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Effect of biochar addition and reduced irrigation regimes on growth, physiology and water use efficiency of cotton plants under salt stress

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ABSTRACT

To alleviate the salinity and drought stresses faced in agricultural production, and to improve crop water use efficiency (WUE) in drought-prone regions, novel management strategies are needed. The combination of biochar amendment and reduced irrigation regimes could mitigate the negative effects of salinity and drought stresses and improve WUE of cotton plants. A split-root pot trial was performed in order to investigate the effects of two biochar amendments [wheat straw pellets biochar (WSP) and soft wood pellets biochar (SWP)] combined with three irrigation schemes [full irrigation (FI), deficit irrigation (DI), and partial root-zone drying irrigation (PRD)] on the growth, physiology and WUE of cotton plants under two salinity levels [0 mM NaCl (S0) and 200 mM NaCl (S1)]. The results showed that salt stress depressed plant growth and physiology, and reduced seed cotton yield and lint yield by 19.33–47.22% and 40.43–58.81%, respectively. However, the biochar amendment alleviated salt stress and increased plant dry biomass allocation ratio by 3.85%–12.54%, 5.07%–14.39% and 9.78%–46.62% in boll, seed cotton and lint cotton under S1, respectively, and also increased lint ginning out turn (GOT), harvest index (HI), WUE at plant and yield level (WUE_p and WUE_y) by 0.21%–28.69%, 5.07%–14.39%, −0.30%–15.71% and 5.83%–32.44%, respectively. Moreover, WSP was superior to SWP in terms of improving plant growth and yield. PRD showed better growth and physiological effects than DI, especially WUE_p and WUE_y were 6.88%–13.73% and 9.16%–22.78% greater under PRD than under DI. The combined application of biochar and PRD counteracted the decrease in WUE caused by biochar application alone under S0. Collectively, WSP combined with PRD could be a promising strategy in sustainable cotton production under drought and salinity stress.

1. Introduction

Drought and salt stress are recognised as the two dominant abiotic stresses worldwide (Acquaah, 2009). Approximately 45% of the world's agricultural land is reported to be exposed to frequent or persistent drought stress (Abdelraheem et al., 2019), and 20% of cultivable land is subject to salinity stress (Abdelraheem et al., 2019; El Sabagh et al., 2021). According to prediction, drought and salt stress will result in 50% loss of cropland by 2025 (El Sabagh et al., 2021), considering the total world population predicted to increase to 9.7 billion by 2025 (Ehrlich and Harte, 2015), there will be a significant lack of food and fiber for humans and animals (Iqbal et al., 2020). Meanwhile, water deficits are gradually increasing due to the climate change (Iglesias and Garrote, 2015; Wang et al., 2012) and water available for irrigation has declined dramatically in many countries (Jensen et al., 2010), which makes it particularly important to increase WUE of crops (Debaeke and Aboudrare, 2004). Therefore, there is an urgent need to find a synergistic approach to solve the current problem of insufficient water resources for agricultural irrigation and severe soil salinization in order to meet crop productivity in future climatic situations.

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Deficit irrigation (DI) and partial root-zone drying irrigation (PRD) are water-saving irrigation techniques that have been widely investigated worldwide (Du et al., 2015; Kang and Zhang, 2004; Wei et al., 2018). DI is a technique that irrigates less water than the potential evapotranspiration to the entire root zone and causes a slight stress with minimum impact on yield (Wei et al., 2018). PRD is a modification of DI, and it relates to keeping a portion of the root system in dry soil while the other part remains irrigated normally, with the root system on the dry side and the wet side being altered with a frequency based on soil moisture and crop water requirements (Kang and Zhang, 2004). Studies on many crops have shown that PRD saves water while not causing a pronounced decrease in yield and thus increases crop WUE (Liu et al., 2006; Kang and Zhang, 2004), and it has been confirmed the advantages of PRD over DI in maintaining yield and improving WUE when irrigated with the same amount of water (Wang et al., 2010). Moreover, PRD has great benefits in promoting plant nutrient uptake and improving crop quality (Davies et al., 2000; Shahnazari et al., 2008; Sun et al., 2014a; Yang et al., 2021). For example, it has been shown in many studies that nitrogen uptake by crops is increased under PRD (Hu et al., 2009; Li et al., 2007; Skinner et al., 1999), and Wang et al. (2009) suggested that PRD makes increased plant nitrogen uptake due to at least two reasons, that is, an increased root system and increased nitrogen availability in the soil. Therefore, PRD strategy has a great potential not only in promoting plant growth, but also in saving water resources and increasing crop WUE (Liu et al., 2006; Liu et al., 2021).

Soil salinity has a detrimental effect on crop development and growth (Abdelraheem et al., 2019; Evelin et al., 2009; Hidri et al., 2016). Salinity affects root morphology and distribution, reduces water and nutritional access and transportation from the roots to the shoots, causes ionic and osmotic imbalance in plants, induces stomatal closure, decreases photosynthesis, inhibits enzymes activity, protein synthesis and cell division, increases reactive oxygen species (ROS) production, and damages membrane integrity (Cheng et al., 2019; Guo et al., 2015; Munns, 2002; Ren et al., 2021; Sarkar et al., 2018; Zhang et al., 2017; Zhang et al., 2019). Moreover, the poisonous effects of Na^+ and Cl^- suppressed the growth of roots, stems, leaves, boll and other organs and even lead to death, ultimately causing a decrease in cotton yield and biomass (Guo et al., 2015; Parida et al., 2005; Ren et al., 2021). Drought and salinity have similar negative effects on cotton growth, especially during the osmotic stress phase, because the osmotic effect caused by either drought or salt stress leads to cellular dehydration, resulting in a decrease in cytosolic and vacuolar volumes (Abdelraheem et al., 2019). Moreover, the combination of drought and salinity stresses leads to more yield losses than a single stress (Mittler, 2006).

Biochar is a carbon-rich, porous, solid, stable product obtained by thermochemical transformation, mainly under anaerobic or oxygen-limited conditions, and it is often applied as a soil additive to modify the hydraulic properties and nutritional status of soils (Gao et al., 2019; Lehmann and Joseph, 2015; Omondi et al., 2016). Biochar amendment has been considered a promising strategy in alleviating soil drought and salt stress. Hammer et al. (2015) revealed that biochar facilitates the growth of lettuce in saline soils, and Zhang et al. (2019) showed that biochar effectively alleviated salt stress in rice seedlings. Yang et al. (2020) also showed biochar promoted the growth physiological characteristics of quinoa under drought and salt stress, indicating that biochar alleviated both drought and salt stress. In addition, as previous studies have found that biochar can provide a large amount of potassium (K) to the soil, which alleviates the negative effect of imbalance in K^+ uptake by plants due to the large amount of NaCl in saline soils (Munns, 2002; Sarkar et al., 2018). Thus, biochar can also be used as a substitute for chemical potassium fertilizers (Van Zwieten et al., 2009; Wu et al., 2019). However, many current researches have primarily demonstrated the single effects of salt stress or biochar amendment or different irrigation regimes on different crops (Dodd, 2007; Zhang et al., 2017), and those studies on biochar have mainly focused on non-saline soils (Azeem et al., 2019). Fewer studies have been conducted to investigate the effects of biochar addition to saline soils (Palansooriya et al., 2019; Zhang et al., 2019). In particular, it is necessary to investigate the impacts of different biochar in combination with different irrigation mechanisms on crop growth, physiological characteristics and WUE under saline soils.

Cotton (*Gossypium hirsutum* L.) is mainly grown in areas where precipitation and irrigation are often scarce and therefore drought and salinity stress are common constraints for the production of the crop (Abdelraheem et al., 2019), though it possesses a great ability to tolerate salinity and drought stress (Brady et al., 2008; Maas and Grattan, 1999; Zhang et al., 2017; Zhang et al., 2016). Therefore, the present experiment was performed to study the impact of different biochar in conjunction with different irrigation regimes on the growth, physiology and WUE of cotton under salt stress. It was hypothesized that biochar amendment combined with PRD irrigation would alleviate the impacts of salt stress and improve WUE of cotton plants.

2. Materials and methods

2.1. Experimental setup

The experimental site is located in the solar glasshouse of the Northwest A&F University (34° 15 N, 108° 04 E) in Yangling, Shaanxi, China. The experiment started in June 2020 and ended in January 2021. The biochar materials used in the trials are wheat straw pellets biochar (WSP) and soft wood pellets biochar (SWP) produced by the UK Biochar Research Center, University of Edinburgh, UK, which are pyrolysed at 550 ℃ under anaerobic conditions. The soils used in the trials were from the 0–25 cm soil layer of a local farm in Yangling, Shaanxi Province. The soil texture is classified as clay loam based on the USDA classification system. It consisted of 8% clay (<0.002 mm), 85% silt (0.05–0.002 mm) and 7% sand (2–0.05 mm). The soil was air-dried and then passed through a 0.5 cm sieve. In detail, the physicochemical properties of the soil and biochar used in the trials are shown in Table 1. The biochar used in the trials was ground in a mortar and passed through a 0.45 mm sieve, then thoroughly mixed with soil at a ratio of 2% (w/w) and packed into pots. These pots are custom-made, with an inner length × width × height of 26 cm × 16 cm × 40 cm and a volume of 16 liters. Each pot was separated into two chambers of equal volume by a plastic plate in order to prevent the exchange of water between the two chambers. At the same time, a small rectangular strip of 3 cm in width and 6 cm in height was taken out of the middle of the top of this plastic plate so that cotton seedlings could be transplanted in this position.

Cotton seeds (*Gossypium hirsutum L.*, var. Lumian No. 37) were sown in nursery seedling tray plate with substrate on 1st June 2020. Cotton seedlings were transplanted into custom-made split-root pots when they reached the three-leaf to four-leaf stage, with one cotton seedling in each

Table 1
Physicochemical properties of biochar and soil.

attribute	soil	WSP	SWP
EC, μScm^{-1}	360	1700	90
pH	7.72	9.94	7.91
CEC, cmol + kg^{-1}	1.95	6.15	3.15
Total N, g kg^{-1}	0.46	13.9	<1
Total K, g kg^{-1}	24.24	15.6	2.5
Total P, g kg^{-1}	0.59	1.4	0.6
Total C, g kg^{-1}	17.79	682.6	855.2
Available K, mg kg^{-1}	86.33	-	-
C/N ratio	38.67	49.11	<855.2
C stability, %	-	96.51	69.62
Zinc, mg kg^{-1}	-	10.50	25.71
Copper, mg kg^{-1}	-	3.63	19.41
Cadmium, mg kg^{-1}	-	3.15	3.48
Nickel, mg kg^{-1}	-	1.00	3.30
Total surface area, m^2 g^{-1}	-	26.40	26.40
Total ash, %	-	21.25	1.25

pot. Prior to transplanting, 4.86 g urea and 5.4 g KH_2PO_4 were applied to each pot to maintain an adequate supply of nutrients throughout the experiment. When transplanting, cotton seedlings with similar size were selected from the nursery trays, and the substrate remaining on the root surface was first cleaned with water, then the main roots of the cotton seedlings were divided equally into two parts with a blade longitudinally. Finally, the split-root cotton seedlings were transplanted into the split-root pots, making sure that both pot compartments has the same amount of roots. Each pot was filled with 18 kg of soil with a bulk density of 1.20 g cm^{-3} or a soil-biochar mixture. Based on the method of Hansen et al. (2016), the water holding capacity (WHC) of soil without biochar, soil with SWP biochar and soil with WSP biochar in the pots was 25%, 26% and 27%, respectively (in mass). After transplanting, a 2-cm layer of perlite was covered on the soil surface in order to minimize the soil evaporation. At the same time, two 35 cm TDR probes were inserted in the center of each chamber of the pots to measure the daily volumetric soil water contents (SWC, %).

A humidity & temperature meter (TH-Logger, China) was used to monitor the environmental factors in the greenhouse, i.e. relative humidity (RH), temperature (T), and vapor pressure deficiency (VPD). The daily RH, T, and VPD in the greenhouse during the experimental period are shown in Fig. 1.

2.2. Treatments

This pot experiment involved three factors and a total of 18 treatments, i.e., three biochar amendments (without biochar as control, SWP biochar and WSP biochar, denoted as CK, SWP, WSP, respectively), two salinity levels (0 mM NaCl and 200 mM NaCl, denoted as S0 and S1, respectively), and three irrigation regimes. Each treatment contained four pots. The cotton seedlings were well irrigated to 90% of WHC over the first month. Thereafter, half the number of pots were randomly selected to be irrigated with the NaCl solution. And these selected pots were irrigated with 100 mL NaCl every two days (4.74 g NaCl per pot every time) for 20 days in order to gradually establish a saline soil environment with a salt concentration of 200 mM in the soil solution. Afterwards, all the cotton plants in the pots were exposed to three irrigation treatments, that is, full irrigation (FI), deficit irrigation (DI), and partial root-zone drying irrigation (PRD), respectively. For the FI-treated pots, all soil compartments were irrigated to 90% of WHC, while for the DI-treated pots, only 70% of the FI-treated irrigation volume was used to irrigate all soil compartments. For the PRD treatment, only one of the compartments in the pots was irrigated with the same amount of irrigation as the DI and the irrigation was switched until the soil moisture content in the other compartment was reduced to 12%. The irrigation treatment lasted for 100 days until the cotton plants were harvested after cotton bolls opened and irrigation was carried out daily from 16:00–18:00. For pots without salinity, time-domain reflectometer (TDR, MINITRASE, USA) was used to monitor SWC. It should be noted that since the TDR equipment is greatly affected by the soil salinity content, we could not use the TDR to monitor the soil moisture content of the S1 treatment, so we monitored the change of soil moisture content in FI and DI pots by weighting. At the same time, the TDR measurement was corrected by the weighing method for the S0 treatment. Consequently only the soil moisture contents of the FI and DI treatment under the S1 treatment were obtained. For the switching time of the PRD under S1 we refer to the corresponding switching time for PRD under S0 treatment.

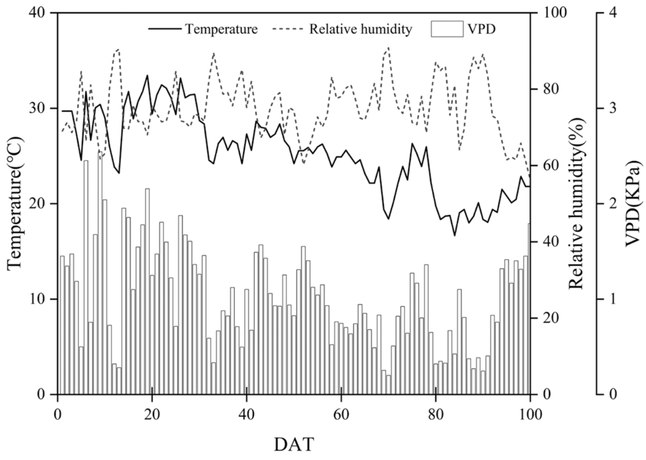

Fig. 1. The daily average relative humidity, temperatures, and vapor pressure deficiency (VPD) during the experimental period in the greenhouse.

2.3. Measurements and calculations

2.3.1. Leaf gas exchange parameters, instantaneous and intrinsic water use efficiency

Photosynthetic rate (A_n), transpiration rate (T_r), intercellular CO_2 concentration (C_i) and stomatal conductance (g_s) were measured using a portable photosynthetic measurement system (LI-6800, Inc., Lincoln, USA) at the upper canopy mature leaves per plant from 9:00–11:00 according to Ma et al. (2021a). Gas exchange parameters (A_n, T_r, C_i, g_s) were measured a total of three times during the irrigation treatment, i.e., on October 21, November 21 and December 20 for the third mature leaf at the top of the cotton plant, taking care to avoid clamping the large veins in the middle of the leaf during the measurement of the leaf chamber. And the leaf chamber temperature of the portable photosynthesis measurement system was set to 25 ℃, photosynthetic active radiation (PAR) was set to 1500 μmol m^{-2} s^{-1} and the CO_2 concentration was 400 ppm according to Ma et al. (2021a). Also, according to Liu et al. (2021), intrinsic and instantaneous water use efficiency (WUE_i and WUE_I) were computed as A_n/g_s and A_n/T_r, respectively.

2.3.2. Leaf morphological traits and chlorophyll

After each measurement of the gas exchange parameters of the leaves, the chlorophyll density (ChlD, μg cm^{-2}) was determined immediately using DUALEX SCIENTIFIC (Force-A, France) on the same leaf as for the gas exchange measurements according to Cerovic et al. (2012). So the ChlD was also measured three times on the same dates as the gas exchange parameters were measured. According to Rezzouk et al. (2020), this DUALEX SCIENTIFIC instrument works with an LED light source whose excitation light wavelengths are kept at 375 nm (UV) and 650 nm (red), which correspond to the maximum absorption efficiency of flavonoids and chlorophyll, respectively. When harvesting, the leaves of each plant were first cut off and then the leaf area (LA, cm^2) of each cotton plant was immediately measured using a leaf area meter (LI-3100, Inc. Lincoln, USA) based on Liu et al. (2021). The leaves were then dried (the detailed method is described in 2.3.3) and the leaf mass per area (LMA, g m^{-2}) was calculated, where LMA was calculated as the ratio of leaf dry biomass (LDB) to LA according to Liu et al. (2022b). In addition, the total chlorophyll content (ChlT, mg $plant^{-1}$) of each plant was calculated as the product of ChlD and LA according to Arunyanark et al. (2008).

2.3.3. Dry biomass, dry biomass allocation ratio and yield components

The cotton plants were harvested on January 21, and the leaves, stalks, roots, and bolls of the cotton plants were collected separately according to Ma et al. (2021a). And when collecting the roots, all the roots were placed in nylon mesh bags for rinsing and the broken roots were still recovered to ensure that all the roots were collected according to the method of Liu et al. (2022a). Subsequently, these different organs were placed in an oven at 75 ℃ and dried to a constant weight. After the bolls were dried, they were separated into the husk, seed cotton, cotton seeds and lint cotton. Afterwards, the root dry biomass (RDB), stem dry biomass (SDB), leaf dry biomass (LDB), husk dry biomass (HDB), seed cotton yield (SCY), boll dry biomass (BDB), lint cotton yield (LCY), cotton seeds biomass (CSB) and boll number (BN) were measured according to Zhu et al. (2020). Finally, the total dry biomass (TDB) was calculated according to the Eq. (1). Based on the descriptions of Rehman et al. (2022) and Manzoor et al. (2022), the lint ginning out turn (GOT) was calculated according to Eq. (2). And Based on Wu et al. (2019), the harvest index (HI) was calculated according to Eq. (3). In addition, we calculated the dry biomass allocation ratio of different organs according to Liu et al. (2021). The dry biomass allocation ratio of root ($[RDB]_a$) was calculated as the ratio of RDB to TDB. Similarly, the dry biomass allocation ratio of stalk ($[SDB]_a$) was calculated as the ratio of SDB to TDB; the dry biomass allocation ratio of leaf ($[LDB]_a$) was calculated as the ratio of LDB to TDB; the dry biomass allocation ratio of husk ($[HDB]_a$) was calculated as the ratio of HDB to TDB; the dry biomass allocation ratio of seed cotton ($[SCY]_a$) was calculated as the ratio of SCY to TDB; the dry biomass allocation ratio of boll ($[BDB]_a$) was calculated as the ratio of BDB to TDB; the dry biomass allocation ratio of lint cotton ($[LCY]_a$) was calculated as the ratio of LCY to TDB; the dry biomass allocation ratio of cotton seeds ($[CSB]_a$) was calculated as the ratio of CSB to TDB.

$$\text{TDB (g)} = \text{LDB} + \text{SDB} + \text{RDB} + \text{HDB} + \text{SCY} \tag{1}$$

$$\text{GOT (\%)} = 100 \times \text{LCY/SCY} \tag{2}$$

$$\text{HI (\%)} = \text{SCY/TDB} \tag{3}$$

2.3.4. *Water use and water use efficiency at plant and yield levels*

After harvesting, plant water use (WU, L) was computed based on the changes of SWC monitored by weighing or TDR in the pot and the amount of irrigation according to Ma et al. (2021a) and Liu et al. (2021). Besides, according to Rashid et al. (2018) and Liu et al. (2021) water use efficiency at seed cotton yield level (WUE_y) and water use efficiency at total dry biomass level (WUE_p) were calculated according to the Eqs. (4)–(5).

$$WUE_y \text{ (g L}^{-1}) = \text{SY/WU} \tag{4}$$

$$WUE_p \text{ (g L}^{-1}) = \text{TDB/WU} \tag{5}$$

2.4. *Data and statistical analysis*

To investigate the effects of biochar ([B]), salt ([S]) and irrigation ([IR]) and their interactions ([B] × [S] × [IR]), all data were analyzed by three-way ANOVA using SPSS 22.0 (IBM, Corporation, USA). And all data were expressed as mean of four replicates ± standard error. Tukey's test was applied to the data at the 5% level of significance. In addition, principal component analysis (PCA) of all data were conducted

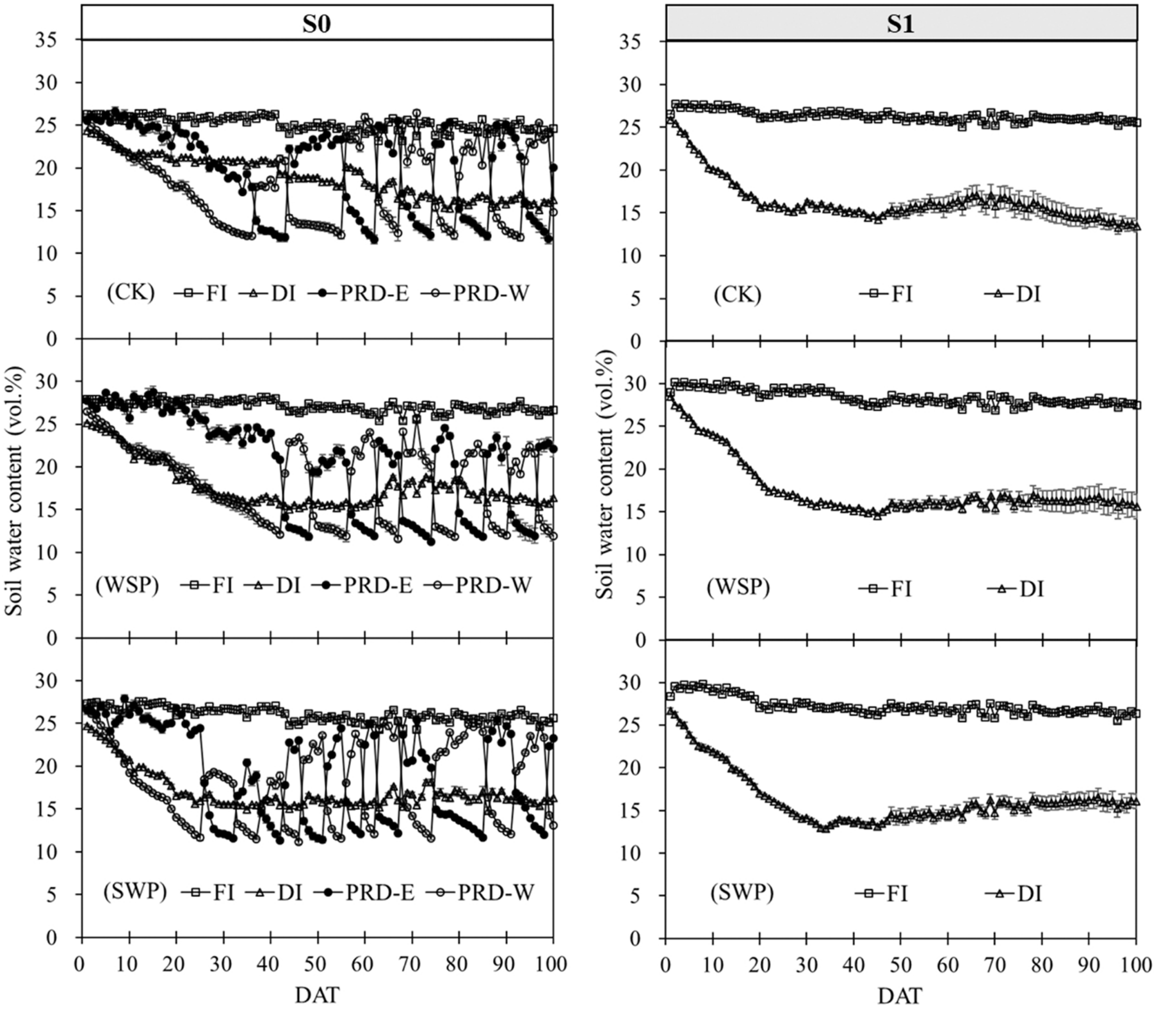

Fig. 2. Changes in soil water content of cotton plants during exposure to three irrigation treatments (FI, DI, PRD), three biochar amendment treatments (CK, WSP, SWP) and two salinity treatments (S0 and S1). PRD-W and PRD-E indicate the western and eastern compartment of the split-root pots under the S0 treatment, respectively. soil water content of the eastern and western compartments of the PRD treatment is not shown under the S1 treatment. The values shown are means ± SE (n = 4), Same as below.

by CANOCO 5.0 (Ithaca, NY, USA).

3. Results

3.1. Changes in soil water contents

The changes of the volumetric water content of the soil under S0 and S1 treatment during the [IR] treatment are shown in Fig. 2. The soil with biochar addition increased the WHC compared to the soil without biochar addition and, in particular, this increase was more significant under WSP amended soil. In FI, soil water contents were maintained near 28% and 27% for WSP and SWP, respectively, while it was near 26% for the CK treatment under S0. In PRD, the variation of soil moisture content on both sides depended on the alternating wet and dry cycles. i.e., the soil water content was approximately close to FI on the wet side and it of the drying side decrease to ca. 12% before switching of the irrigation. The soil water contents of [CK, FI], [WSP, FI] and [SWP, FI] under S1 were maintained at about 25%, 27% and 26%, respectively.

3.2. Leaf gas exchange parameters and water use efficiency at the leaf level

The gas exchange parameters (A_n, g_s, T_r, C_i), intrinsic water use efficiency (WUE_i), and instantaneous water use efficiency (WUE_l) were significantly affected by [S], [B], and [IR] (Fig. 3; Table 5). Compared to the S0 treatment, although A_n, T_r, g_s, C_i were decreased by 2.06%–16.16%, 5.77%–37.91%, 9.91%–36.15% and 7.50%–22.78%, respectively, WUE_l and WUE_i were significantly increased by 7.19%–35.83% and 10.13–31.35% under S1 treatment. The addition of biochar lessened the decrease in A_n, T_r, g_s, C_i under S1 in relation to S0. In particular, WSP biochar was more favorable in mitigating this adverse impact of salt stress in terms of gas exchange compared to SWP biochar. A_n was increased by 0.81%–27.57% with biochar addition compared to CK regardless of other factors. Under S0 combined with FI, the application of biochar resulted in the reduction of WUE_l and WUE_i compared toCK. However, this reduction was reversed under S1 combined with FI, i.e., the application of biochar under S1 treatment increased WUE_l and WUE_i by 5.30%–5.92% and 2.90%–7.83% compared toCK. Furthermore, A_n, T_r, g_s, C_i of cotton plants had the highest values under FI compared to DI and PRD. A_n increased by 0.89%–8.15% under PRD, while Tr, gs, and Ci decreased compared to DI. As a result, both WUE_l and WUE_i were higher under PRD than FI and DI. Moreover, there was a remarkable interactive effect of [S]× [B] on A_n, [S]× [IR] on T_r, and [S]× [B]× [IR] on T_r and WUE_l (Table 5).

3.3. Leaf morphological traits and chlorophyll content

As shown in Table 2, the LA was significantly reduced by 24.41%–57.12% under S1 compared to S0. And compared with FI, the LA under DI and PRD decreased by 6.29%–38.24% and 2.96%–32.32%, respectively. The addition of biochar increased LA by 20.90%–37.60% compared to CK under S1. Salt stress and deficit irrigation increased the ChlD, but biochar amendment decreased the ChlD. The ChlD was increased by 12.73%–25.05% under S1 compared with S0. The ChlD under DI and PRD were increased by 1.60%–11.78% and 1.85%–8.62% compared to FI, respectively. The ChlT was reduced by salinity, but the ChlT was increased by biochar amendment under S1. Overall, both salt stress and deficit irrigation (DI and PRD) resulted in an increase in LMA, and biochar addition resulted in a decreasing trend in LMA (except for [S0, SWP, DI]).

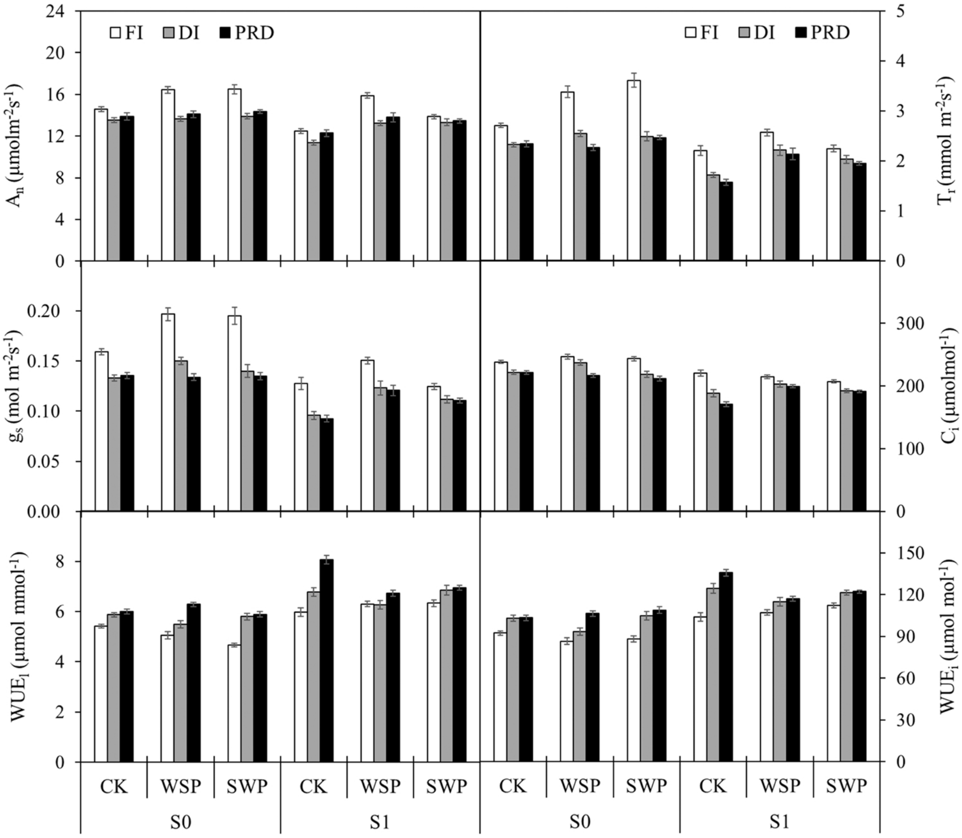

Fig. 3. Gas exchange parameters (A_n, g_s, T_r, C_i), intrinsic and instantaneous water use efficiency (WUE_i and WUE_l) of cotton plants subjected to three irrigation treatments (FI, DI, PRD), three biochar amendment treatments (CK, WSP, SWP) and two salinity treatments (S0 and S1). The three-way ANOVA for salt, biochar, and irrigation and the interaction effects between them are presented in Table 5.

Table 2
The leaf area (LA), chlorophyll density (ChlD), total chlorophyll content (ChlT), and leaf mass per area (LMA) of cotton plants subjected to three irrigation treatments (FI, DI, PRD), three biochar amendment treatments (CK, WSP, SWP) and two salinity treatments (S0 and S1), and the three-way ANOVA for salt ([S]), biochar ([B]), and irrigation ([IR]) and the interaction effects between them. The values shown in the table are means ± SE (n = 4), Same as below.

Salt	Biochar	Irrigation	LA (cm^{-2})	ChlD (μg cm^{-2})	ChlT (mg plant^{-1})	LMA (g m^{-2})
S0	CK	FI	7278.27 ± 440.83	26.06 ± 0.35	189.93 ± 12.76	60.76 ± 1.59
		DI	4655.37 ± 158.16	27.25 ± 0.59	127.10 ± 6.78	63.39 ± 1.48
		PRD	5081.41 ± 198.14	26.70 ± 0.74	135.87 ± 7.72	61.25 ± 0.70
	WSP	FI	7457.44 ± 225.57	25.04 ± 0.87	186.37 ± 5.84	58.11 ± 0.85
		DI	4605.56 ± 167.79	25.44 ± 0.54	117.21 ± 5.46	59.85 ± 2.35
		PRD	5347.38 ± 165.04	25.50 ± 0.51	136.15 ± 2.47	58.92 ± 0.29
	SWP	FI	7573.84 ± 213.43	24.79 ± 0.93	187.89 ± 9.90	57.71 ± 2.52
		DI	4898.44 ± 250.95	26.60 ± 0.81	130.21 ± 7.29	64.09 ± 1.32
		PRD	5125.92 ± 213.63	26.93 ± 1.03	138.07 ± 8.46	58.46 ± 0.44
S1	CK	FI	3121.26 ± 298.31	31.26 ± 0.38	97.76 ± 9.82	64.55 ± 2.16
		DI	2789.07 ± 230.61	33.06 ± 1.12	92.96 ± 11.11	65.03 ± 5.94
		PRD	2677.62 ± 165.22	33.39 ± 1.05	89.87 ± 7.87	66.44 ± 5.32
	WSP	FI	3773.64 ± 122.05	28.23 ± 1.28	106.05 ± 1.32	63.08 ± 3.77
		DI	3481.24 ± 179.32	31.55 ± 0.87	110.22 ± 8.32	63.81 ± 2.88
		PRD	3293.95 ± 83.08	30.23 ± 1.02	99.67 ± 4.92	63.49 ± 1.62
	SWP	FI	3796.76 ± 277.31	30.00 ± 0.72	114.08 ± 9.40	61.62 ± 3.48
		DI	3558.04 ± 98.00	30.49 ± 0.37	108.57 ± 4.25	61.71 ± 0.61
		PRD	3684.46 ± 175.20	31.28 ± 0.41	115.31 ± 6.20	62.27 ± 3.60
ANOVA factor						
Salt ([S])			***	***	***	*
Biochar ([B])			***	***	ns	ns
Irrigation ([IR])			***	**	***	ns
[S]× [B]			*	ns	ns	ns
[S]× [IR]			***	ns	***	ns
[B]× [IR]			ns	ns	ns	ns
[S]× [B]× [IR]			ns	ns	ns	ns

Notes: *, **, *** indicates significant at $P < 0.05$, $P < 0.01$, $P < 0.001$ level, respectively, 'ns' indicates no significance.

3.4. Dry biomass of different organs, dry biomass allocation ratio, and yield elements

It was clearly seen that the TDB of cotton plants had maximum (164.92 g plant^{-1}) and minimum (60.56 g plant^{-1}) values under [S0, WSP, FI] and [S1, CK, DI], respectively (Table 3). Moreover, the dry biomass of different organs was significantly influenced by [B], [S], and [IR]. In detail, compared to S0, salt stress decreased the dry biomass of cotton plants in root, stem, leaf, husk, seed cotton, boll and TDB by 4.09%–37.79%, 34.63%–53.70%, 19.66%–54.42%, 27.30%–47.72%, 17.81%–47.22%, 20.51%–47.36%, and 27.41%–47.52%, respectively. Similarly, compared to FI, deficit irrigation made them decrease by 11.03%–36.57%, 1.16%–36.54%, 1.82%–28.57%, 8.46%–34.62%, 6.82%–33.09% and 6.43%–26.30%, respectively. Moreover, under S1, biochar amendment resulted in significant increases in RDB, SDB, LDB, HDB, SCY, BDB and TDB by 15.88%–41.12%, 28.73%–45.11%, 14.84%−30.15%, 29.10%–51.61%, 33.59%–58.22%, 32.46%–55.21% and 25.64%–40.11%, respectively. Furthermore, SCY and BDB were increased by 2.14%–7.12% and 0.49%–6.76% under WSP relative to SWP.

On the other hand, although salt stress reduced the TDB and dry biomass of different organs, salinity made the dry biomass allocation ratio of cotton plants in roots, seed cotton and boll ([RDB]$_a$, [SCY]$_a$ and [BDB]$_a$) were increased by 17.16%–58.08%, 1.18%–23.47% and 0.76%–19.48% compared to S0, respectively. Moreover, under S1, biochar amendment increased [SCY]$_a$ and [BDB]$_a$ by 5.07%–14.39% and 3.85%–12.54%, respectively, but decreased [RDB]$_a$ by −0.83%–7.61% ([S1, WSP, PRD] increased [RDB]a by 0.83% compared to [S1, CK, PRD]). Compared to FI, [RDB]$_a$ was increased by 32.25%–41.92% and 40.90%–44.71% under DI and PRD under S0, and by 5.18%–11.70% and 11.72%–21.92% under S1, respectively.

3.5. Harvest indicator, water consumption and water use efficiency at plant and yield levels

The WU, WUE$_y$, and WUE$_p$ of cotton plants were influenced by [B], [S], and [IR] (Table 5, Fig. 4). Compared to S0, salt stress decreased WU by 42.98%–52.19%, but increased WUE$_y$ and WUE$_p$ by 3.25%–61.26% and 2.65%–34.88%. The biochar amendment increased WU by 17.38%–26.50%. At the same time, biochar amendment decreased WUE$_y$ and WUE$_p$ under S0, but increased WUE$_y$ and WUE$_p$ under S1. Interestingly, compared to FI, DI resulted in an increase in WUE$_y$ and WUE$_p$ under S0 (except [S0, SWP, DI] in WUE$_p$), but at the same time resulted in a decrease in WUE$_y$ and WUE$_p$ under S1. Nevertheless, PRD led to an increase in WUE$_y$ and WUE$_p$ compared to DI and FI, both under S0 and S1. Also, WUE$_y$ and WUE$_p$ of cotton plants had minimum values under [S1, CK, DI] and maximum values under [S1, WSP, PRD]. Further, there was an interaction between [S]× [B] for WU, WUE$_y$, WUE$_p$ and [S]× [IR] for WU, WUE$_y$, respectively (Table 5).

3.6. PCA analysis of growth and physiological characteristics of cotton plants

The PCA plot of cotton plants grown in non-saline-stressed (S0) and salinity-stressed (S1) is shown in Fig. 5. PC1 and PC2 explained 56.50% and 15.15% of the total variables, respectively. The PCA plot reveals that S0 and S1 are clearly divided into two distinct clusters, where the S0 cluster towards the right side and the S1 cluster towards the left side. S1 are clustered in the same direction as the vectors for LMA, ChlD, HI and WUE at different levels (red vectors). However, S0 are clustered towards the LA, ChlT, WU, [LDB]$_a$, gas exchange parameters (green vectors), different organ dry biomass (black vectors) and yield components (yellow vectors). Moreover, the distribution under S1 cluster are more dispersed while S0 cluster are more concentrated. This indicates that the performance of the treatments under S1 conditions have greater differences in LMA, ChlD, HI and WUE at different levels compared to the treatments under S0 conditions in the ChlT, WU, [LDB]$_a$, gas exchange parameters, different organ dry biomass and yield components. The ChlD had a significant negative correlation with LA and ChlT, while it had a positive correlation with LMA.

Furthermore, we analyzed the PCA plots of [B] and [IR] on the growth and physiology under S0 and S1, respectively (Fig. 6). It can be clearly seen that the clustering of different [B] treatments under S0 have a larger overlap, whereas the clustering of different [B] treatments under S1 are more dispersed. Correspondingly, PC1, PC2 under S0 and S1 explained 39.51%, 19.16% and 47.16%, 13.21% of the total variables, respectively.

For S0, the clustering of CK tend to be more towards the upper left corner in line with the orientation of ChlD and LMA vectors. However, the clustering of WSP tend to be distributed in the lower right corner in the same direction as the yield components (yellow vectors). The clustering of SWP are basically distributed in the middle of the CK cluster and the WSP cluster. Moreover, the clustering of different [IR] treatment

Table 3
The root dry biomass (RDB), stem dry biomass (SDB), leaf dry biomass (LDB), husk dry biomass (HDB), seed cotton yield (SCY), boll dry biomass (BDB, BDB=HDB+SCY), lint cotton yield (LCY), cotton seeds biomass (CSB, CSB=SCY-LCY), boll number (BN), and total dry biomass (TDB, TDB=RDB+SDB+LDB+BDB) of cotton plants as influenced by salt ([S]), biochar ([B]), and irrigation ([IR]) and the interaction effects between them.

Salt	Biochar	Irrigation	RDB (g plant^{-1})	SDB (g plant^{-1})	LDB (g plant^{-1})	HDB (g plant^{-1})	SCY (g plant^{-1})	BDB (g plant^{-1})	LCY (g plant^{-1})	CSB (g plant^{-1})	BN (plant^{-1})	TDB (g plant^{-1})
S0	CK	FI	6.44 ± 0.78	61.42 ± 3.86	44.16 ± 2.60	10.43 ± 0.42	29.79 ± 0.72	40.22 ± 1.01	12.76 ± 0.16	17.03 ± 0.58	7.25 ± 0.63	152.23 ± 7.78
		DI	6.83 ± 0.20	45.88 ± 1.94	29.98 ± 1.21	8.89 ± 0.61	23.82 ± 1.12	32.71 ± 1.64	9.35 ± 0.30	14.47 ± 1.11	6.00 ± 0.41	115.39 ± 4.47
		PRD	7.58 ± 0.79	50.63 ± 2.67	31.13 ± 1.29	9.81 ± 0.49	26.13 ± 1.86	35.94 ± 2.34	11.47 ± 0.33	14.67 ± 1.95	6.75 ± 0.48	125.27 ± 3.89
	WSP	FI	7.19 ± 0.33	67.81 ± 1.30	43.39 ± 1.86	12.43 ± 0.64	34.12 ± 0.84	46.54 ± 1.43	17.24 ± 0.83	16.88 ± 0.69	9.50 ± 0.87	164.92 ± 3.92
		DI	7.46 ± 0.29	57.40 ± 3.10	27.53 ± 1.32	10.35 ± 0.17	26.40 ± 0.41	36.75 ± 0.52	13.30 ± 1.08	13.11 ± 0.75	8.00 ± 0.41	129.14 ± 4.19
		PRD	8.73 ± 0.45	60.33 ± 1.00	30.77 ± 0.77	11.66 ± 0.32	30.53 ± 0.36	42.19 ± 0.57	15.60 ± 0.27	14.94 ± 0.12	8.50 ± 0.50	142.01 ± 1.74
	SWP	FI	6.54 ± 0.26	66.54 ± 5.78	43.83 ± 2.99	12.02 ± 0.67	32.41 ± 1.56	44.43 ± 2.23	16.14 ± 0.37	16.27 ± 1.32	9.50 ± 0.65	161.34 ± 9.19
		DI	6.91 ± 0.51	53.95 ± 1.89	31.31 ± 1.17	10.14 ± 0.47	25.49 ± 1.40	35.63 ± 1.67	12.26 ± 0.39	13.23 ± 1.12	8.50 ± 0.29	127.80 ± 4.76
		PRD	7.85 ± 0.66	54.75 ± 0.83	29.96 ± 1.21	11.34 ± 0.74	28.62 ± 0.90	39.95 ± 1.59	14.75 ± 0.20	13.87 ± 0.76	8.50 ± 0.29	132.51 ± 3.99
S1	CK	FI	5.42 ± 0.45	30.55 ± 1.69	20.13 ± 1.94	6.50 ± 0.58	19.23 ± 1.34	25.73 ± 1.59	6.39 ± 0.49	12.84 ± 0.92	5.50 ± 0.29	81.82 ± 5.29
		DI	4.25 ± 0.41	21.24 ± 0.91	17.85 ± 1.12	4.65 ± 0.27	12.57 ± 0.66	17.22 ± 0.58	3.85 ± 0.37	8.72 ± 0.42	4.25 ± 0.25	60.56 ± 1.87
		PRD	5.07 ± 0.18	25.07 ± 1.58	17.56 ± 0.68	5.58 ± 0.52	15.39 ± 0.88	20.96 ± 1.35	5.68 ± 0.37	9.71 ± 0.90	4.50 ± 0.29	68.65 ± 3.40
	WSP	FI	6.81 ± 0.22	44.32 ± 2.07	23.94 ± 2.15	8.87 ± 0.33	27.52 ± 0.46	36.39 ± 0.78	10.23 ± 0.39	17.29 ± 0.48	6.25 ± 0.25	111.46 ± 4.16
		DI	5.60 ± 0.24	28.12 ± 1.60	22.12 ± 0.91	6.76 ± 0.40	19.55 ± 0.50	26.31 ± 0.80	7.68 ± 0.55	11.87 ± 0.48	5.00 ± 0.41	82.15 ± 2.41
		PRD	7.15 ± 0.40	35.26 ± 1.28	20.63 ± 0.12	8.19 ± 0.59	24.34 ± 0.75	32.54 ± 1.32	9.83 ± 0.35	14.51 ± 0.54	6.00 ± 0.41	95.57 ± 2.90
	SWP	FI	6.28 ± 0.39	39.32 ± 0.67	23.12 ± 0.62	8.40 ± 0.43	25.69 ± 1.07	34.09 ± 1.43	9.08 ± 0.51	16.61 ± 0.58	6.25 ± 0.48	102.80 ± 2.13
		DI	5.70 ± 0.53	28.86 ± 1.63	21.97 ± 0.78	7.04 ± 0.24	19.14 ± 1.26	26.18 ± 1.47	6.73 ± 0.57	12.42 ± 0.76	4.75 ± 0.25	82.71 ± 2.06
		PRD	6.86 ± 0.21	34.72 ± 1.60	22.85 ± 1.14	8.24 ± 0.52	23.52 ± 0.70	31.76 ± 1.21	8.79 ± 0.54	14.73 ± 0.33	6.00 ± 0.71	96.19 ± 2.31
ANOVA factor												
Salt ([S])			***	***	***	***	***	***	***	***	***	***
Biochar ([B])			***	***	*	***	***	***	***	***	***	***
Irrigation ([IR])			***	***	***	***	***	***	***	***	***	***
[S]× [B]			ns	ns	*	ns	***	**	ns	***	ns	*
[S]× [IR]			*	ns	***	ns	ns	ns	ns	ns	ns	*
[B]× [IR]			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
[S]× [B]× [IR]			ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Notes: *, **, *** indicates significant at P < 0.05, P < 0.01, P < 0.001, respectively, 'ns' indicates no significance.

under S0 are clearly distributed on the left and right sides. That is, the FI treatment are mainly distributed on the right side with the same direction as the gas exchange parameters and yield components. However, the DI treatment and PRD treatment are mainly distributed on the left side in the same direction as dry biomass allocation rate and WUE. Besides, compared to the DI treatment which are more inclined to the upper left corner, the clustering of PRD treatment are mainly distributed in the lower left corner in line with the orientation of the vectors for WUE_y, $[BDB]_a$, $[SCY]_a$, HI and RSR.For S1, biochar amendments, especially WSP amendment, effectively improved the growth and physiological characteristics of cotton plants compared to CK. This is mainly evidenced by the fact that the clustering of WSP treatment and SWP treatment are mainly distributed on the left side of the PCA plot in line with the orientation of the vectors for dry biomass and gas exchange parameters. However, the clustering of CK treatment is mainly distributed on the right side in the same direction as the vectors for LMA and ChlD. Besides, the clustering of WSP treatment were more predominantly distributed in the upper left corner in line with the orientation of the vectors for Tr, gs and Ci compared to SWP treatment. Considering the [IR] factor jointly, the clustering of FI treatment are mainly distributed in the upper part of the PCA plot, and the clustering of DI treatment and PRD treatment are mainly distributed in the lower side. And it can be clearly seen that the clustering of [S1, WSP, PRD] treatment indicates higher $[SCY]_a$, $[BDB]_a$, WUE_y, WUE_p, HI and GOT. However, the clustering of [S1, CK, PRD] treatment indicate higher LMA, ChlD, WUE_l and WUE_i.

4. Discussion

In order to investigate the effects of biochar amendment and different irrigation regimes on the growth, physiology and water use efficiency of cotton plants under salt stress, we determined the leaf gas exchange parameters, plant growth, dry biomass accumulation and partitioning, yield components, plant water use and WUE as influenced by the treatments. Below, details of the effects of the biochar amendment and irrigation treatment on cotton plants under slat stress were discussed.

4.1. *Effects of biochar and different irrigation regimes on leaf gas exchange under salt stress*

Similar to the previous findings (Moles et al., 2016; Zhang et al.,

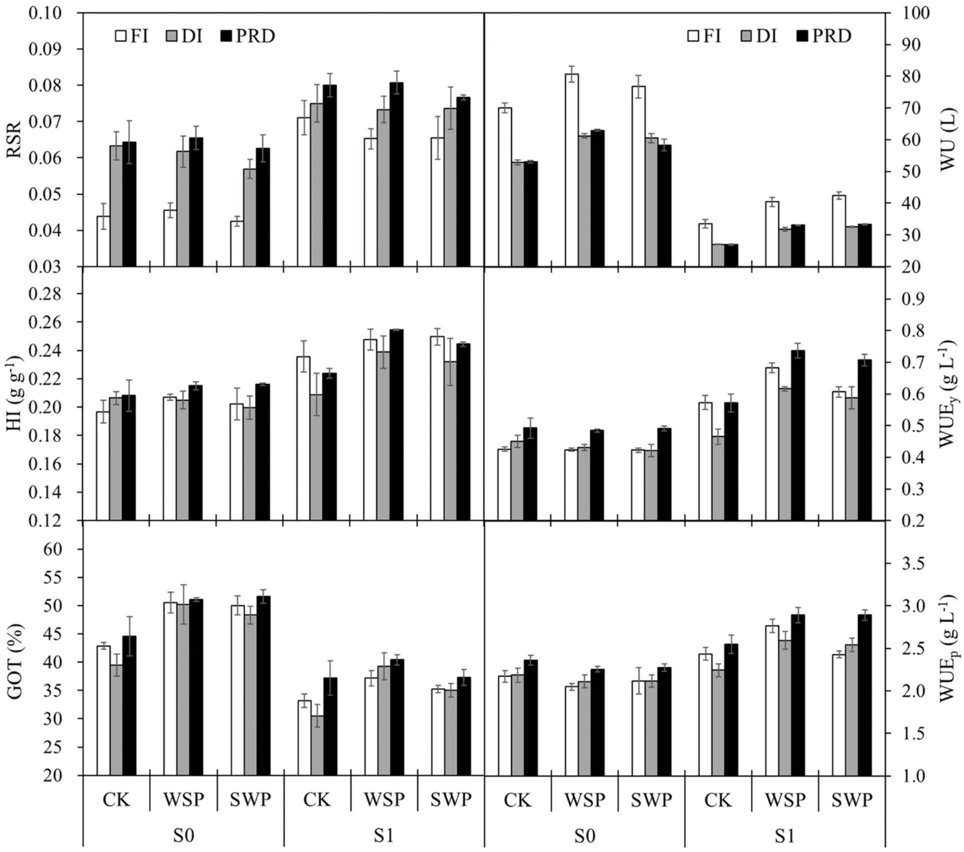

Fig. 4. Root to shoot ratio (RSR), harvest index (HI), lint ginning out turn (GOT), water use (WU), water use efficiency for seed cotton yield (WUE_y) and water use efficiency for total dry biomass (WUE_p) of cotton plants subjected to three irrigation treatments (FI, DI, PRD), three biochar amendment treatments (CK, WSP, SWP) and two salinity treatments (S0 and S1). The three-way ANOVA for salt, biochar, and irrigation and the interaction effects between them are presented in Table 5.

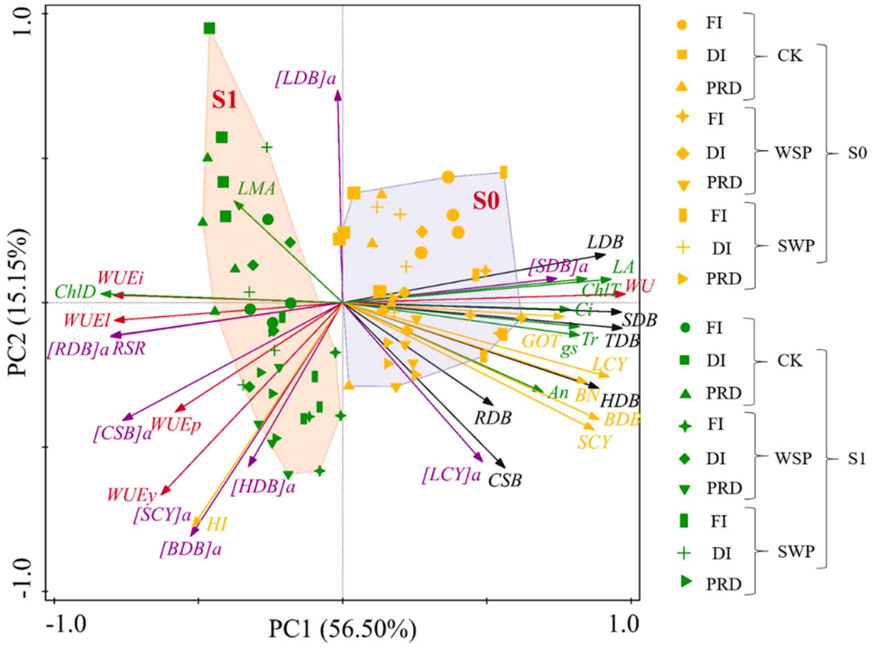

Fig. 5. Plot of principal component analysis (PCA) of cotton plants grown in non-saline-stressed (S0) and salinity-stressed (S1) without biochar (CK) or with biochar amendment (WSP; SWP) under three irrigation treatments (FI, DI, PRD). Green vectors are related to leaf morphological characteristics and gas exchange parameters (LA, LMA, ChlD, ChlT, A_n, T_r, g_s, C_i), purple vectors are related to the dry biomass allocation rate of different organs and root to shoot ratio ($[RDB]_a$, $[SDB]_a$, $[LDB]_a$, $[HDB]_a$, $[SCY]_a$, $[BDB]_a$, $[LCY]_a$, $[CSB]_a$, RSR), red vectors are related to water consumption and water use efficiency at different levels (WU, WUE_i, WUE_l, WUE_p, WUE_y), black vectors are related to different organ dry biomass (RDB, SDB, LDB, HDB, CSB, TDB), and yellow vectors are related to yield components, harvest index and lint ginning out turn (SCY, BDB, LCY, BN, HI, GOT).

2018; Zheng et al., 2009; Zhou et al., 2022), in our experiments, A_n and T_r were significantly reduced under salt stress regardless of other factors, accompanied by a decrease in g_s and C_i, indicating that the reduction of A_n was mainly due to the stomatal limitation (Von Caemmerer and Farquhar, 1981). In fact, under salt stress, the decrease in g_s and T_r would facilitate plants to reduce water loss and decrease salt uptake as a biophysical strategy for plants to respond to stress (Chaves et al., 2009; Moles et al., 2016). Meanwhile, it was observed that, in relation to S0,

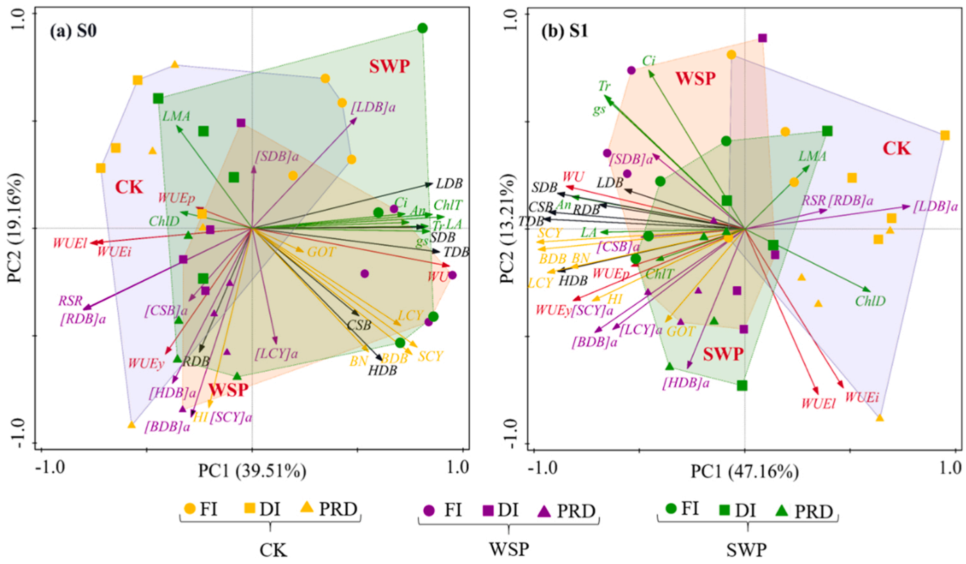

Fig. 6. Plot of principal component analysis of cotton plants grown in (a) non-saline-stressed (S0) and (b) salinity-stressed (S1) without biochar (CK) or with biochar amendment (WSP; SWP) under three irrigation treatments (FI, DI, PRD). Green vectors are related to leaf morphological characteristics and gas exchange parameters (LA, LMA, ChlD, ChlT, A_n, T_r, g_s, C_i), purple vectors are related to the dry biomass allocation rate of different organs and root to shoot ratio ($[RDB]_a$, $[SDB]_a$, $[LDB]_a$, $[HDB]_a$, $[SCY]_a$, $[BDB]_a$, $[LCY]_a$, $[CSB]_a$, RSR), red vectors are related to water consumption and water use efficiency at different levels (WU, WUE_i, WUE_l, WUE_p, WUE_y), black vectors are related to different organ dry biomass (RDB, SDB, LDB, HDB, CSB, TDB), and yellow vectors are related to yield components, harvest index and lint ginning out turn (SCY, BDB, LCY, BN, HI, GOT).

WUE_l and WUE_i were increased under S1 treatment, in agreement with the results of Moles et al. (2016), who discovered there was a significant increase in WUE and WUE_i at 300–450 mM NaCl in relation to 0 mM NaCl. This is mainly due to the fact that salt stress caused greater reduction of g_s and T_r than A_n, resulting in a significant increase of WUE_l and WUE_i (Ma et al., 2021a). And Liao et al. (2022) showed that the accumulation of osmolytes (e.g., proline and glycine betaine), which could sustain the water status and A_n of the leaf and improve WUE_i. On the other hand, in agreement with the results of Guo et al. (2021), we also discovered that biochar improved the gas exchange parameters of cotton plants under S0 treatment, and the addition of biochar effectively prevented the reduction of gas exchange parameters (A_n, T_r, g_s and C_i) caused by salt stress, which could have also led to an increase in RDB, SDB, LDB, BDB and TDB. Interestingly, biochar amendment caused a decrease in WUE_l and WUE_i while increasing A_n, T_r and g_s under S0 treatment, which is in accordance with the results of Liu et al. (2021).

In addition, DI and PRD caused a significant decrease in gas exchange parameters compared to FI, but A_n was slightly greater under PRD compared to DI though with even further lowered T_r and g_s, resulting in greater WUE_l and WUE_i under PRD, in line with the results revealed in some previous reports (Davies et al., 2000; Du et al., 2007; Du et al., 2006; Yongjun et al., 1999). In fact, we found that the PRD treatment possessed higher A_n compared to the DI treatment both under S1 treatment and S0 treatment, indicating that the PRD enhanced salt tolerance and drought resistance in relation to the DI strategy under the same irrigation volume. The reasons for this could be: (I) the PRD strategy caused non-uniform salt concentration in the soil compared to the uniform salt concentration under DI treatment, which could have improved the salt stress resistance of plants (Kong et al., 2017). It has been suggested that the roots in the high salt concentration side could sense the high salt concentration and up-regulated the expression of genes related to sodium efflux (*SOS1, SOS2, PMA1,* and *PMA2*) and water uptake (*PIP1* and *PIP2*) in the roots on the low salt concentration side, thus enhancing Na^+ efflux and water uptake in the roots of the low-salt concentration side, reducing the Na^+ content in the roots and alleviating osmotic stress (Kong et al., 2012, 2016). In addition, the salinity on the high-salt side could induce expression of key genes for cytokinin synthesis in the roots of the low-salt side, which increased the indole acetic acid (IAA) content and promoted the root growth, favoring the root system to absorb more nutrients and improving the salt tolerance of plants (Kong et al., 2016). (II) the PRD strategy might induce the up-regulation of the expression of jasmonic acid (JA) synthesis genes, increasing the JA content in the leaves. And the JA is transported to the roots of the wetting side through the phloem, which increased the plasma membrane intrinsic protein (*PIP*) gene expression and improved the hydraulic conductance of the roots, which made the roots absorb more water and improved the drought resistance (Luo et al., 2019).

4.2. *Effects of biochar and different irrigation regimes on cotton leaf characteristics under salt stress*

Chlorophyll is an important pigment associated with photosynthesis. Chlorophyll content can indicate the nitrogen nutritional status of crops (Akhtar et al., 2014). In accordance with the results of Ma et al. (2021a) on cotton and Yang et al. (2020) on quinoa, our experiments showed that the ChlD of cotton leaves was elevated under salt stress regardless of other factors (Table 3). A possible explanation is that mild salt stress (200 mM NaCl) stimulated the expression of genes encoding NO_3^- transporters in cotton roots and shoots (Guo et al., 2019), upregulated the activity and/or number of NO_3^- transporters (Miranda-Apodaca et al., 2020), and thereby increased the nitrogen uptake of leaves (Razzaghi et al., 2012). As a result, the nitrogen content in cotton leaves was elevated under salt stress, and ChlD was also increased due to the positive correlation between nitrogen content and chlorophyll (Akhtar et al., 2014). However, the reports of Akhtar et al. (2015) indicated that the nitrogen content and ChlD of potato leaves were reduced under salt stress, likewise Mehmood et al. (2020) also suggested that the ChlD in soybean leaves was reduced under salt stress. The discrepancy between the different experiments could be attributed to the different salt tolerance of the crops examined. According to previous reports, the plants are divided into five groups according to their sensitivity to salinity stress, i.e., sensitive, moderately sensitive, moderately tolerant, tolerant, and yields unacceptable for most crops. And cotton is believed to be a salt-tolerant crop (Brady et al., 2008; Maas and Grattan, 1999). Although the authors set the salinity level to be lower compared to our experiments, it is likely that those salinity level results in the inhibition of chlorophyll synthesis or the degradation of chlorophyll by chlorophyllase (Santos, 2004) due to oxidative stress or photo-protection mechanisms (Elsheery and Cao, 2008; Smirnoff, 1996; Taïbi et al., 2016). On the other hand, here the DUALEX SCIENTIFIC instrument used to measure chlorophyll works primarily by detecting visible and near-infrared radiation transmitted through leaves (Rezzouk et al., 2020), which makes the ChlD potentially affected by leaf thickness and/or density. If the leaves were denser or thicker, it is possible to result in higher values of chlorophyll per unit leaf area (Yousfi et al., 2009). Although we did not directly measure leaf thickness, the results showed that salt stress increased LMA (Table 2), which indicates an enhanced leaf thickness and density (Garnier and Laurent, 1994; Poorter et al., 2009), resulting in an increased chlorophyll content per unit leaf area under salt stress. In fact, it has been previously demonstrated that moderate salinity stress leads to thicker or denser leaves due to more compacted mesophyll (Zhang et al., 2022), which in turn increases ChlD (Passioura and Munns, 2000; Yousfi et al., 2009). Similar to the results of our study, Eisa et al. (2012) also indicated that salt stress increased the LMA of quinoa, and Cerovic et al. (2015) revealed that salt stress increased the nitrogen content and the ChlD of leaves.

In accordance with the previous findings (Puangbut et al., 2017; Ronga et al., 2019), our study indicated that the ChlD of cotton leaves was also increased under DI and PRD. This was attributed to the fact that plants have thicker leaves and/or higher LMA when exposed to drought stress (Arunyanark et al., 2008; Rao and Wright, 1994; Wright et al., 1993). Similar to the results of Sun et al. (2014b), here the plants possessed higher LMA under DI treatment and/or PRD treatment compared to FI treatment. Furthermore, the biochar amendment tended to decrease ChlD, probably because of the fact that the higher C/N ratio of biochar led to N immobilization in the soil, which in turn reduces the N uptake by plants (Lehmann et al., 2003). Besides, biochar relies on the electrostatic attractive force to adsorb inorganic nitrogen such as ammonium and nitrate nitrogen from the soil, thus reducing the availability of nitrogen for plant uptake hereby decreasing the concentration of nitrogen in the plants (Nguyen et al., 2017). Furthermore, we noticed that biochar addition decreased LMA (except for [S0, SWP, DI] treatment) while increasing LA, so this could have also caused ChlD decreases under biochar addition. Consistent with this, an earlier study proposed that biochar addition promoted more assimilates to be transported from leaves to reproductive organs, resulting in lower non-structural carbohydrate (e.g., starch, sucrose, and hexose) in leaves and therefore lower LMA (Hu et al., 2015). More intriguingly, under salt stress, biochar amendment (especially WSP biochar) resulted in greener leaves at later growth stages compared to those without biochar amendment, suggesting that the application of biochar might have delayed the senescence of the plants under salt stress. This seems to have a huge significance in applying biochar in saline areas, yet the underlying mechanisms need to be explored in future research.

4.3. *Effects of biochar and different irrigation regimes on dry biomass and dry biomass allocation under salt stress*

Similar to previous studies (Graber et al., 2010; Lashari et al., 2013; Quilliam et al., 2013), our experiments showed that biochar improved the growth of cotton plants and seed cotton yield, especially under salt stress where the increase in seed cotton yield was more pronounced with biochar addition compared to no biochar addition. Moreover, compared with SWP biochar, WSP biochar had a more favorable impact on plant growth, which may be contributed to its higher ash content (Table 1), and the mineral nutrients in the ash can supply some of the nutrients to the soil (Yuan et al., 2016). Furthermore, in our experiments, we found that the root and shoot dry biomass of cotton plants were reduced under salt stress, which is consistent with the results of previous studies (Chen et al., 2009; Hammer et al., 2015). The reason behind this could be that the salts in the soil solution reduced the soil water potential and decreased the water uptake by the plants, causing physiological drought in the plants (Mahajan and Tuteja, 2005). Moreover, the higher Na^+ concentration in the soil leads to an increase in Na^+ concentration in the plant, which is toxic to cells and induces osmotic stress, and consequently hinders the uptake of nutrient ions, such as K^+ and Ca^{2+}, and inhibits plant growth (Munns et al., 2006). However, the addition of biochar into soil was effective in alleviating the reduction of dry biomass caused by salt stress. The reason for this could be, on the one hand, the addition of biochar increased the soil water holding capacity and improved the water status of plants (Akhtar et al., 2014; Saifullah et al., 2018; Yang et al., 2020); on the other hand, the adsorption of biochar to Na^+ that reduces the concentration of Na^+ in the soil solution, reducing Na^+ uptake by plants (Hammer et al., 2015). Moreover, the addition of biochar brought more nutrients such as exchangeable potassium to the soil, which also improved the nutrient status of plants (Wu et al., 2019). This effect of biochar in alleviating salinity stress was also found in previous studies (Hammer et al., 2015; Lashari et al., 2013; Lashari et al., 2015; Thomas et al., 2013). Although the dry biomass of shoot and root was decreased under salt stress, $[RDB]_a$, $[SCY]_a$, $[BDB]_a$, $[CSB]_a$ were increased and $[SDB]_a$, $[LCY]_a$ were decreased under S1 treatment compared to S0 in our experiments. Similarly, Zhang et al. (2022) also found that NaCl treatment increased the dry biomass allocation ratio in the roots and reduced the dry biomass allocation ratio in the leaves of tomato plants. These results suggest that dry biomass allocation was more toward organs such as roots and bolls under salt stress, but reduced dry biomass transport to the stems or leaves. This may be due to salt stress-induced decrease in zeatin riboside in the leaves, allowing an increase in cytokinin in the leaves (Albacete et al., 2008), which may lead to a shift in dry biomass allocation from the leaves to the bolls under salt stress (Ma et al., 2021b). Meanwhile, the auxin indole-3-acetic acid (IAA) content in the leaves was reduced but accumulated in the roots, which increased the auxin/cytokinins ratio and promoted the elongation and growth of root cells (Sachs, 2005). This could be the reason that $[RDB]_a$, $[SCY]_a$, $[BDB]_a$, $[CSB]_a$ were increased and $[SDB]_a$ was decreased under salt stress. As for why it decreased $[LCY]_a$ but increased $[CSB]_a$, it could be due to that the assimilates were distributed differently within the cotton boll, with more assimilates being distributed into the seeds rather than the fibers (Gao et al., 2020a; Tang et al., 2017; Ul-Allah et al., 2021). Yet, a more detailed mechanistic explanation for this has not been reported, and we speculate that it may be related to the differential expression of sucrose synthase (SuSy) under salt stress, causing differences in carbon partitioning between fibers and seeds (Ruan et al., 1997). Nevertheless, based on previous reports, we can affirm that the differences in dry biomass allocation in different organs of plants are mainly governed by phytohormones (Sachs, 2005). In addition, in our experiment, we found that biochar addition decreased $[LDB]_a$ but increased $[BDB]_a$, regardless of other factors. This was mainly due to the fact that biochar addition increased the K^+ content in the soil, and the increased K^+ content could have promoted carbohydrate metabolism in the leaves by decreasing the starch, sucrose and hexose content in the leaves (Hu et al., 2015), which facilitated the translocation of sucrose from the leaves to the cotton boll and increased the boll biomass (Ju et al., 2021).

Earlier studies have shown that the RSR tends to increase when plants are subjected to certain degree of salt stress (Albacete et al., 2008; Lovelli et al., 2012; Moles et al., 2016). In good agreement with this, here we also found an increase in the RSR under salt stress (Table 4). This could be due to the inhibition of shoot growth while root growth was maintained when the plants subjected to salt stress. Consequently, this strategy of increasing the distribution of photosynthetic products to the sink (e.g., roots, bolls, and cotton seeds) and decreasing the distribution to the source (e.g., leaves and stem) also facilitates that plants better escape osmotic stress under salt treatment (Lynch, 1995). On the other hand, our results revealed that biochar addition slightly decreased the RSR compared to the CK treatment under salt stress (Fig. 4), corroborating earlier findings (Drake et al., 2015; Thomas et al., 2013). A possible explanation is that biochar enhanced the WHC of the soil and alleviated the osmotic stress caused by salinity (Lehmann and Rondon, 2006; Oguntunde et al., 2004; Schulz and Glaser, 2012). Moreover, biochar has improved soil porosity and decreased soil bulk density, which would be favorable for water and nutrient acquisition (Ajayi et al., 2016; Asai et al., 2009; Bruun et al., 2014; Obia et al., 2016), facilitating the growth of shoots rather than roots.

As expected, RSR was significantly increased under PRD and DI compared to FI, and RSR was slightly increased under PRD relative to DI (Fig. 4 and Table 5), affirming the conclusion of Wang et al. (2012), who observed that the RSR of maize plants was increased in PRD compared to

Table 4
The dry biomass allocation ratio in roots, stem, leaf, husk, seed cotton, boll, lint cotton, and cotton seeds ([RDB]$_a$, [SDB]$_a$, [LDB]$_a$, [HDB]$_a$, [SCY]$_a$, [BDB]$_a$, [LCY]$_a$, and [CSB]$_a$) of cotton plants as influenced by salt ([S]), biochar ([B]), and irrigation ([IR]) and the interaction effects between them.

Salt	Biochar	Irrigation	[RDB]$_a$ (%)	[SDB]$_a$ (%)	[LDB]$_a$ (%)	[HDB]$_a$ (%)	[SCY]$_a$ (%)	[BDB]$_a$ (%)	[LCY]$_a$ (%)	[CSB]$_a$ (%)
S0	CK	FI	4.19 ± 0.32	40.27 ± 0.74	28.98 ± 0.41	6.88 ± 0.25	19.68 ± 0.81	26.55 ± 0.97	8.44 ± 0.42	11.23 ± 0.41
		DI	5.95 ± 0.34	39.75 ± 0.38	25.98 ± 0.38	7.68 ± 0.30	20.64 ± 0.44	28.32 ± 0.51	8.15 ± 0.45	12.49 ± 0.48
		PRD	6.03 ± 0.52	40.49 ± 2.11	24.84 ± 0.51	7.82 ± 0.24	20.83 ± 1.10	28.65 ± 1.33	9.20 ± 0.54	11.63 ± 1.27
	WSP	FI	4.36 ± 0.18	41.14 ± 0.58	26.28 ± 0.72	7.53 ± 0.29	20.69 ± 0.22	28.22 ± 0.48	10.44 ± 0.31	10.25 ± 0.47
		DI	5.81 ± 0.39	44.36 ± 1.00	21.31 ± 0.54	8.03 ± 0.20	20.50 ± 0.61	28.53 ± 0.79	10.27 ± 0.66	10.23 ± 0.89
		PRD	6.14 ± 0.28	42.48 ± 0.41	21.66 ± 0.38	8.21 ± 0.26	21.50 ± 0.29	29.72 ± 0.49	10.98 ± 0.18	10.52 ± 0.13
	SWP	FI	4.07 ± 0.13	41.03 ± 1.29	27.19 ± 1.08	7.50 ± 0.47	20.22 ± 1.13	27.72 ± 1.60	10.08 ± 0.47	10.14 ± 0.79
		DI	5.38 ± 0.23	42.23 ± 0.42	24.51 ± 0.30	7.93 ± 0.11	19.95 ± 0.82	27.88 ± 0.75	9.61 ± 0.28	10.34 ± 0.69
		PRD	5.89 ± 0.33	41.39 ± 0.94	22.59 ± 0.33	8.53 ± 0.36	21.59 ± 0.09	30.13 ± 0.36	11.15 ± 0.25	10.44 ± 0.27
S1	CK	FI	6.63 ± 0.42	37.42 ± 0.59	24.44 ± 1.04	7.94 ± 0.45	23.57 ± 1.08	31.51 ± 0.80	7.80 ± 0.17	15.78 ± 0.98
		DI	6.97 ± 0.44	35.05 ± 0.77	29.44 ± 1.31	7.65 ± 0.26	20.88 ± 1.50	28.53 ± 1.42	6.42 ± 0.75	14.46 ± 0.92
		PRD	7.41 ± 0.28	36.45 ± 0.57	25.66 ± 0.95	8.09 ± 0.53	22.39 ± 0.33	30.48 ± 0.80	8.30 ± 0.57	14.09 ± 0.85
	WSP	FI	6.13 ± 0.24	39.75 ± 0.86	21.39 ± 1.44	7.96 ± 0.19	24.77 ± 0.72	32.73 ± 0.85	9.23 ± 0.59	15.53 ± 0.17
		DI	6.83 ± 0.32	34.14 ± 1.02	26.92 ± 0.66	8.23 ± 0.40	23.88 ± 1.14	32.11 ± 1.38	9.41 ± 0.87	14.47 ± 0.63
		PRD	7.47 ± 0.27	36.88 ± 0.30	21.64 ± 0.68	8.54 ± 0.38	25.47 ± 0.05	34.01 ± 0.37	10.29 ± 0.22	15.18 ± 0.27
	SWP	FI	6.13 ± 0.51	38.27 ± 0.54	22.48 ± 0.15	8.16 ± 0.30	24.96 ± 0.58	33.12 ± 0.76	8.81 ± 0.32	16.15 ± 0.31
		DI	6.85 ± 0.51	34.82 ± 1.25	26.60 ± 1.06	8.54 ± 0.43	23.19 ± 1.67	31.73 ± 2.05	8.15 ± 0.74	15.04 ± 1.00
		PRD	7.12 ± 0.06	36.09 ± 1.31	23.80 ± 1.38	8.55 ± 0.35	24.44 ± 0.16	32.99 ± 0.51	9.11 ± 0.39	15.33 ± 0.27
ANOVA factor										
Salt ([S])			***	***	ns	*	***	***	***	***
Biochar ([B])			ns	*	***	*	*	**	***	ns
Irrigation ([IR])			***	ns	***	**	ns	*	***	ns
[S]× [B]			ns	ns	ns	ns	ns	ns	ns	*
[S]× [IR]			ns	***	***	ns	ns	ns	ns	ns
[B]× [IR]			ns	ns	ns	ns	ns	ns	ns	ns
[S]× [B]× [IR]			ns	ns	ns	ns	ns	ns	ns	ns

Notes: *, **, *** indicates significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, 'ns' indicates no significance.

Table 5
Output of three-way ANOVA for gas exchange parameters (A_n, g_s, T_r, C_i), intrinsic and instantaneous water use efficiency (WUE_i and WUE_l), root to shoot ratio (RSR), harvest index (HI), lint ginning out turn (GOT), water use (WU), water use efficiency for seed cotton yield (WUE_y) and water use efficiency for total dry biomass (WUE_p) as influenced by salt ([S]), biochar ([B]), and irrigation ([IR]) and the interaction effects between them.

ANOVA factor	A_n	T_r	g_s	C_i	WUE_l	WUE_i	RSR	HI (g/g)	GOT (%)	WU (L $plant^{-1}$)	WUE_y (g L^{-1})	WUE_p (g L^{-1})
Salt ([S])	***	***	***	***	***	***	***	***	***	***	***	***
Biochar ([B])	***	***	***	*	*	*	ns	*	***	***	***	*
Irrigation ([IR])	***	***	***	***	***	***	***	ns	*	***	***	***
[S]× [B]	*	ns	ns	ns	ns	ns	ns	ns	ns	*	***	***
[S]× [IR]	ns	*	ns	ns	ns	ns	ns	ns	ns	***	**	ns
[B]× [IR]	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
[S]× [B]× [IR]	ns	*	ns	ns	*	ns	ns	ns	ns	ns	ns	ns

Notes: *, **, *** indicates significant at $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, 'ns' indicates no significance. The data are shown in Fig. 3.

FI and DI. One reason behind this could be that PRD strategy stimulated the growth of cotton roots (Du et al., 2007), resulting in increased root dry biomass and RSR under PRD compared to DI. This was also confirmed by our findings on RDB and [RDB]$_a$ (Table 3; Table 4).

4.4. *Effects of biochar and different irrigation regimes on yield components and harvest index under salt stress*

Consistent with the results of previous studies (Ali et al., 2004; Farooq et al., 2020; Iqbal et al., 2021b), in the present study drought stress induced by PRD and DI and salt stress decreased LA and BN; whereas the addition of biochar effectively improved LA, BN and yield traits. Moreover, the association among the variables as exemplified in the PCA plot indicated that ChlD has a significant negative correlation with A_n, TDB, LCY, SCY, BN, and BDB (Fig. 5; Fig. 6), meaning that the increase in chlorophyll density in leaves does not reflect the increase in photosynthetic assimilation rate, TDB and yield components (LCY, SCY, BN, BDB). On the contrary, there was a remarkable positive correlation between ChlT and TDB and yield components under S0 or S1, consistent with the findings of Arunyanark et al. (2008). Therefore, we believe that ChlT should be used as an indicator to measure or predict plant biomass and yield, rather than ChlD.

Harvest index (HI) is an essential parameter in determining crop yield (Deguchi et al., 2010). Similar to the studies conducted in the field (Iqbal et al., 2021a), our results also indicated that HI was improved under PRD in relation to FI treatment. In fact, previous studies have shown that long-term and moderate drought stress increases HI, but short-term and severe drought stress reduces HI (Jefferies, 1992, 1995). Moreover, biochar application led to an increasing trend of HI, this was particularly true for plants grown under salt stress, in accordance with the results of Wu et al. (2019). This may be due to the fact that the addition of biochar increased the K^+ content in the soil (Wu et al., 2019) and changed the biomass distribution, increasing the biomass of reproductive organs and decreasing the ratio of leaf to reproductive organ biomass (Hu et al., 2015). The ultimate goal of cotton yield is lint yield, and ginning out turn (GOT) is an important component of cotton fiber yield since it is in direct correlation with lint yield. (Hayat and Bardak, 2020). Our experiments showed that GOT was positively correlated with LCY and SCY (Fig. 5; Fig. 6), in accordance with the results of Saleem et al. (2010). Besides, GOT was significantly increased under the PRD treatment in comparison to FI treatment and DI treatment, which is in agreement with the results of Iqbal et al. (2021b). This may be related to the compensatory effect caused by PRD treatment making more photosynthetic products transported from the leaves to the cotton boll (Du et al., 2007; Hu et al., 2009), but a deeper explanation of this has not been reported and needs further investigations. Moreover, SCY had a positive correlation with BN and BDB, and a negative correlation with GOT, which is also in accordance with the results of Farooq et al. (2020).

4.5. Effects of biochar and different irrigation regimes on WU, WUE$_y$ and WUE$_p$

Numerous studies have indicated that, DI and PRD strategies could improve WUE in comparison to FI strategy (Kang and Zhang, 2004; Olanrewaju et al., 2009; Wei et al., 2018). Likewise, here our results indicated DI and PRD increased WUE$_p$ under S0 treatment compared to FI. The increase of WUE$_p$ could be attributed to either a reduced WU or an sustained/increased A_n or both (Wang et al., 2010). Moreover, the higher LMA of the leaves under DI and PRD in relation to FI could have also linked to the increased WUE$_p$ (Liu and Stützel, 2004; Wright et al., 1994) (Fig. 6a). In addition, it is noteworthy that, when irrigated with the same amount of water, PRD had higher WUE$_p$ and WUE$_y$ than DI, affirming previous results (Du et al., 2007; Du et al., 2008). The underlying physiological mechanisms for increased WUE under PRD treatment is mainly due to the partial stomatal closure induced by the root-to-shoot chemical signal ABA, which reduces transpirational water loss along with the maintenance of photosynthesis rate (Liu et al., 2005), hereby resulting in an increased WUE at different scales. Furthermore, as PRD could activate antioxidant defense mechanisms, it may mitigate the deleterious effects of drought stress on the photosynthetic machinery (Dbara et al., 2016). Also PRD could stimulate the expression of abiotic stress-responsive transcription factors (i.e., *MdbZIP2, MdoMYB121, MdbZIP48, and MdoMYB155*) in order to increase WUE while maintaining yield (Ghafari et al., 2020).

Additionally, it was also evident that plants under salt stress possessed higher WUE$_y$ and WUE$_p$ compared to no salinity treatment disregarding other factors. This may be due to the fact that the LA responds more sensitively to salt stress than TDB (Chartzoulakis, 2005), which allows the plant to preferentially limit leaf extension and reduce T_r under salt stress, while TDB responds more sluggishly to salinity, thus allowing WUE to be elevated under salt stress.

Different conclusions have been reached about the effect of biochar on WUE (Akhtar et al., 2014; Aller et al., 2017; Jeffery et al., 2015; Omondi et al., 2016; Ramlow et al., 2019; Wang et al., 2022), mainly because the impact of biochar on WUE relies on soil type, physicochemical properties and nutrient content of biochar (Gao et al., 2020b; Lehmann et al., 2002; Zimmerman et al., 2011). Our experiments showed that biochar amendment caused a decrease in WUE$_y$ and WUE$_p$ under S0 treatment, and this decrease in WUE may be related to soil type and biochar application rate. It has been shown that biochar causes a decrease in WUE in clay loam soil (Aller et al., 2017; Liu et al., 2021); however, biochar increases WUE in sandy soil or sandy loam soil (Akhtar et al., 2014; Faloye et al., 2019; Kammann et al., 2011; Uzoma et al., 2011; Wang et al., 2022). As the soil used in our experiment was clay loam, thus the conclusions obtained are in line with that reported from previous studies. Additionally, Gao et al. (2020b) discovered that there was an enhancing impact on WUE when biochar was applied at less than 20 t ha^{-1}, while higher biochar application rates showed less improvement in WUE. The 2% (w/w) biochar application rate used in our experiment was almost equivalent to that of 40 t ha^{-1} or 50 t ha^{-1} in the field trial, which could have potentially negatively affected WUE (Gao et al., 2020b). Nevertheless, although the 2% biochar application caused a slight decrease in WUE$_y$ and WUE$_p$ under the S0 treatment, it was interesting that a positive and significant effect of biochar on WUE$_y$ and WUE$_p$ was noticed under the S1 treatment. Such positive impact of biochar amendment on WUE was also reported by Thomas et al. (2013), who revealed that biochar mitigated the effects of salt stress significantly at higher addition rates (50 t ha^{-1}). Further, we found that WUE$_y$ and WUE$_p$ under SWP treatment were lower than those under WSP treatment when the plants grown at S1. This could be due to the higher carbon content of SWP biochar than WSP biochar (Table 1), resulting in a higher C:N ratio of SWP, which in turn leads to the immobilization of nitrogen in the soil and reduces the availability of nitrogen (Sun et al., 2018), and consequently the growth and physiological processes of the plants were negatively affected (Li et al., 2015). This also confirms the notion that the higher the carbon content contained in the biochar, the lower the WUE when amended with biochar (Gao et al., 2020b). Therefore, biochar containing low carbon content produced from such as herbaceous raw materials (straw from wheat, rice, corn, etc.) could be more beneficial in improving WUE than biochar produced with high carbon content raw materials (e.g., wood).

A suitable irrigation strategy combined with biochar can improve WUE (Liu et al., 2021; Safahani Langeroodi et al., 2019). In our current study, PRD improved WUE under the treatment with biochar amendment compared to FI and DI, possibly because the alternating cycle of soil wetting and drying caused by PRD regime result in a reduction of biochar hydrophobicity, which makes the biochar more hydrophilic under the PRD regime (Aller et al., 2017; Das and Sarmah, 2015; Kinney et al., 2012). Besides, PRD treatment could also improve the soil porosity, decrease the soil bulk density, and increase the WHC and soil water supply capacity (Głąb et al., 2016; Sasal et al., 2006; Wu et al., 2022). Furthermore, the 'Birch effect' induced by the PRD regime would accelerate the decomposition and mineralization of nitrogen in the soil, releasing more inorganic N that is accessible to plants (Birch, 1958; Jarvis et al., 2007), which promotes plant growth and improves WUE in turn (Pandian et al., 2016; Wang et al., 2018). Interestingly, unlike the higher WUE$_y$ results for DI and PRD treatment than FI treatment under S0, the WUE$_y$ of DI treatment was lower than that of FI and PRD under S1, although the irrigation amount was the same for DI and PRD treatment. This suggests that the dual stress caused by salinity and soil water deficit significantly inhibited plant growth and reduced WUE$_y$ under DI treatment (Table 5), in accordance with the results of Agbna et al. (2017).

5. Conclusions

In order to alleviate the salinity stress, drought stress and low water use efficiency faced in today's agricultural production, this experiment was designed to investigate the effects of different biochar additions combined with reduced irrigation regimes on the growth, physiology and WUE of cotton plants under salt stress. We concluded from the experiment that both salt stress and reduced irrigation had an inhibitory effect on the growth and physiology of cotton plants. Salt stress increased WUE$_p$ and WUE$_y$ although it reduced gas exchange parameters, leaf area, chlorophyll content, plant water use, dry biomass and yield of cotton plants. Meanwhile, salinity changed the source-sink relationship, causing a decrease in dry biomass allocated to the stems and an increase in dry biomass allocated to roots, bolls and seed cotton, thus increasing the root to shoot ratio and harvest index. The biochar amendment effectively alleviated salt stress and improved gas exchange parameters and leaf area, resulting in lower dry biomass allocation ratio in the roots and higher dry biomass allocation ratio in the bolls, thus improving dry biomass and yield, leading to an increase in lint ginning out turn, harvest index and WUE$_y$ by 0.21%–28.69%, 5.07%–14.39% and 5.83%–32.44%, respectively. Moreover, WSP amendment was superior to SWP amendment in promoting plant growth and yield enhancement. Although biochar had negative and positive effects on WUE$_p$ or WUE$_y$ under non-salt stress and salt stress, respectively, the combined application of PRD and biochar effectively counteracted the reduction in WUE caused by biochar amendment alone under non-salt stress. Also PRD significantly improved WUE at different levels, and PRD had a better promotion effect on plant growth than DI. Therefore, the combined application of WSP biochar with PRD may be a promising method in future agricultural production in terms of salt stress mitigation and WUE enhancement. However, it is important to note that the present conclusions were drawn on the basis of pot experiment in greenhouse and there is still a need to verify the research findings under field conditions.

CRediT authorship contribution statement

Jingxiang Hou: Methodology, Experimental implementation, Data analysis, Manuscript writing. **Jiarui Zhang:** Experimental assistance, Data analysis. **Xuezhi Liu:** Software and Modifications. **Yingying Ma:** Evaluation and Revisions. **Zhenhua Wei:** Experimental supervision and Evaluation. **Heng Wan:** Experimental assistance. **Fulai Liu:** Evaluation and revision, Methodology, Financial support, Supervision and management, Revision and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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