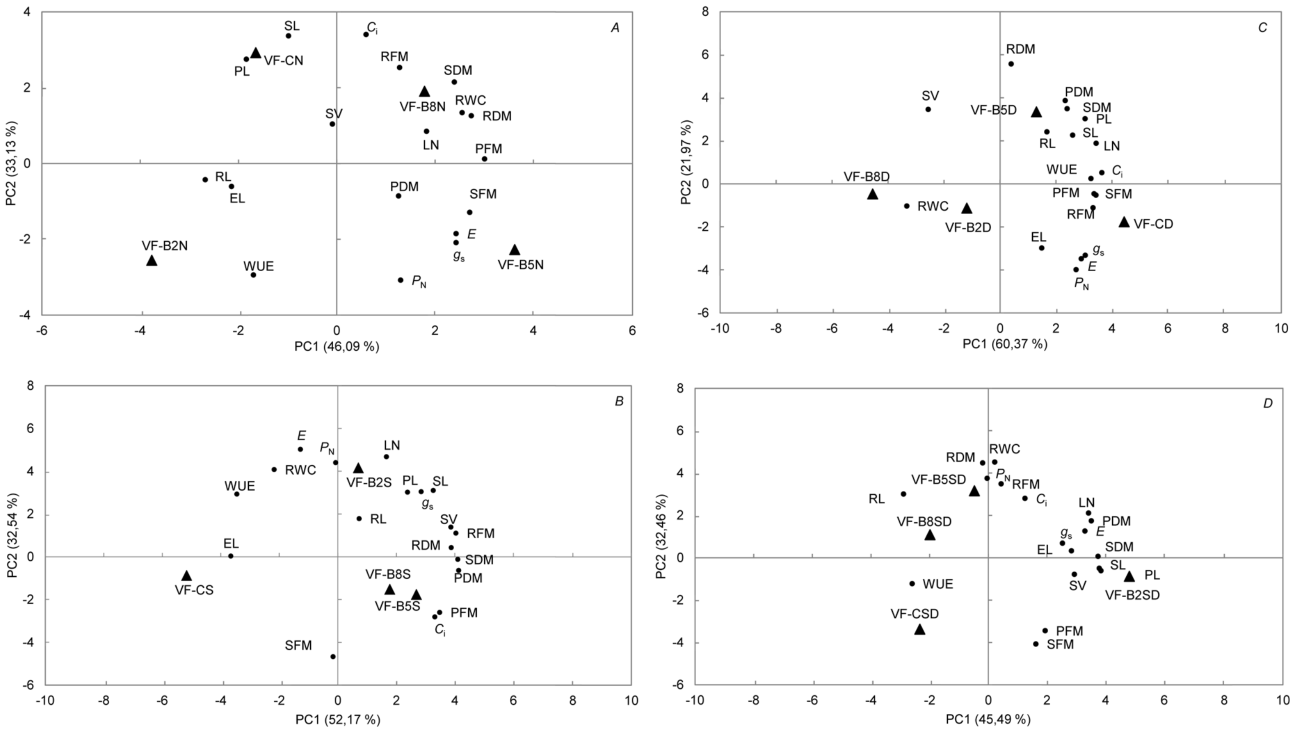

Fig. 1. Plots from the PCA demonstrating the contribution of the different parameters to the variation to different axes and the grouping of plants grown under normal conditions (*A*) or affected by a single effect of salinity (*B*), drought (*C*), and the interactive effect of both salinity and drought stresses (*D*) according to PC1 and PC2. VF – *Vicia faba* plants; C – control, *i.e.*, 0% of biochar; B2, B5, and B8 presented different contents of biochar (2, 5, and 8%, respectively); S – salinity; D – drought; SD – combined salinity and drought conditions; RL – root length; SL – shoot length; PL – plant length; RFM – root fresh mass; SFM – shoot fresh mass; PFM – plant fresh mass; RDM – root dry mass; SDM – shoot dry mass; PDM – plant dry mass; RWC – relative water content; SV – SPAD value; LN – leaf number; C_i – intercellular CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; P_N – net CO_2 assimilation rate; WUE – water-use efficiency; EL – electrolytes leakage.

Fig. 1*A* revealed four groups. Group 1 included controls VF-CN, cultivated in soil without adding biochar, which was situated separately in the positive and negative sides of PC2 and PC1, respectively. These plants were characterized by high PL. Group 2, formed by *Vicia faba* plants sowed in soil amended with 2% of biochar (VC-B2N), was localized on the bottom of the score's plots and correlated negatively to both axes PC1 and PC2. Group 3, composed of *Vicia faba* plants cultivated in soil amended with 5% biochar (VF-B5N), was located in the right bottom of the score's plots and correlated negatively to PC2 and positively to PC1. These plants were characterized by having the highest photosynthetic parameters. Finally, group 4 (plants cultivated in soil containing 8% biochar, VF-B8N) was situated in the upper right side of the scores plot and correlated positively to PC1 and PC2. This group was distinguished by exhibiting the highest PFM, LN, RDM, SDM, and C_i. Thus, plants cultivated in soil amended with 8% biochar showed the highest photosynthetic activity and biomass production. Therefore, results obtained from this evaluation allow us to conclude that C_i and PFM could be used as the discriminating parameters of the response of plants to different biochar concentrations under non-stressed conditions.

Morphological and physiological parameters under salinity conditions: Under salinity conditions, control and treated plants [sowed in soil without biochar (0%) or in 2 (B2), 5 (B5), and 8% (B8) of B, respectively] were designed as follows: VF-CS, VF-B2S, VF-B5S, and VF-B8S. The PCA plot setup for different parameters of *Vicia faba* plants grown under soils mixed with different biochar concentrations and treated with 100 mM of NaCl is summarized in Fig. 1*B*. PC1 and PC2 axes explained 84.7% of the total variance (52.2 and 32.5%, respectively). As shown in Table 1, SDM and P_N were the top contributing variables to the descriptions of PC1 and PC2 with contribution values of 10.49 and 15.46, respectively. By analyzing the scores plot in the area defined by PC1 and PC2, *Vicia faba* plants were divided into three groups. Group 1 (the plants planted with 2% biochar, VF-B2S) was situated in the upper right side of the scores plot and correlated positively to PC1 and PC2. High values of P_N, E, LN, and g_s were characteristic for this group. Group 2, formed by VF plants planted at 5 and 8% biochar (VF-B5S and VF-B8S) was localized on the bottom right of the scores plot, and correlated positively to PC1 and negatively to PC2. This group was distinguished especially by the highest values of PFM and C_i. Group 3, formed by plants grown in unamended soil (VF-CS), was situated on the bottom left of the scores plot and correlated negatively to both PC1 and PC2. Under salinity conditions, we noted good discrimination of the response of faba beans to different biochar concentrations according to SDM and P_N (Table 1, Fig. 1*B*).

Morphological and physiological parameters under drought conditions: The sets of data, consisting of all parameters measured in faba beans planted under drought conditions and in different biochar soil mixtures (VF-CD, VF-B2D, VF-B5D, and VF-B8D) were submitted to the multivariate statistical analysis techniques (Fig. 1*C*). The PC1 and PC2 explained 82.4% of the total variance. The first axis (PC1 = 60.4%) was highly correlated to C_i. The second axis (PC2 = 21.97%) was determined by RDM. The samples were divided into three groups. Group 1, which was located on the top of the scores plot and correlated positively to both axes, was composed of *Vicia faba* plants grown under 5% of biochar amendment (VC-B5D), which was characterized by the highest PDM, SDM, SL, and the largest LN. Group 2 was located on the right side of the scores plot, and it was positively correlated to PC1 and negatively to PC2 consisting of the *Vicia faba* cultivated in unamended soil (VF-CD). The highest levels of PFM were characteristic for this group. The third group was negatively correlated to both axes; it was formed by *Vicia faba* planted soil amended with 2 and 8% biochar (VC-B8D and VF-B2D).

Morphological and physiological parameters under combined salinity and drought conditions: The PCA plot setup for different parameters of faba beans planted under combined salinity and drought conditions (SD) and grown under soils mixed with different biochar concentrations (VF-CSD, VF-B2SD, VF-B5SD, and VF-B8SD) is summarized in Fig. 1*D*. The PC1 and PC2 axes explained 78.0% of the total variance (45.5 and 36.5%, respectively). PC1 and PC2 correlated to SDM and RWC, respectively. The samples were divided into three groups. Group 1 was situated in the bottom right side of the scores plot and correlated positively to PC1 and negatively to PC2; it included plants cultivated in soil amended with 2% biochar (VF-B2SD). These plants exhibited the highest SL and SDM. Group 2 was situated on the bottom left of the scores plot and correlated negatively to both PC1 and PC2; it was formed by *Vicia faba* plants grown in unamended soil (VF-CSD). Group 3 was situated on the upper left side of the scores plot and correlated negatively to PC1 and positively to PC2; it was constituted by legumes planted in soil amended with 5 and 8% B (VC-B5SD and VC-B8SD).

Correlations between physiological and chemical parameters: Correlations between the various physiological and chemical parameters were analyzed to study relations in plants grown under different biochar contents. Table 2 shows the coefficients of *Pearson*'s correlation between all parameters in faba bean plants. Data demonstrated very good correlations between PL and SL, DSM and SFM, PFM and PDM, and g_s and P_N (r = 0.973, 0.919, 0.927, and 0.929, respectively). Additionally, we noticed a significant positive correlation between RWC and SL, g_s and SL, and g_s and LN (r = 0.760, 0.782, and 0.761, respectively). A poor positive correlation was also detected between RFM and RL, RWC and RDM, and g_s and SV (r = 0.274, 0.382, and 0.100, respectively). However, a negative correlation was observed between C_i and RL, P_N and RL, and EL and SFM (r = –0.235, –0.024, and –0.789, respectively).

Table 2. *Pearson*'s correlation coefficients (r) among the analyzed parameters of plants grown under different types of stresses and different contents of biochar. RL – root length; SL – shoot length; PL – plant length; RFM – root fresh mass; SFM – shoot fresh mass; PFM – plant fresh mass; RDM – root dry mass; SDM – shoot dry mass; PDM – plant dry mass; SV – SPAD value; RWC – relative water content; LN – leaf number; C_i – intercellular CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; P_N – net CO_2 assimilation rate; WUE – water-use efficiency; EL – electrolytes leakage.

Parameters	RL	SL	PL	RFM	SFM	PFM	PDM	SDM	PDM	SV	RWC	LN	C_i	E	g_s	P_N	WUE	EL
RL	1																	
SL	–0.044	1																
PL	0.189	0.973	1															
RFM	–0.022	0.559	0.545	1														
SFM	–0.028	0.809	0.789	0.600	1													
PFM	–0.028	0.815	0.794	0.690	0.993	1												
RDM	0.274	0.482	0.537	0.676	0.432	0.490	1											
SDM	–0.008	0.786	0.771	0.739	0.919	0.940	0.531	1										
PDM	0.093	0.844	0.851	0.724	0.906	0.927	0.576	0.956	1									
SV	0.134	0.421	0.445	0.606	0.340	0.397	0.458	0.472	0.519	1								
RWC	–0.042	0.760	0.737	0.417	0.702	0.696	0.382	0.623	0.656	0.245	1							
LN	0.164	0.854	0.877	0.507	0.717	0.724	0.623	0.722	0.770	0.380	0.758	1						
C_i	–0.235	–0.355	–0.404	0.068	–0.438	–0.386	–0.056	–0.245	–0.291	–0.148	–0.613	–0.316	1					
E	0.148	0.556	0.581	0.433	0.662	0.662	0.446	0.550	0.582	0.325	0.745	0.756	–0.456	1				
g_s	–0.142	0.782	0.735	0.362	0.772	0.751	0.446	0.628	0.704	0.100	0.701	0.761	–0.328	0.726	1			
P_N	–0.024	0.699	0.681	0.283	0.688	0.664	0.370	0.520	0.644	0.106	0.785	0.719	–0.529	0.786	0.929	1		
WUE	–0.443	0.230	0.123	–0.251	0.186	0.132	–0.460	0.057	0.089	–0.251	0.409	–0.003	–0.464	0.139	0.361	0.480	1	
EL	0.202	–0.561	–0.505	–0.760	–0.789	–0.826	–0.372	–0.824	–0.784	–0.566	–0.566	–0.405	0.323	–0.455	–0.433	–0.402	–0.181	1

Hierarchical cluster analysis: The collected data were submitted to hierarchical cluster analysis (HCA) to detect the effect of biochar addition on the growth of faba bean plants under normal, salinity, drought, and combined stress. The result of the heatmap cluster analysis shows that there are two types of dendrogram: a plant grown under different levels of biochar dendrogram with a horizontal position and a parameters dendrogram with a vertical position (Fig. 2). The heat map derived from one-way HCA grouped plants grown under different types of stresses or not into three groups. The first group consisted of plants grown in different biochar contents under salinity conditions or combined salinity and drought stresses: VF-CSD, VF-B3SD, VF-B5SD, VF-B5S, VF-B2SD, and VF-B8S). The second group consisted of VF-B2S and VF-CS. The third group included VF-CN, VF-B8N, VF-B2N, VF-B5N, VF-CD, VF-B5N, VF-CD, VF-B8D. The heat map is a colored representation of data. The red stands indicate the low values of the studied parameters, the black indicate the intermediate values, and the green indicate the high values. Based on dendrogram parameter grouping, group 1 exhibited a high level of C_i, PL, and SL. On the other hand, groups 2 and 3 were characterized by an important value of PL and C_i.

Classification of different treatments under normal conditions: To classify the biochar concentrations used in this study, the most selective physiological descriptors were considered for the valuation of the physiological behavior of faba bean plants grown under normal conditions. Thus, C_i and PFM parameters presented the maximum contribution to the description of PC1 and PC2, respectively, were employed (Table 1). Our results showed that the values of C_i of legume plants grown under different concentrations of biochar (VF-CN, VF-B2N, VF-B5N, and VF-B8N) were 172, 140, 169, and 190 μmol(CO_2) mol^{-1}, respectively (Fig. 3*A*). In addition, the values of PFM varied between 29 for VF-N to 31 g per plant for VF-B8N (Fig. 3*B*). The use of *Duncan*'s test for C_i and PFM allowed us to classify the treatments into three and two groups, respectively. Faba bean plants noted as (a) exhibited the highest value of these parameters. However, the plant indicated as (c) exhibited the lowest values. The result showed that 8% biochar was the best concentration to increase the growth of legume plants under normal conditions. This result was confirmed by PCA analysis, where VF-B8N was situated on the positive side of PC1 and PC2 axes (Fig. 2*A*).

Classification of different treatments under salinity conditions: The most discriminating descriptors were used for evaluating the response of faba bean plants to salinity stress. SDM and P_N parameters presented the maximum contributions to the description of PC1 and PC2, respectively, as shown in Table 1, and were considered

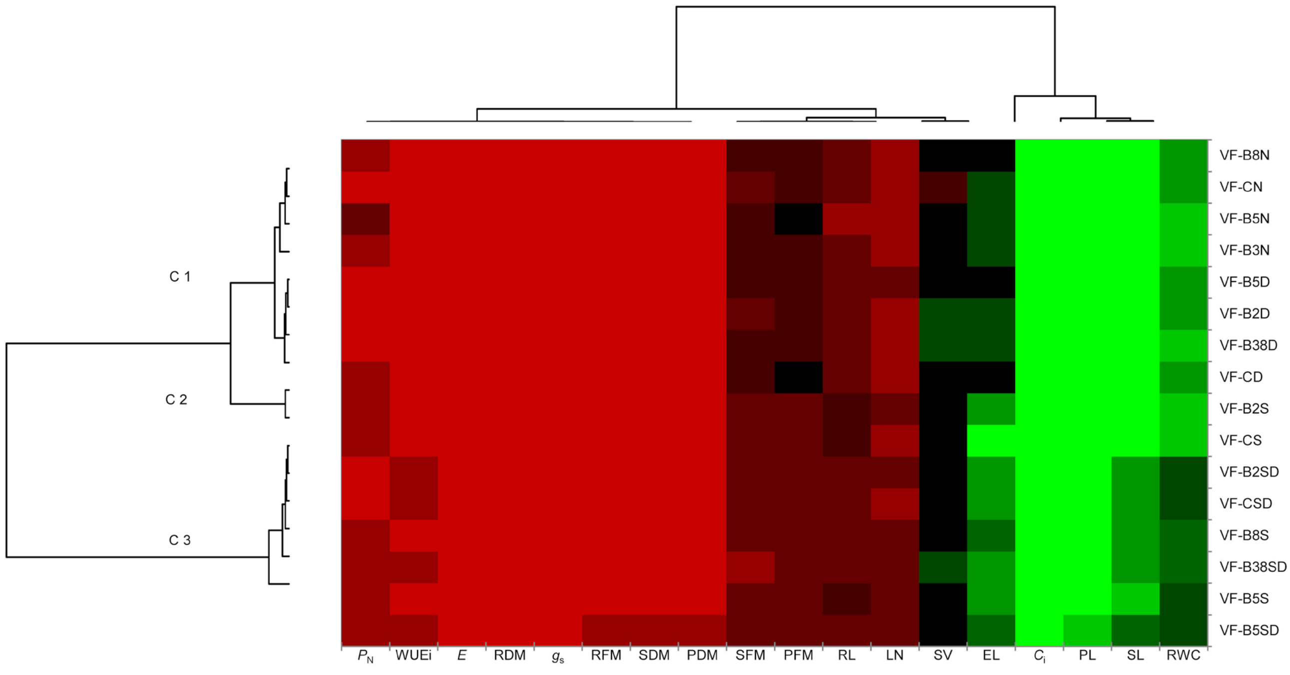

Fig. 2. Heat map cluster of significant parameters interacting in *Vicia faba* plants grown under normal conditions (VF-CN, VF-B2N, VF-B5N, and VF-B8N) and as affected by the single effect of salinity (VF-CS, VF-B2S, VF-B5S, and VF-B8S), drought (VF-CD, VF-B2D, VF-B5D, and VF-B8D), and the interactive effect of both salinity and drought conditions (VF-CSD, VF-B2SD, VF-B5SD, and VF-B8SD). The red stands indicate the low values of the studied parameters, the black indicate the intermediate values, and the green indicate the high values. RL – root length; SL – shoot length; PL – plant length; RFM – root fresh mass; SFM – shoot fresh mass; PFM – plant fresh mass; RDM – root dry mass; SDM – shoot dry mass; PDM – plant dry mass; RWC – relative water content; LN – leaf number; SV – SPAD value; C_i – intercellular CO_2 concentration; E – transpiration rate; g_s – stomatal conductance; P_N – net CO_2 assimilation rate; WUE – water-use efficiency; EL – electrolytes leakage.

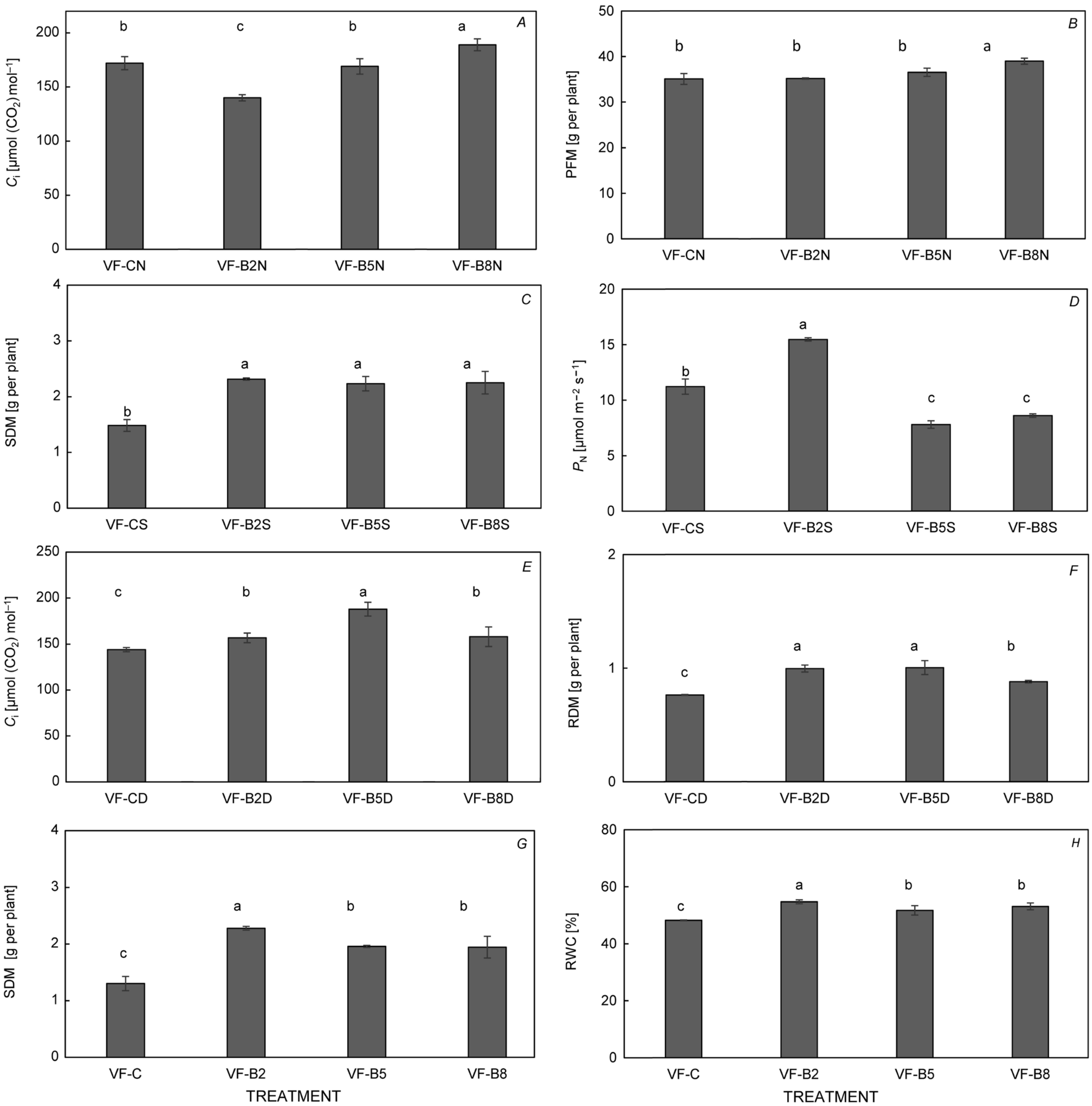

Fig. 3. The selected parameters under normal conditions: C_i (*A*) and PFM (*B*); salinity conditions: SDM (*C*) and P_N (*D*); drought conditions: C_i (*E*) and RDM (*F*); and combined salinity and drought conditions: SDM (*G*) and RWC (*H*). C_i – intercellular CO_2 concentration; PFM – plant fresh mass; RDM – root dry mass; SDM – shoot dry mass; P_N – net CO_2 assimilation rate; RWC – relative water content. All values are means ± SD. The data followed by *different letters* are significantly different at $p \leq 0.05$.

for the classification of different treatments under salinity stress. Fig. 3*C*,*D* illustrates the behavior of plants in terms of SDM and P_N, respectively. The SDM values varied between 1.48 g per plant for VF-CS to 2.31 g per plant for VF-B2S (Fig. 3*C*). The P_N ranged from 7.8 μmol(CO_2) m^{-2} s^{-1} for VF-B5S to 15.46 μmol(CO_2) m^{-2} s^{-1} for VF-B2S. A significant fluctuation was revealed between different treatments. The statistical analysis based on *Duncan*'s test for SDM allowed dividing the legumes into two groups. Plants designated by the letter (a) were considered the most tolerant to salinity conditions. However, the legume noted by the letter (b) was considered the most sensitive. The distribution based on P_N values subdivided plants into three groups (Fig. 3*D*). The most tolerant was mentioned with the letter (a), the plants indicated by the letter (c) were considered the most sensitive. These results showed that plants grown in soil amended with 2% biochar surmounted these severe salt conditions. So, we can conclude that adding 2% biochar is the best concentration to alleviate salinity stress. This result was approved by PCA analysis, where VF-CS and VF-B2S were diametrically opposite (Fig. 2*B*).

Classification of different treatments under drought conditions: The most discriminating physiological parameters selected by the statistical analysis, C_i and RDM, were used for evaluating the response of faba bean plants grown under drought conditions (Table 1, Fig. 3*E*,*F*). The values of C_i measured in this study varied between 144 and 188 µmol(CO_2) mol^{-1} for VF-CD and VF-B5D, respectively (Fig. 3*E*). However, the RDM ranged from 0.75 to 1.1 g per plant for VF-CD and VF-B5D, respectively (Fig. 3*F*). The statistical analysis based on *Duncan*'s test for both descriptors allowed dividing the plants into three groups. The most tolerant plants to drought stress were designed by the letter (a) and the most sensitive were indicated by the letter (c). Therefore, the obtained results allow us to conclude that the best biochar concentration to alleviate the harmful effects of drought was 5% (VF-B5D). This result was confirmed by the PCA analysis, where VF-B5D was situated on the positive side for PC1 and PC2 of the plots (Fig. 2*C*).

Classification of different treatments under combined salinity and drought conditions: For the classification of different treatments, parameters presented the maximum contributions to the description of PC1 and PC2 (RDM and RWC), were used to evaluate the response of faba bean plants to combined salinity and drought conditions (Table 1, Fig. 2*D*). Plants grown in soil amended with 2% biochar (VF-B2SD) exhibited the highest values of RDM (2.3 g per plant) and RWC (55%) compared with other treatments (Fig. 3*G*,*H*). *Duncan*'s test divided the biochar treatments into three groups. Plants designed by the letter (a) were considered the most tolerant to combined stress. Nevertheless, legumes indicated by the letter (c) were considered the most sensitive. Therefore, this study allows us to conclude that adding 2% biochar in the soil can alleviate the effects of combined stress on the growth of faba bean plants.

Discussion

Areas of the world with salt-affected soils are expected to increase in the upcoming years, with the most obvious effects of salt stress occurring in arid and semi-arid regions (Benmoussa *et al*. 2022, Nefissi Ouertani *et al*. 2022b). Limited crop production due to the degradation of fertile land will affect food availability to a steadily increasing world population. Salinity significantly inhibited the leaf number, plant heights and masses, chlorophyll content, photosynthetic parameters, RWC, and relative growth rate of faba bean seedlings (Neji *et al*. 2021, Nefissi Ouertani *et al*. 2022a, Rajhi *et al*. 2023b). The presence of salt in the soil diminishes the capacity of plant to absorb water and this conducts to trouble in the growth rate and is assigned to the osmotic or water-deficit effect of salinity (Nefissi Ouertani *et al*. 2021). Additionally, the ionic effects due to the diffusion of high amounts of salt in the plant tissues cause the damage of the cells (Rajhi *et al*. 2011, Takahashi *et al*. 2015, Nefissi Ouertani *et al*. 2021, Zhang *et al*. 2022). Habitually, salinity engenders nutritional disorders, limits the uptake of essential plant nutrients (potassium, calcium, magnesium, and phosphorus), and eventually induces further alteration in growth leading to crop yield losses (Rezaie *et al*. 2019). Drought stress is intimately associated with plant water accessibility (Farid *et al*. 2019). The capacity of plants to adjust the water balance considerably impacts the growth of plants (Singh *et al*. 2012, Kim *et al*. 2020). Drought stress affects cell division, turgor stress, and mineral translocation in plants (Sah *et al*. 2020) and directly disturbs plant growth, production, and yield (Wei *et al*. 2021). Interactive effects of salinity and drought had more destructive consequences on plant growth than the single drought or salinity effect (Goharrizi *et al*. 2020, Zhang *et al*. 2020).

Consequently, there is an urgent need to determine new agricultural practical and efficient strategies to keep a moderate level of soil moisture and ions balance for corps under individual or combined effects of stresses. Different approaches have been used to alleviate the impacts of stresses on crops and to increase the fertility of soils, including the biochar amendment. Thus, we aimed in this study to evaluate the effect of biochar addition on the single and interactive effects of salinity and drought treatments on the growth of faba bean seedlings. It is important to know whether biochar application could be used as an effective management to damaged soil under these conditions.

To deeply analyze the different plants' responses to the studied biochar concentrations, a multivariate analysis was used (Rajhi *et al*. 2021). The major advantage of the utilization of multivariate analysis is the allowance of a simultaneous analysis of multiple parameters and the increase of the accuracy in the ranking of treatments. In fact, we could select the best biochar concentration that alleviates the single or combined effects of both stresses. In Tunisia, the faba bean is among the most valuable grain legume pulses (Rajhi *et al*. 2022d,e).

The most useful indices for evaluating the impact of biochar on the growth of faba bean plants under normal or stressed conditions were related to photosynthesis (C_i, P_N) and biomass parameters (PFM, SDM, and RDM). The photosynthesis parameters play a crucial role in regulating crop yield (Hussain *et al*. 2018). These were affected by the individual and interactive effects of salinity and drought (Rajhi *et al*. 2020, Zhang *et al*. 2020). That has been ascribed to the closure of the stomata which leads to the decrease of the CO_2 diffusion within the leaves and through the inhibition of photosynthetic enzymes due to the lower CO_2 concentration (Farooq *et al*. 2017). In addition, the C_i plays an important role in assessing the effect of salt on photosynthetic efficiency (Zhang *et al*. 2020). Saline soils increase the concentrations of Na^+ and Cl^- in plant leaves which can lead to the reduction of cell expansion and photosynthetic activity and provoke the senescence of leaves and inhibition of the crop yield (Munns and Gilliham 2015). During this study, the photosynthetic traits were improved with the addition of biochar under salinity or combined salinity and drought. This result is consistent with the data presented by Rezaie *et al*. (2019) and Yang *et al*. (2020). That might be explained by the amelioration of the water status of the plants due to the ability of biochar to increase soil water content, absorb the excess of Na^+ and increase K^+ uptake in plants (Usman *et al*. 2016, Saifullah *et al*. 2018).

Also, our results showed that biochar addition improves fresh plant mass, root dry mass, and shoot dry mass parameters. Higher dry mass confirmed the role of biochar in diminishing the negative effect of environmental stress on faba bean growth (Rezaie *et al.* 2019). Nevertheless, an opposite result was observed in soybean seedlings when they grow at different biochar concentrations under salinity and drought conditions (Zhang *et al.* 2020). These authors showed that the biomass parameters did not change with the addition of this amendment. This result could be explained by a limited availability of nutrients as well as the possible phytotoxic effect of biochar.

Conclusions: Salinity and drought stresses negatively affected the *Vicia faba* plant growth. The addition of biochar at different concentrations under normal, salinity, drought, and combined conditions, improved the photosynthetic parameters in studied legumes. In conclusion, our result demonstrates that the addition of 2% (B2) biochar could significantly mitigate the negative effect of the single effect of salinity and combined salinity and drought. On the other hand, the addition of 5% (B5) biochar could alleviate the individual effect of drought compared to their respective controls. This result confirms the positive effect of biochar addition due to its ability to (*1*) desorb salt, and (*2*) increase the water-holding capacity of amended soils and consequently improve the biochemical, physiological, and photosynthetic traits of *Vicia faba* plants. These biochar concentrations are recommended for the growth of *Vicia faba*, and it is also important to evaluate these concentrations under field conditions. Thus, a better understanding of biochar addition on a physiological basis and root traits for soybean growth under drought and salinity stress will be beneficial for sustainable agriculture.

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