# Partial root-zone drying irrigation improves growth and physiology of tobacco amended with biochar by modulating phytohormonal profile and antioxidant system

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## Abstract

*Background and aims* Biochar is a porous-pyrogenic carbon that can improve crop productivity in suboptimal conditions, yet the combined effects of biochar and soil water deficit under partial root-zone drying irrigation (PRD) on plant growth and physiology remain largely elusive. This study therefore investigated the ecophysiological responses of tobacco amended with biochar to different irrigation regimes including PRD.

*Methods* Tobacco plants were grown in split-root pots to implement PRD under Ferralsol and Anthrosol amended with wheat-straw (WSBC) and softwood (MWBC) biochar, and subjected to three irrigation regimes. Key parameters of plant growth and physiology were determined.

*Results* Compared to plants grown under full irrigation (FI), deficit irrigation (DI) and PRD decreased leaf area (LA), leaf dry matter (LDM) and leaf relative water content (RWC) but increased leaf N content ($[N]_{leaf}$); also decreased leaf photosynthetic rate, maximum rate of carboxylation by rubisco and stomatal conductance, while significantly enhanced the intrinsic water-use efficiency (20% and 45%). Compared to non-biochar, WSBC increased LA and LDM but lowered RWC and $[N]_{leaf}$. DI and PRD significantly increased leaf abscisic acid ($[ABA]$) and zeatin riboside ($[ZR]$) while reduced gibberellic acid ($[GA3]$) and indole-3-acetic acid ($[IAA]$) concentrations; PRD possessed greater [ABA] and [ABA]/[GA3] but lower [GA3] and [GA3]/[ZR] than DI, which was further magnified by WSBC. Additionally, superoxide dismutase and peroxidases activities were up-regulated by WSBC especially under PRD.

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*Conclusion* Collectively, incorporating WSBC and PRD might be an effective strategy to improve water productivity by optimizing phytohormonal profile and antioxidant system thereby growth and physiology of tobacco.

Highlights • Partial root-zone drying irrigation (PRD) and biochar addition altered tobacco phytohormonal profile and antioxidant system.

- Wheat-straw biochar amplified the PRD-induced increased leaf abscisic acid and lowered gibberellic acid concentrations.
- Wheat-straw biochar up-regulated superoxide dismutase and peroxidases activities.
- Combined PRD/wheat-straw biochar application improved tobacco growth and Water-use efficiency.

**Keywords** Partial root-zone drying irrigation · Biochar · Enzymatic antioxidant system · Endogenous phytohormones · Stomatal conductance · Water-use efficiency

## Abbreviations

| | |
|---|---|
| WSBC | wheat straw biochar |
| MWBC | soft wood biochar |
| LA | leaf area |
| LDM | leaf dry matter |
| RWC | leaf relative water content |
| $[N]_{leaf}$ | leaf nitrogen content |
| [ABA] | leaf abscisic acid concentration |
| [ZR] | leaf zeatin riboside concentration |
| [GA3] | leaf gibberellic acid concentration |
| [IAA] | leaf indole-3-acetic acid concentration |
| ROS | reactive oxygen species |
| SOD | superoxide dismutase |
| POD | peroxidase |
| CAT | catalase |
| $A_n$ | leaf photosynthetic rate |
| $g_s$ | stomatal conductance |
| $T_r$ | transpiration rate |
| $WUE_i$ | intrinsic water use efficiency |
| $WUE_n$ | instantaneous water use efficiency |
| PCA | principal component analysis |

## Introduction

Globally, climate change is exacerbating both biotic and abiotic stresses that detrimentally affect crop production, food safety and marketability (Begum et al. 2020; Awan et al. 2020). Drought stress is considered the utmost damaging abiotic factor for plant growth that occurs more frequently and intensely in the future along with worldwide warming (Leng and Hall 2019). Drought-induced restrictions in plant growth and physiology undoubtedly lead to significant yield losses (Liu et al. 2021a). Nevertheless, plants are capable of mitigating drought stress by invoking various regulatory networks to enhance water productivity (Ulfat et al. 2021; Khan et al. 2021). However, the mechanisms behind these modifications have not been fully evaluated.

Phytohormones, such as abscisic acid (ABA), being considered as the pivotal regulator of resistance against abiotic (e.g., drought) stresses (Awan et al. 2020). The synthesis of ABA signaling by plant roots sensing soil drying is the first adaptive answer to drought stress (Wei et al. 2020; Ababaf et al. 2021). Via the xylem system, ABA then is transported to the leaves for narrowing stomatal aperture and curtailing water loss, thereby enhancing water use-efficiency (WUE) (Liu et al. 2006, 2021b). Besides, phytohormone homeostasis might serve as a critical contributor to stomatal regulation under stressed conditions (Ma et al. 2021). However, the closure of plant stomata would parallelly lead to $CO_2$ starvation for photosynthesis, which will result in the over-accumulation of reactive oxygen species (ROS; Begum et al. 2020; Khan et al. 2021). Excessive ROS can destabilize plant membranes stability and cellular functioning by blocking photosynthesis and redox reaction pathways (Ahmad et al. 2019; Kohli et al. 2019; Ahanger et al. 2021), which accordingly activates the posterior defense mechanisms in plants (Khan et al. 2021). The enzymatic and non-enzymatic defense systems within plants suppress or scavenge the overload-ROS and protect them from drought stress-induced oxidative damage (Ghobadi et al. 2013; Begum et al. 2020). For instance, superoxide dismutase (SOD) plays an unparalleled role in enzymatic antioxidants as it can metabolize $O^{2\bullet-}$ (oxygen radicals) to $H_2O_2$ (hydrogen peroxide) (Farhangi-Abriz and Torabian 2017). Afterwards, the $H_2O_2$ is further eliminated by peroxidase (POD) and catalase

(CAT) into $H_2O$ and $O_2$ (Begum et al. 2020). Substantial evidence revealed that higher antioxidant enzyme activities could effectively combat drought-induced oxidative damage on plants (Hu et al. 2010; Ababaf et al. 2021; Khan et al. 2021). Therefore, appropriate management strategies are desperately required to mitigate the drought detrimental effects on plants by strengthening some defense systems in plants.

Tobacco as an important economic crop worldwide (Poltronieri 2016; Liu et al. 2021a), has been shown decreasing trends in yield and quality in recent years due to drought stress (Begum et al. 2020). Therefore, advanced water management strategies needed to be implemented to effectively mitigate the unfavorable effects of water deficiency on plants (Liu et al. 2006; Yang et al. 2021). Recently, as an efficient biological water-saving irrigation technology, partial root-zone drying irrigation (PRD) has been widely adopted in water-restricted agriculture (Liu et al. 2006, 2021a; Du et al. 2008; Yang et al. 2021). The PRD deliveries the irrigation water in an alternated spatio-temporal manner for constructing drying/rewetting cycles in the soil profile to induce the biochemical and physiological modifications in the soil-plant systems (Zhang et al. 2020a; Yang et al. 2021). A number of studies have shown that PRD could substantially depress stomatal conductance ($g_s$) while greatly maintain photosynthetic rate ($A_n$), thereby leading to a markedly higher WUE compared to conventional deficit irrigation (DI), despite both using a similar irrigation volume (Liu et al. 2006; Yang et al. 2021). Many possible mechanisms have been proposed to explain the outstanding effects of PRD (Wang et al. 2017; Guo et al. 2021). ABA-based chemical signaling synthesized by roots sensing drought is regarded as the principal mechanism to increase WUE by effectively closing stomata (Liu et al. 2006, 2021b; Wang et al. 2017). Besides, PRD can mitigate water deficit-induced oxidative injury to cellular metabolism by activating antioxidant systems, such as increasing the activities of SOD, POD, and CAT (Hu et al. 2010). Nevertheless, it remains largely unknown how PRD affects WUE by regulating stomatal behavior as mediated by enzymatic antioxidant and phytohormone signaling under water deficit conditions.

Biochar, also called "black gold" in agriculture, is a carbon-based substance obtained from pyrolysis of biomass residues with or without in the presence of oxygen (Lehmann et al. 2011; Liu et al. 2020).

Recently, biochar is widely recognized as a user-friendly sustainable option to upgrade soil quality and crop productivity (Jeffery et al. 2015). The porous structure, high surface area and enriched oxygen functional groups of biochar help to prepare cohesion and adhesion forces to retain more water and nutrients in soil while reducing nutrients leaching (Suliman et al. 2017). The conserved water in biochar-soil porous particles can maintain the leaf turgor pressure and photosynthesis, thus promising to alleviate the adverse effects of drought on plants (Khan et al. 2021). Biochar may potentially enhance the sustainability of agro-ecosystems by optimizing plant growth strategy (Omondi et al. 2016; Faloye et al. 2019; Liu et al. 2020). For instance, biochar-induced changes in root growth and morphological traits can promote water and nutrient absorption, thereby enhancing crop yield (Xiang et al. 2017; Liu et al. 2021c). Besides, some studies also reported biochar could modify the ROS scavenging enzymes and provides an efficient electron transferring mechanism to tackle the toxic effects of ROS in plants (Farhangi-Abriz and Torabian 2017; Mansoor et al. 2021). However, biochar effectiveness in the agricultural sector depends on biochar-soil-plant interactions (Hansen et al. 2016; Liu et al. 2021a). For example, positive effects are noticeable in fertile soils while absent in infertile soils (Noguera et al. 2010; Liu et al. 2021a). Many studies have focused on the response of crop productivity to biochar in many plants, but there are research gaps and insufficient information on the effects of biochar on tobacco plants under reduced irrigation regimes, particularly PRD.

Therefore, the objective of this study was to investigate the ecophysiological responses such as photosynthetic characteristics, leaf gas exchange, plant water relations, endogenous phytohormonal levels and enzymatic antioxidants of tobacco plants to biochar incorporation and reduced irrigation regimes including DI and PRD. To achieve this, we imposed *Nicotiana tabacum* L. (tobacco) to three different irrigation treatments in combination with two different biochar amendments incorporated into two contrasting soil types. It was hypothesized that the changes in the soil environment caused by PRD and biochar application could alter phytohormonal levels and antioxidant enzyme activities, effectively regulating leaf gas exchange, thereby improving plant growth and water productivity.

## Materials and methods

### Experimental setup and treatments

During April and October 2019, tobacco plants were grown in a nature-lig greenhouse at Northwest A&F University, China. Uniform tobacco seedlings (*Nicotiana tabacum* L. var. Qinyan No. 96) with 6 leaves, offered by China Shaanxi Tobacco Co., were transferred into 16-liter rectangle split-root containers with 18 kg of either dried soils or biochar/soil mixtures based on the standard of one plant in each pot, for details see Liu et al. (2021a). Two soil types and two biochar amendments with different properties were used to build experimental soils for potted plants. Briefly, soils were obtained from Northwest and Central China and classified as Anthrosol (in a field near Yangling, 0-25cm depth) and Ferralsol (in a Changsha field from 60-100 cm), respectively (Liu et al. 2021a). The UK Biochar Research Centre of UK (UKBC) offered the biochar materials made by wheat straw pellets (WSBC) and soft-wood pellets (MWBC) at 550 °C. The basic soil and biochar physio-chemical characteristics were presented in Liu et al. (2021b). Biochar/soil mixtures were formed by thoroughly adding the air-dried finely powdered biochars (< 0.45 mm) into the soils (< 0.5 cm) at a 2% (*w/w*) ratio. The biochars were crushed into a smaller particle size to ensure a relatively homogeneous distribution in the soil. Seven-days after transplanting, all plants were fertigated at the rate of 0.1 g N (as urea), 0.1 g P (as $KH_2PO_4$), and 0.15 g K (as $KH_2PO_4 + K_2SO_4$) per kg soil to provide substrate nutrients for plant growth. All plants were grown in the greenhouse for 115 days with a mean temperature of 26.7 °C, mean relative humidity of 72.7 %, and mean vapour pressure deficient (VPD) of 0.97 KPa throughout the experiment, as shown in Liu et al. (2021a). The light intensity in the greenhouse was a minimum of 0 Klux (nighttime) and a maximum of *ca*. 50 Klux (daytime) during the experiment period (Zhang et al. 2017).

A full-factorial block design was employed for the split-root pot experiment comprising 18 treatments with three replicates including two soil types, three biochar amendments, and three irrigation treatments, thereby resulting in 54 pots totally. All plants received adequate water during the first 30 days after transplantation, i.e., daily watered to 100 % of field capacity (FC), to replenish the water requirements for plant growth. Afterwards, the plants were watered based on three water treatments at the rooting stage during the field growth period of tobacco (i.e., with approx. 10-12 leaves), viz. (1) full watering in the entire root-zone (FI, watered to 90 % of FC); (2) water deficit throughout the entire root-zone (DI, irrigated by 70 % water amount applied in FI); and (3) water deficit in partial root-zone (PRD, where the same water volume as DI was imposed on only one soil-zone and the irrigation was alternately switched between both soil sectors as soon as the soil water content in the drying side dropped to approximately 10%). All plants were daily watered at 16:00 h based on the water loss metered by time-domain reflectometer moisture probes (TDR, MINITRASE, Soil Moisture Equipment Corp., USA). The daily changes of soil water content in the pots were shown in Liu et al. (2021a).

### Measurements

#### *Leaf gas exchange and chlorophyll content*

Applying a LI-6800 portable gas exchange meter (LI-COR, Lincoln, SEC, USA) to generate light-saturated photosynthetic $CO_2$ response ($A_n$-$C_i$) curves on the upper canopy fully expanded leaves at 35 (the rosette stage), 49 (the fast-growing stage), and 77 (the leaf maturity stage) days after onset of the irrigation treatments, according to the protocol by Xiong and Flexas (2021). During the measurement, leaf chamber temperature and leaf-to-air flow rate were respectively programmed at 25 °C and 500 μmol s$^{-1}$, and the reference concentration of $CO_2$ ([$CO_2$]) was controlled by a $CO_2$ controller within the gas exchange analyzer. Before commencing the $A_n$-$C_i$ curve measurements, the target leaves were acclimatized for 15-20 minutes to enable photosynthesis and stomatal conductance to stabilize and Rubisco to be fully activated (Moualeu-Ngangue et al. 2017), the auto-progress of the $CO_2$ response curve was then adopted (Xiong and Flexas 2021). The first point was measured under 400 ppm [$CO_2$], following by 300, 200, 100, 50, 400, 600, 800, 1000, 1200, 1500 ppm to obtain $A_n$-$C_i$ curves under 1500 μmol m$^{-2}$ s$^{-1}$ light conditions (Moualeu-Ngangue et al. 2017).

Leaf net photosynthetic rate ($A_n$), stomatal conductance ($g_s$) and transpiration rate ($T_r$) at 400 ppm [$CO_2$] condition were extracted from the measured $A_n$-$C_i$ curves regards as the gas exchange parameters

under the currently ambient $CO_2$. Subsequently, intrinsic ($A_n/g_s$) and instantaneous ($A_n/T_r$) water use-efficiency ($WUE_i$ & $WUE_n$ respectively, hereafter) were calculated. Simultaneously, a SPAD-502Plus (Soil Plant Analysis Development) was used to determine the chlorophyll content index (SPAD value) on the same dates and leaves used for measuring leaf gas exchange, according to Ma et al. (2021).

### Estimation of the photosynthetic characteristics from the $A_n$-$C_i$ curves

Based on the FvCB model of $C_3$ plants by Ethier and Livingston (2004), the $A_n$-$C_i$ curves (Fig. 1) were fitted with a usable Microsoft Excel Tool "A/ $C_i$ Response Curve Fitting 10.0" at http://landflux. org/Tools.php to evaluate photosynthetic characteristics, according to Rashid et al. (2018). The estimated parameters including the maximum carboxylation rate of Rubisco ($V_{cmax}$; µmol m$^{-2}$ s$^{-1}$), RuBP-regeneration maximum electron transport rate ($J$, µmol e$^{-1}$ m$^{-2}$ s$^{-1}$), day-time respiration ($R_d$, µmol m$^{-2}$ s$^{-1}$), mesophyll conductance ($g_m$, mol m$^{-2}$ s$^{-1}$), $CO_2$ compensation point ($\Gamma$, µmol mol$^{-1}$) and maximum photosynthetic capacity at 1500 µmol m$^{-2}$ s$^{-1}$ irradiance and 1500 ppm [$CO_2$] ($A_{nmax}$, µmol m$^{-2}$ s$^{-1}$), and the $J/V_{cmax}$ was calculated (Rashid et al. 2018).

### Plant water relation and endogenous phytohormonal concentrations

During the gas exchange measurements, a *ca.* 2cm × 2cm leaf sample obtained from the 2nd or 3rd fully expanded leaf adjacent (downward) to the leaf applied for measuring gas exchange was used to measure leaf relative water content (RWC). Briefly, the target sample was immersed in distilled water for 3 h in a dim environment after determining the fresh weight (FW). Subsequently, the turgid leaves were quickly wiped dry with tissue paper before determining the turgid weight (TW). The dry biomass of leaves (DW) was determined after the turgid leaves were oven-dried at 75 °C for 48 h. The RWC was calculated by the following formula:

$$RWC(\%) = [(FW - DW)/(TW - DW)] \times 100$$

After RWC determination, the fully expanded seventh leaf counted from the top of the plant, was cut into two parts, immediately encased in aluminum foil, flash-frozen in liquid nitrogen, and saved at -80°C until the measurements of leaf endogenous phytohormones, including ABA ([ABA]), GA3 ([GA3]), IAA ([IAA]) and ([ZR]) concentration and leaf antioxidant enzyme activities. Each frozen leaf sample was grinded into a fine powder with liquid nitrogen for the phytohormones concentration measurements (Ma et al. 2021). Briefly, a 500 mg powder sample was weighed into a 10 mL Eppendorf tube and then assayed for leaf [ABA], [GA3], [IAA] and [ZR] using an ELISA method (Enzyme-Linked Immuno Sorbent Assay) according to the approach of Ma et al. (2021).

### Leaf antioxidant enzyme activities analysis

Frozen leaf tissue of *ca.* 0.5 g was crushed to a fine powder with liquid nitrogen and then homogenized in a cooled mortar with 5 ml extraction buffer (consisting of 50 mM of pre-cooled phosphate buffer pH 7.0 and 0.4% polyvinylpyrrolidone), according to Hu et al. (2010). The homogenate was centrifuged at 10,000 × $g$ and 4 °C for 30 min and the supernatant was collected for the assays of the antioxidant enzyme activities (Hu et al. 2010; Ababaf et al. 2021). The activity of SOD was determined by tracking the suppression of photochemical reduction of nitro blue tetrazolium (NBT) (Li et al. 2020). The amount of enzyme causing half-maximal inhibition of NBT reduction at 560 nm absorbance was defined as the unit of SOD activity (Li et al. 2020). POD activity was assayed using oxidizing guaiacol to produce 4-o-methoxyphenyl, and POD activity unit was identified as the enzyme amount needed for an increase in absorbance of 0.01 per min at 470 nm, based on Li et al. (2020). CAT activity was determined via monitoring the decrease of absorbance at 240 nm through decomposing $H_2O_2$, its unit was the enzyme amount that decreases by 0.1 absorbance per min, according to Li et al. (2020).

### Plant growth and leaf N content

At tobacco leaf maturity stage (90% leaves turning yellow), seven upper leaves per plant (*i.e.,* 1-7th leaves counted from tobacco top) were collected. The collected leaves were utterly milled to a fine powder for leaf N concentration ([N], mg g$^{-1}$) measurement after being roasted to a constant weight
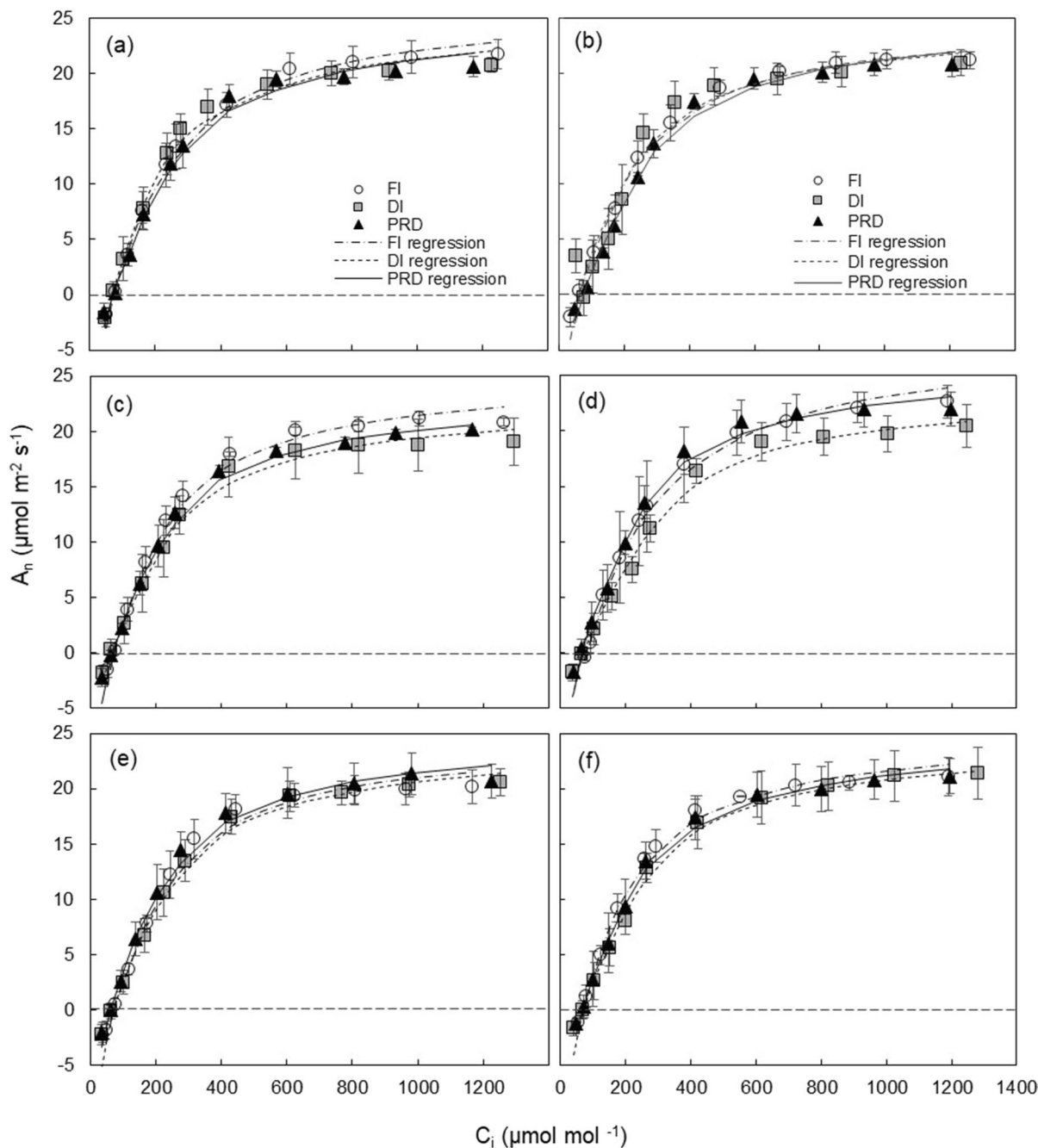
**Fig. 1** Photosynthetic $CO_2$ response ($A_n$-$C_i$) curves of tobacco leaves as affected by full (FI), deficit (DI) and partial root zone drying (PRD) irrigation under Ferralsol and Anthrosol amended without (0BC; a, b) or with wheat-straw (WSBC; c, d) or softwood (MWBC; e, f) biochar. $C_i$ is the intercellular $CO_2$ concentration. Error bars are $\pm$ standard error ($n = 3$).

(LDM) at 50-55°C. A portable leaf area meter (LI-3100, LI-Cor, Inc. Lincoln, NE, USA) was used for leaf area measurement at each leaf harvesting, and then the total upper leaves area (LA) was calculated

(Liu et al. 2021a). Subsequently, leaf mass per unit area (LMA; LDM/LA) and leaf N content per unit area ($[N]_{leaf}$, g m$^{-2}$; $[N] \times$ LMA) were calculated separately. At the end of the experiment, plant

height and stem diameter were measured manually using scale.

## Statistical analysis

To uncover the overall treatment impact over the long-term, the measured values of leaf gas exchange, photosynthetic parameters from $A_n$-$C_i$ curves, RWC and SPAD were averaged across the different measurement dates to compare among the different treatments. Prior to statistical analysis, data for all parameters were subjected to the Shapiro-Wilk test and the Levene's test, and logarithmic transformation was performed, if needed. Data were then subjected to three-way analysis of variance (ANOVA) to assess the effects of soil types (S), biochar addition (B) and irrigation treatments (IR), as well as their interactions on the independent variables. In addition, one-way ANOVA and Duncan's multiple range test at 5% confidence level was further used when there are significant interactions between independent variables to test for significant differences between treatments. The ANOVA analyses were performed in IBM SPSS Statistics ver. 23.0 (SPSS Inc, New York, USA). Linear regression models were performed to identify the correlation among the key parameters. All differences between treatments were considered significant when $p < 0.05$. Moreover, principal component analysis (PCA) was performed individually for Ferralsol and Anthrosol on the measured variables with CANOCO 5.0 (Software for Ordination, Microcomputer Power, Ithaca, NY, USA).

## Results

### Plant growth

Ferralsol possessed taller but thinner plants than Anthrosol, and biochar addition increased plant height and stem diameter compared to non-biochar controls (Table 1). The LA and LDM of tobacco plants were higher 22% and 23% in Anthrosol than in Ferralsol, respectively, and both were significantly increased by biochar, especially with WSBC (14 % and 17%, respectively) (Table 1). Compared to FI, reduced irrigation regimes (DI and PRD) significantly reduced plant height particularly under Ferralsol (9%), stem diameter (9-12%), LA (17-18%), LDM (24-25%) and

LMA (8-9%), and these traits were higher for PRD-treated plants than DI plants (Table 1).

### Leaf N content, leaf chlorophyll content and leaf relative water content

The S, B and IR treatments significantly influenced $[N]_{leaf}$ (Table 1), being higher in Ferralsol than Anthrosol. Compared to non-biochar controls, WSBC significantly lowered $[N]_{leaf}$ by 17%. Significantly higher 15% $[N]_{leaf}$ was observed in PRD-plants than FI and DI treated plants. The SPAD value responded similarly to S, B and IR as $[N]_{leaf}$ (Table 1). Moreover, there were significant interactions of S×IR and B×IR for SPAD (Table 1) where significantly depressed SPAD value was observed in the plants treated by reduced irrigation regimes (especially DI) under WSBC amended Ferralsol compared to non-biochar soils.

Tobacco leaf RWC was significantly affected by S and IR treatments (Table 1). Regardless of B and IR treatments, RWC was overall greater in Ferralsol over Anthrosol. As expected, compared to FI, reduced irrigation regimes significantly lowered RWC (3% and 6% for DI and PRD, respectively), which highlighted the evidently lower RWC in PRD plants than DI-plants.

### Photosynthetic $CO_2$–response characteristics

The application of WSBC amplified the difference of $A_n$-$C_i$ curves between DI and PRD compared to non-biochars (Fig. 1a, b), particularly in Anthrosol, yet the MWBC did not (Table 1c). Among the photosynthetic parameters derived from $A_n$-$C_i$ curves, only $V_{cmax}$ and $g_m$ were significantly affected by only IR treatment (Table S1). Reduced irrigation regimes significantly depressed both $V_{cmax}$ and $g_m$ in comparison with FI, while PRD tended to have higher $V_{cmax}$ and $g_m$ over DI.

### Leaf gas exchange

Irrigation treatment (IR) significantly influenced leaf gas exchange parameters (Table 2). Compared to FI, DI and PRD lowered $A_n$ by 17% and 14%, $g_s$ by 30% and 41%, and $T_r$ by 20% and 29%, respectively, which also showed PRD plants possessed relatively greater $A_n$ and evidently lower $g_s$ and $T_r$ than DI plants (Fig. 2a-c). There was a significant S×IR interaction

**Table 1** Treatment effects and output of three-way analysis of variance (ANOVA) for tobacco morphophysiological traits.

| Soil | Biochar | Irrigation | Plant Height (cm) | Stem Diameter (mm) | LA (m⁻²) | LDM (g plant⁻¹) | LMA (g m⁻²) | [N]leaf (g m⁻²) | SPAD value | RWC (%) |
|---|---|---|---|---|---|---|---|---|---|---|
| Ferralsol | OBC | FI | 177.00±10.02bc | 17.61±0.25 | 0.46±0.03def | 39.89±1.23 | 86.76±2.43 | 1.10±0.03 | 46.17±0.48bcd | 91.38±1.92 |
| | | DI | 174.67±0.88bcd | 15.94±0.10 | 0.38±0.01g | 27.86±1.46 | 74.03±3.09 | 1.32±0.08 | 46.92±1.46abc | 86.42±0.44 |
| | | PRD | 159.67±7.69defg | 16.57±0.21 | 0.40±0.02fg | 30.48±1.04 | 76.25±0.89 | 1.44±0.13 | 49.92±0.85a | 84.89±2.16 |
| | WSBC | FI | 187.67±3.84ab | 19.85±0.96 | 0.56±0.03bc | 49.55±3.60 | 87.69±2.61 | 1.08±0.11 | 40.79±0.66fg | 90.28±1.14 |
| | | DI | 165.00±4.73cde | 17.81±0.54 | 0.52±0.01cd | 38.42±0.92 | 73.30±1.60 | 0.94±0.04 | 40.36±1.04g | 87.86±1.78 |
| | | PRD | 160.00±9.02defg | 18.03±0.17 | 0.49±0.04cde | 37.46±4.61 | 77.11±6.35 | 1.19±0.17 | 43.66±0.82def | 82.17±1.31 |
| | MWBC | FI | 192.67±5.78a | 19.09±0.24 | 0.50±0.03cd | 41.64±4.31 | 82.14±3.67 | 1.31±0.29 | 44.33±1.80cd | 91.10±0.78 |
| | | DI | 172.33±2.33cd | 16.91±0.30 | 0.41±0.02fg | 31.48±1.27 | 77.21±2.22 | 1.26±0.16 | 46.30±0.37bcd | 83.28±2.23 |
| | | PRD | 169.33±2.33cd | 17.13±0.65 | 0.43±0.02efg | 33.62±2.18 | 78.27±3.38 | 1.42±0.05 | 47.21±0.41abc | 85.49±0.98 |
| Anthrosol | OBC | FI | 147.67±4.33fgh | 19.69±0.24 | 0.62±0.03ab | 55.02±4.52 | 88.35±3.18 | 1.03±0.04 | 39.57±0.36g | 86.89±2.81 |
| | | DI | 142.00±3.06h | 16.92±0.29 | 0.51±0.01cd | 40.33±0.26 | 79.76±0.93 | 1.18±0.07 | 46.71±1.09bcd | 84.42±1.18 |
| | | PRD | 146.00±3.61gh | 17.54±0.47 | 0.52±0.01cd | 37.75±1.87 | 73.23±2.83 | 1.27±0.10 | 47.68±0.91ab | 82.70±1.05 |
| | WSBC | FI | 168.67±1.33cd | 20.44±0.94 | 0.67±0.04a | 57.53±6.56 | 84.75±5.10 | 0.87±0.07 | 40.64±0.44fg | 87.11±1.48 |
| | | DI | 142.67±4.70h | 18.29±0.11 | 0.51±0.02cd | 41.72±4.75 | 81.21±4.91 | 0.94±0.11 | 39.86±1.73g | 84.44±1.53 |
| | | PRD | 162.00±2.00cdef | 19.33±0.21 | 0.54±0.01cd | 45.21±2.43 | 84.10±5.63 | 1.03±0.12 | 44.02±1.36cde | 80.82±0.82 |
| | MWBC | FI | 163.00±0.58cdef | 19.84±0.56 | 0.66±0.02a | 50.93±4.44 | 77.47±4.81 | 0.93±0.07 | 41.17±0.78efg | 83.13±2.86 |
| | | DI | 148.30±4.41fgh | 17.23±0.40 | 0.52±0.02cd | 39.74±3.00 | 76.33±3.21 | 1.07±0.06 | 45.26±0.46bcd | 86.20±0.94 |
| | | PRD | 151.33±1.86efgh | 17.67±0.42 | 0.51±0.03cd | 39.07±5.69 | 75.70±7.08 | 1.10±0.10 | 46.47±0.27bcd | 82.92±2.83 |
| **Output of three-way ANOVA** | | | | | | | | | | |
| Soil (S) | | | *** | *** | *** | *** | ns | ** | ** | ** |
| Biochar (B) | | | * | *** | *** | ** | ns | ** | *** | ns |
| Irrigation (IR) | | | *** | *** | *** | *** | * | * | *** | *** |
| S×B | | | ns | ns | ns | ns | ns | ns | ns | ns |
| S×IR | | | ** | ns | ns | ns | ns | ns | * | ns |
| B×IR | | | ns | ns | ns | ns | ns | ns | * | ns |
| S×B×IR | | | ns | ns | ns | ns | ns | ns | ns | ns |

Mean values followed by ± standard error ($n = 3$). LA, leaf area; LDM, leaf dry matter; LMA, leaf mass per unit area; RWC, leaf relative water content; [N]leaf, leaf N concentration per unit area. OBC, WSWB and MWSB denote no biochar, wheat straw or mixed softwood biochar. FI, DI and PRD are, respectively, full, deficit and partial root-zone drying irrigation. Different lowercase letters mean significant differences ($p < 0.05$) among different soils amended by biochars within the same irrigation regime. Different lowercase letters mean significant differences ($p < 0.05$) among different treatments when there are significant interactions. '*', '**', '***' indicates significance level at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively, 'ns' is non-significant.

**Table 2** Output of three-way analysis of variance (ANOVA) for plant parameters.

| Traits | Source of variation | | | | | | |
|---|---|---|---|---|---|---|---|
| | Soil (S) | Biochar (B) | Irrigation (IR) | S×B | S×IR | B×IR | S×B×IR |
| $A_n$ | ns | ns | * | ns | ns | ns | ns |
| $g_s$ | ns | ns | *** | ns | * | ns | ns |
| $T_r$ | ns | ns | *** | ns | ns | ns | ns |
| $WUE_i$ | ns | ns | ** | ns | ns | ns | ns |
| $WUE_n$ | ns | ns | ns | ns | ns | ns | ns |
| [ABA] | ns | *** | *** | ns | *** | *** | ns |
| [GA3] | *** | *** | *** | *** | ns | * | * |
| [IAA] | *** | *** | *** | *** | ns | ** | *** |
| [ZR] | ns | *** | *** | *** | ns | ** | ns |
| SOD | *** | ns | ns | ns | ns | ns | ns |
| POD | ns | * | ns | ns | ns | * | ns |
| CAT | ns | * | ns | ns | ns | ns | ns |

The table shows the level of significance for tobacco leaf net photosynthetic rate ($A_n$), stomatal conductance ($g_s$), transpiration rate ($T_r$), intrinsic water-use efficiency ($WUE_i$), instantaneous water-use efficiency ($WUE_n$); leaf abscisic acid ([ABA]), gibberellic acid ([GA3]), indole-3-acetic acid ([IAA]) and zeatin riboside ([ZR]) concentration; superoxide dismutase (SOD), peroxidases (POD) and catalase (CAT) activity as affected by soil type (S), biochar amendment (B), irrigation treatments (IR) and their interactions. '*', '**', '***' indicates significance level at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively, 'ns' is non-significant. The data is presented in Figs. 2, 3, 4, 5.

(Table 2) being a more pronounced reduction of $g_s$ within Anthrosol than Ferralsol under reduced irrigation regimes as compared with FI. Compared to FI, the much more lowered of $g_s$ than $A_n$ under reduced irrigation regimes contributed to a significantly enhanced $WUE_i$ (20% for DI and 45% for PRD), which highlighted PRD achieved a clearly higher 21% $WUE_i$ over DI (Fig. 3a; Table 2).

Concentrations of ABA, GA3, IAA and ZR in leaf

Compared to without biochar controls, WSBC significantly increased [ABA] by 12%, [GA3] by 10% and [IAA] by 20%, while MWBC significantly lowered [IAA] by 14% and [ZR] by 13%, respectively, particularly pronounced under reduced irrigation of Ferralsol (Fig. 4; Table 2). Compared to FI, reduced irrigation regimes (DI and PRD) significantly increased [ABA] (21% and 55%) and [ZR] (13% and 25%) but lowered [GA3] (10% and 16%) and [IAA] (10% and 21%), and these changes were more pronounced under PRD than DI (Fig. 4a-c; Table 2). Moreover, compared to non-biochar controls, combined PRD/WSBC application significantly enhanced [ABA] of Ferralsol-plants and [GA3] of both soil types plants as indicated by a significant S×B×IR interaction for [ABA] and [GA3]

(Table 2). Regardless of B and IR treatments, Ferralsol overall possessed 7% lower [GA3] while 17% higher [IAA] than Anthrosol (Fig. 4b, c; Table 2).

Leaf antioxidant enzyme activity

The soil types (S) significantly affected SOD activity (Fig. 5a; Table 2), being higher 11% in Anthrosol over Ferralsol. Compared to FI and 0BC controls, PRD and WSBC increased SOD activity (Table 2). Compared to controls, significantly increased POD activity was found on [PRD-MWBC] plants, and WSBC significantly enhanced POD activity of DI-plants, as indicated by a significant B×IR interaction (Fig. 5b; Table 2). The application of biochar significantly depressed CAT activity, particularly MWBC decreased it by 49%, compared to non-biochar controls (Fig. 5c; Table 2).
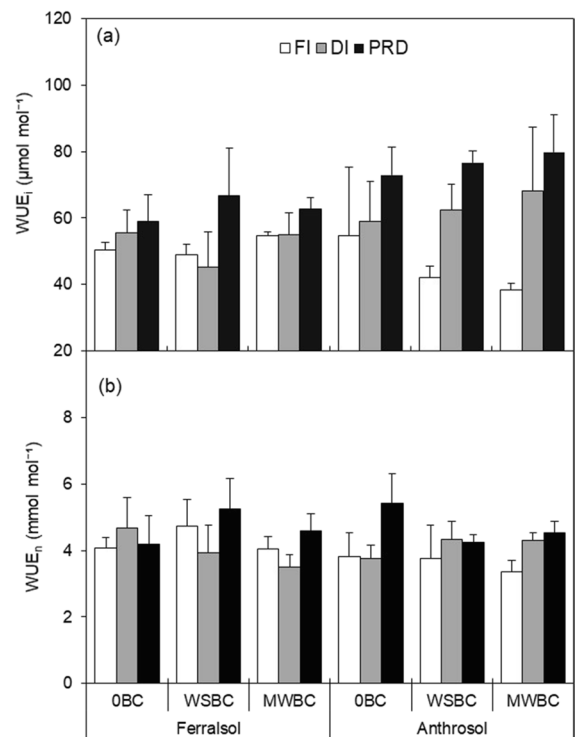
Relationships of stomatal and mesophyll conductance with phytohormone levels

Across the soil and biochar treatments, positive linear correlations between $A_{nmax}$ with $V_{cmax}$, and with $J$ were observed (Fig. 6a), and $g_m$ correlated negatively with $J/V_{cmax}$ (Fig. 6b). Moreover, both $g_s$ and
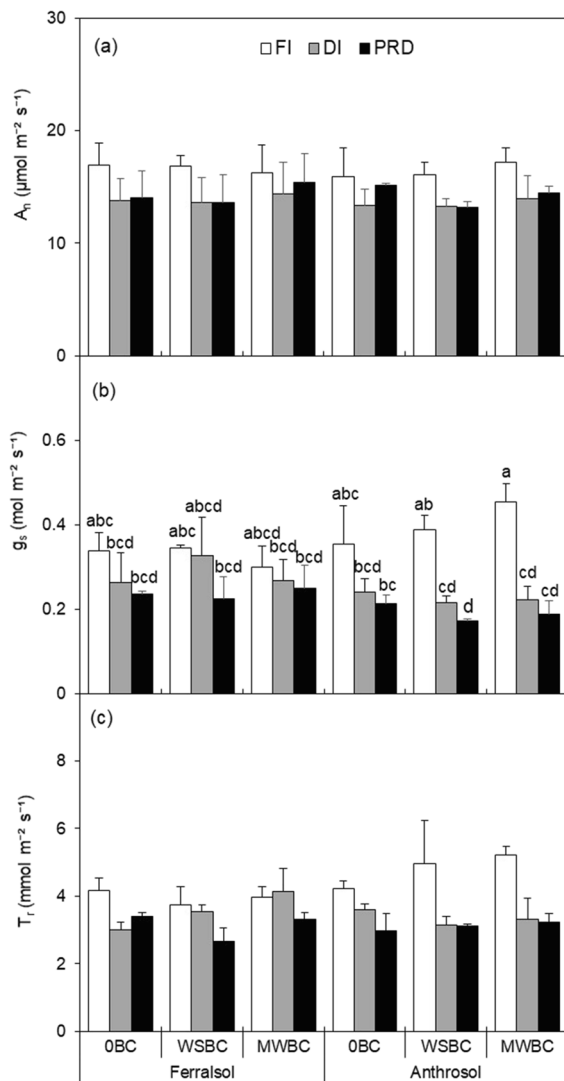
Fig. 2 (a) Net photosynthetic rate ($A_n$), (b) stomatal conductance ($g_s$), (c) transpiration rate ($T_r$) of tobacco leaves as affected by full (FI), deficit (DI) and partial root zone drying (PRD) irrigation under Ferralsol and Anthrosol amended without (0BC) or with wheat-straw (WSBC) or with softwood (MWBC) biochar. Data are mean ± standard error ($n = 3$). Different lowercase letters mean significant differences ($p < 0.05$) among different treatments when there are significant interactions.



Fig. 3 (a) Intrinsic water-use efficiency ($WUE_i$) and (b) instantaneous water-use efficiency ($WUE_n$) of tobacco leaves as affected by full (FI), deficit (DI) and partial root zone drying (PRD) irrigation under Ferralsol and Anthrosol amended without (0BC) or with wheat-straw (WSBC) or with softwood (MWBC) biochar. Data are mean ± standard error ($n = 3$).

[ABA] and [GA3] were increased linearly with depressing RWC and [N]$_{leaf}$, respectively (Fig. 8).

PCA analysis of tobacco growth and physiology

The PCA plots revealed that the different [B] and [IR] treatments created more pronounced clustering for Anthrosol (Fig. 9b) than for Ferralsol (Fig. 9a), correspondingly explained 41.2% (PC1 of 27.5% and PC2 of 13.7%) and 40.5% (PC1 of 24.4% and PC2 of 16.1%) of the total variations, respectively.

The [IR] separated plant growth and physiological traits into distinct clusters. Specifically, reduced irrigation regimes (DI and PRD) clustered more to left towards higher $WUE_i$, $WUE_n$, ABA, ABA/GA3, ZR, [N]$_{leaf}$, SPAD and $J/V_{cmax}$, while FI clustered more to the right in the same direction as plant height, stem diameter, $A_n$, $g_s$, $T_r$, GA3, GA3/ZR, CE, $g_m$, $V_{cmax}$, particularly for Anthrosol

$g_m$ were positively correlated with $A_n$ (Fig. 7a). The $g_s$ declined linearly with increasing [ABA] and [ABA]/[GA3] (Fig. 7b, c), while increased linearly with increasing [GA3]/[ZR] (Fig. 7d). Likewise, $g_m$ showed similar but relatively weaker correlations to phytohormone levels as $g_s$ (Fig. 7b-d). Moreover,
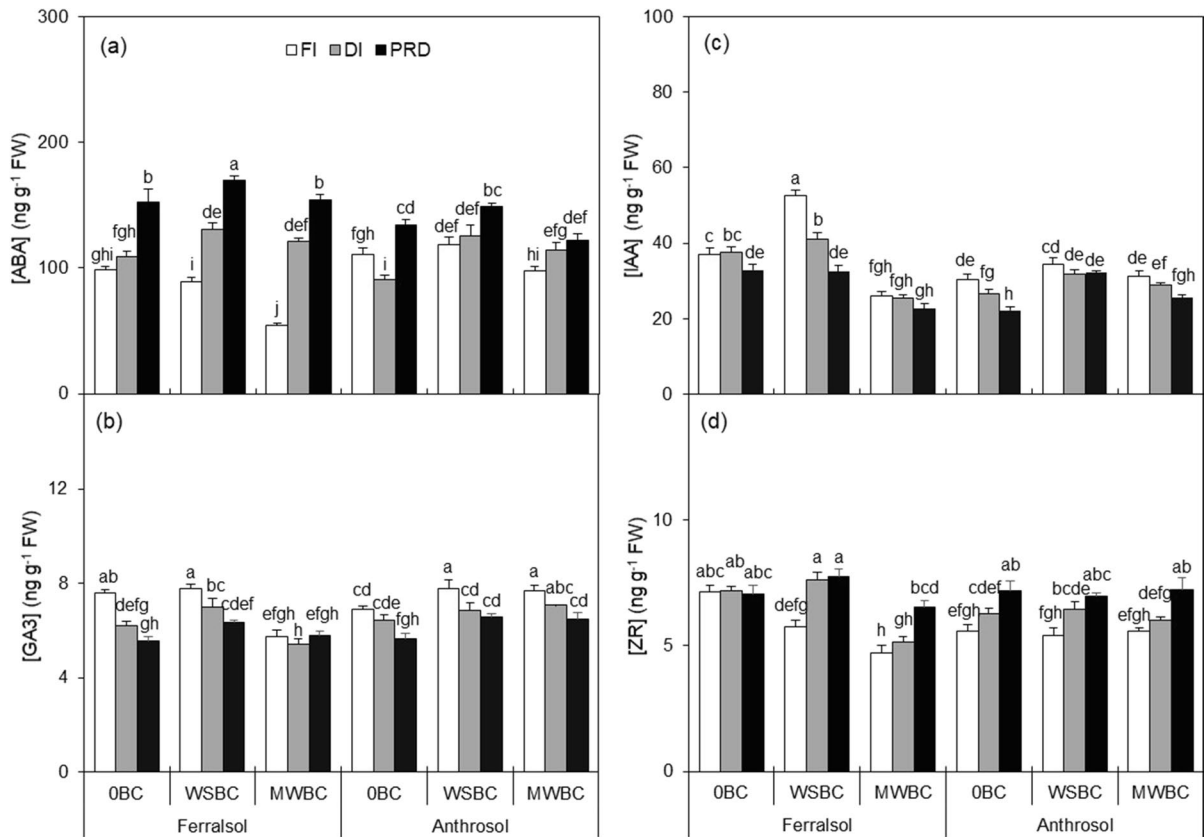
**Fig. 4** (a) Leaf abscisic acid ([ABA]), (b) gibberellic acid ([GA3]), (c) indole-3-acetic acid ([IAA]) and (d) zeatin riboside ([ZR]) concentration of tobacco leaves as affected by full (FI), deficit (DI) and partial root zone drying (PRD) irrigation under Ferralsol and Anthrosol amended without (0BC) or with wheat-straw (WSBC) or with softwood (MWBC) biochar. Data are mean ± standard error ($n = 3$). Different lowercase letters mean significant differences ($p < 0.05$) among different treatments when there are significant interactions.

(Fig. 9). Moreover, PRD formed an apparent clustering toward $WUE_i$ and $WUE_n$ under Anthrosol (Fig. 9b), while minor effect under Ferralsol (Fig. 9a).

The PCA further indicated that biochar especially WSBC addition under FI possessed higher plant height, stem diameter, GA3/ZR, $g_m$, $V_{cmax}$ under both soil types (Fig. 9). Biochar addition under reduced irrigation (DI and PRD) appears to have lower $WUE_i$, $WUE_n$, under Anthrosol (Fig. 9b), while for Ferralsol it is difficult to draw general trends for biochar addition under reduced irrigation regimes as the clustering were relatively indistinct (Fig. 9a).

## Discussion

To minimize the drought damage on plants, proper soil and water management strategies shall be introduced in the agricultural sector (Liu et al. 2020). Accordingly, the present study aimed to explore the role of biochar coupled with PRD in influencing the key regulatory networks in tobacco plants responding to water stress.
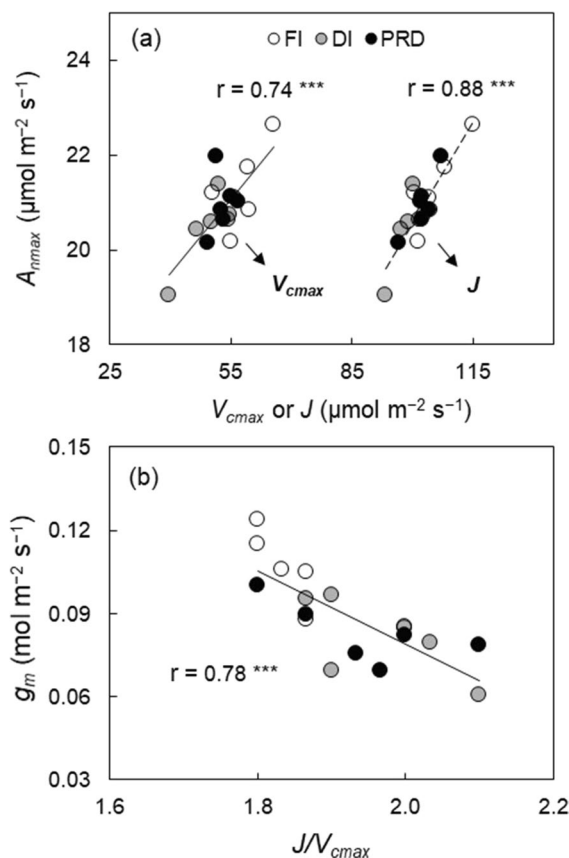
### Biochar and reduced irrigation regimes affected tobacco growth, physiology and WUE

Reduced irrigation regimes adversely affected the tobacco growth as exemplified by the decreases in
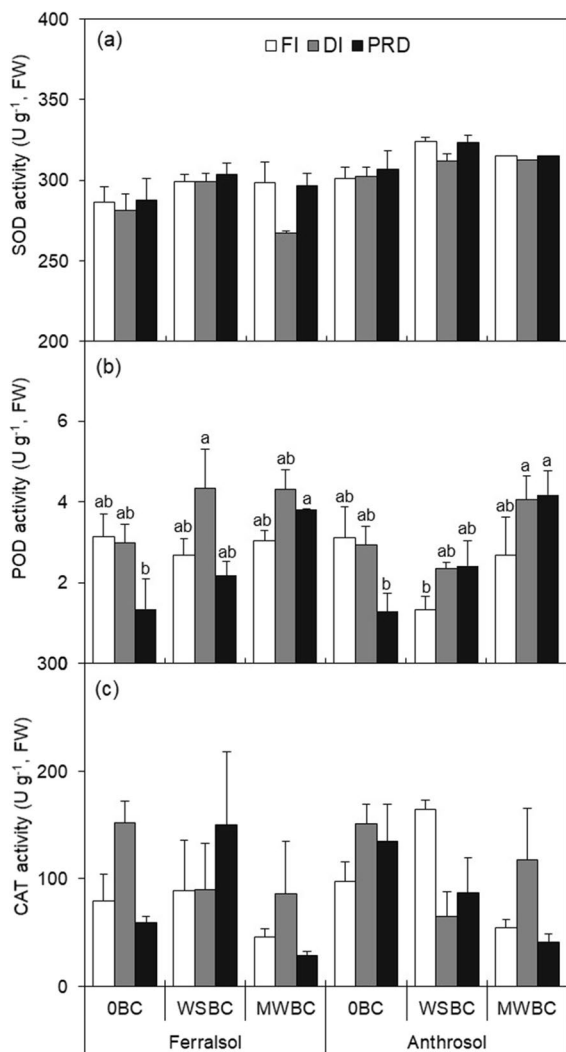
**Fig. 5** (a) Activity of superoxide dismutase (SOD), (b) per-oxidases (POD) and (c) catalase (CAT) of tobacco leaves as affected by full (FI), deficit (DI) and partial root zone drying (PRD) irrigation under Ferralsol and Anthrosol without (0BC) or with wheat-straw (WSBC) or with softwood (MWBC) bio-char. Data are mean ± standard error ($n = 3$). Different lower-case letters mean significant differences ($p < 0.05$) among different treatments when there are significant interactions.



**Fig. 6** Correlations of (a) maximum net photosynthesis at 1500 ppm of [$CO_2$] ($A_{nmax}$) with maximum rate of carboxy-lation by rubisco ($V_{cmax}$) or with electron transport rate for RuBP regeneration ($J$), and (b) mesophyll conductance ($g_m$) with $J/V_{cmax}$ of tobacco plants grown in pots treated with full (FI), deficit (DI) and partial root-zone drying (PRD) irrigation across biochar and soil treatments ($n = 3$). '*', '**', '***' indi-cates significance level at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively, 'ns' is non-significant.

plant height, stem diameter, LA and LDM (Table 1), which aligns with the results by Begum et al. (2020). Water deficiency generally restricts cellular division, histone kinase activity and cell cycle turnover, which ultimately restricts crop growth and development (Ahanger et al. 2021). Moreover, LMA, the trade-off between photo-assimilates and leaf area, was also lowered by reduced irrigation (Table 1) which may

have further restricted water availability within the plant (Xu and Zhou 2008; Liu et al. 2021b). These unfavorable effects on tobacco growth under reduced irrigation regimes may be linked to the limitations in some physiological processes (Begum et al. 2020). Here, the reduced irrigation (DI and PRD) signifi-cantly lowered RWC compared to FI (Table 1) that would depress leaf cell expansion and leaf turgor pressure (Liu et al. 2006). Besides, the depressed [N]$_{leaf}$ and SPAD value (Table 1) and increased resist-ance to $CO_2$ diffusion by lowering $g_s$ and $g_m$ (Rashid et al. 2018) also could decrease carbon assimila-tion capacity, thereby plant growth. However, the
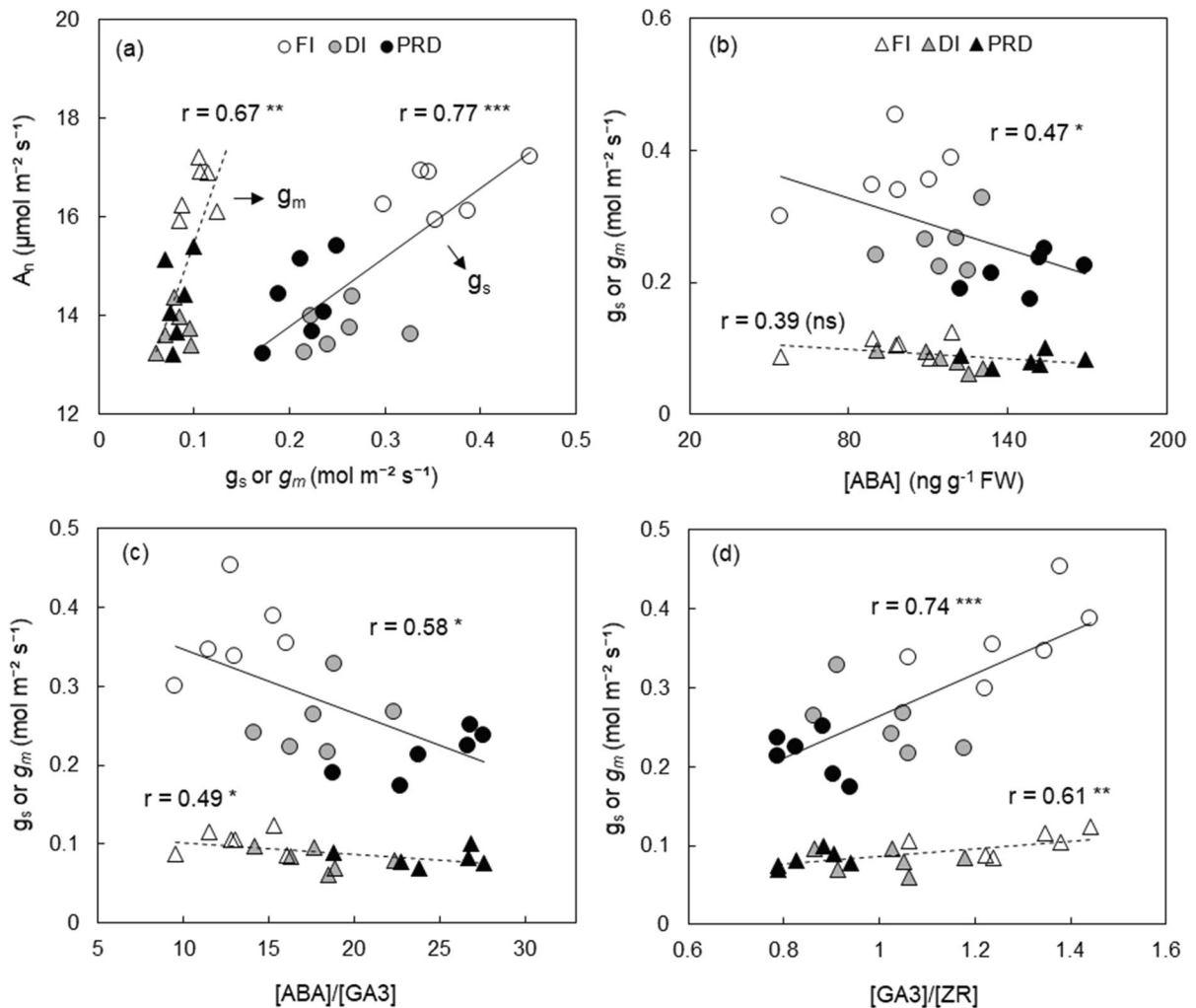
**Fig. 7** Correlations of (a) leaf net photosynthetic rate ($A_n$) with stomatal conductance ($g_s$) or with mesophyll conductance ($g_m$), (b) $g_s$ or $g_m$ with leaf [ABA], (c) $g_s$ or $g_m$ with [ABA]/[GA3], and (d) $g_s$ or $g_m$ with [GA3]/[ZR] of tobacco grown in pots treated with full (FI), deficit (DI) and partial root-zone drying (PRD) irrigation across biochar and soil treatments ($n$ = 3). '*', '**', '***' indicates significance level at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively, 'ns' is non-significant.

application of biochar, especially with WSBC, improved the growth performance of tobacco, such as increased LA and LDM (Table 1), consistent with Khan et al. (2021) in rapeseed, which helps to ameliorate the adverse impacts of drought (Mansoor et al. 2021). A number of studies suggested that the incorporation of biochar can enhance the pools and availability of nutrient (Khan et al. 2021; Liu et al. 2021c) and improve the soil water-holding capacity through modification of soil physio-chemical properties and plant-available water content (Hansen et al. 2016; Liu et al. 2021a; Mansoor et al. 2021), thereby

improving the plant growth under unfavorable conditions.. Here, the significantly lowered $[N]_{leaf}$ and SPAD upon WSBC addition might contribute to the increased biomass accumulation (Table 1) due to the dilution effect (Guo et al. 2021). Also, the reasons for increased biomass were due to improvement of water capacity at durable wilting points and safeguarding of water content through its immense porosity (Mansoor et al. 2021). Notably, despite biochar addition did not improve WUE, the PCA analysis revealed that reduced irrigation regimes clustered toward higher $WUE_i$ and $WUE_n$ compared to FI (Fig. 9), which was
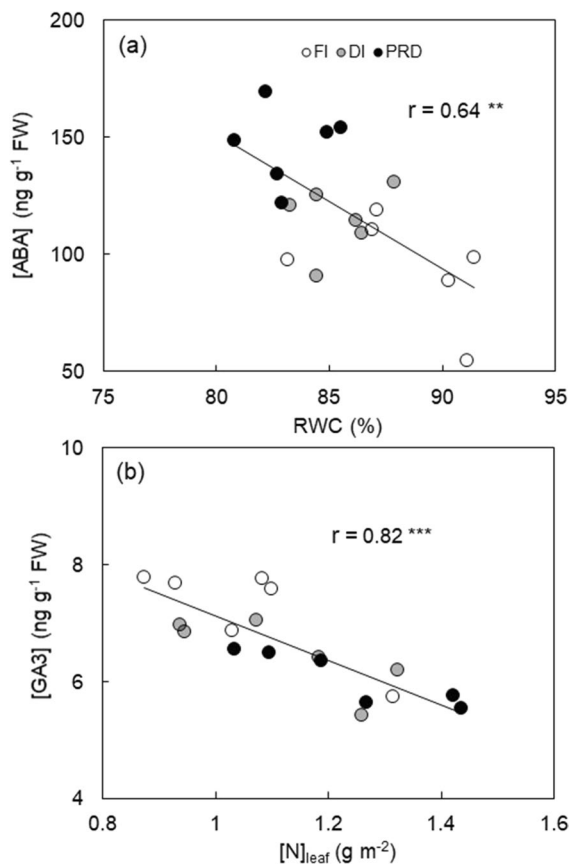
**Fig. 8** Correlations of (a) leaf [ABA] with leaf relative water content (RWC) and (b) leaf [GA3] with leaf N content per unit area ([N]$_{leaf}$) of tobacco plants grown in pots treated with full (FI), deficit (DI) and partial root-zone drying (PRD) irrigation across biochar and soil treatments ($n = 3$). '*', '**', '***' indicates significance level at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$ respectively, 'ns' is non-significant.

related to the reduced $g_m$ and $g_s$ under water deficit (Fig. 2b; Table S1). Many studies indicated that the reduced $g_s$ under drought stress was mainly attributed to the xylem-borne ABA signaling-induced partial stomatal closure (Liu et al. 2006; Zhang et al. 2020b; Yang et al. 2021). Although xylem sap ABA concentration was not determined here, the significant negative correlation between leaf [ABA] and $g_s$ would support ABA role in closing the stomatal aperture (Zhang et al. 2018; Fig. 7b). Besides, other phytohormones and their inner-balance could also be engaged in stomatal regulation under stressed conditions (Fig. 9; Ma et al. 2021; Li et al. 2021). Consistent with this, here, the negative correlations between $g_s$ and $g_m$ with [ABA]/[GA3] but positive relationships

with [GA3]/[ZR] (Fig. 7c, d) highlights the role of phytohormones and their homeostasis in regulating stomatal response to water stress.

Within reduced irrigation regimes, tobacco plants exposed to PRD presented better growth performances as greater LA and LDM and higher WUE$_i$ and WUE$_n$ than DI (Table 1; Fig. 3). We ascribe such advantages to the specific physiological responses in the soil-plant system (Wang et al. 2017; Liu et al. 2021a). For instance, PRD-triggered the soil drying/rewetting cycles could motivate "Birch effect" for soil organic matter mineralization (Birch 1958), leading to a flush of plant bioavailable N (Wang et al. 2017; Table 1), thereby promoting A$_n$ (Fig. 2a). Moreover, the repetitive drying/wetting cycles of soil under PRD can generate stronger and greater ABA signaling (Fig. 8), effectively provokes partial stomatal closure and thus reduces $g_s$ (Liu et al. 2008), consistent with our findings in this study (Fig. 7b). Compared to DI, PRD increased [ABA]/[GA3] but decreased [GA3]/[ZR], which could further co-induce stomatal closure, thus reducing $g_s$ and $g_m$ (Fig. 7c, d). Interestingly, biochar significantly amplified the magnitude of stomatal regulation by hormones through increasing [ABA] and [GA3] and decreasing [ZR] (Fig. 4; Liu et al. 2021b). Adding biochar to soil normally reduces soil acidity, followed by altering rhizosphere biochemistry processes and root exudates, thereby enhancing ABA signaling in the roots (Guo et al. 2021). Hence, PRD coupled with biochar could efficiently trigger partial stomatal closure by up-regulating phytohormone signaling (Fig. 9). Overall, these alterations in the soil environment caused by biochar could ultimately ameliorate the water deficit adverse effects on plants.

Photosynthetic characteristics of tobacco in response to biochar and reduced irrigation regimes

Generally, two controlling steps exist for the limitation of photosynthesis in C$_3$ plants: (1) CO$_2$ concentration in chloroplasts as defined by the diffusive conductivity of CO$_2$; and (2) the trade-off between $J$ and $V_{cmax}$ (Hikosaka et al. 2006). In this study, the lowered CO$_2$ diffusion conductance mediated by mesophyll tissues and stomata pathways, and the biochemical efficiency restrictions, viz. lowered $V_{cmax}$ and $J$, might have jointly contributed to the depressed photosynthesis under reduced irrigation regimes (Figs. 6a and 7a). Reduced irrigation-induced the decreased $V_{cmax}$ and

**Fig. 9** PCA plots of different traits of tobacco plants grown under (a) Ferralsol and (b) Anthrosol amended without (0BC) or with wheat-straw (WSBC) or soft-wood (MWBC) biochar and were subjected to full (FI), deficit (DI) and partial root-zone drying (PRD) irrigation.

$J$ (Table S1; Fig. 9) might be dominated by the limitations of Rubisco and chlorophyll content (Table 1), consistent with Xiong and Flexas (2021). In turn, the relatively increased $J/V_{cmax}$ under reduced irrigation regimes (Fig. 9) may mirror the greater RuBP carboxylation limitation, which correspondingly restricted $g_m$ (Fig. 6b). Therefore, lowered $CO_2$ concentration at the carboxylation site and RuBP carboxylation rate might co-mediated the depressed photosynthesis in the water stressed plants (Fig. 7a). Those results support the earlier observations in tobacco plants responding to abiotic stress by Yamori et al. (2011). Notably, here, PRD mitigated the water stress-induced limitation in photosynthesis by increasing $g_m$ and $V_{cmax}$, despite reducing $g_s$ (Table S1; Fig. 2b), thereby largely sustaining higher $A_n$ compared to DI (Fig. 2a). The improved leaf N nutrition may partially explain this predominance of PRD (Table 1; Wang et al. 2010) that may optimize N partitioning in photosynthetic components so that up-regulates the proteins related to RuBP carboxylation (Rashid et al. 2018; Xiong and Flexas 2021). Because $g_m$ is very sensitive to the changes in leaf N content in tobacco (Xiong and Flexas 2021), which

essentially explained our observations in the study. Oddly enough, biochar addition was not as expected to change the photosynthetic characteristics (Fig. 9). The exact reasons for this are still unclear, and therefore, it is necessary to explore the underlying mechanisms in future research.

### Reduced irrigation regimes and biochar altered antioxidant system of tobacco plants

In fact, ROS as a single-electron reaction product of a class of oxygen, is always located within cells of plant, and the lower ROS are considered necessary for involvement in plant signaling, growth and development (Hu et al. 2010). However, a build-up of ROS above a certain threshold can lead to lipid peroxidation, which directly affects normal cellular functions and exacerbates oxidative stress by generating lipid-derived radicals (Begum et al. 2020; Ahanger et al. 2021). One of the adaptive responses to the over-accumulation of ROS is the activation of the antioxidant defense system (Hu et al. 2010; Khan et al. 2021), which involves increasing antioxidant

enzymes activity (Farhangi-Abriz and Torabian 2017; Begum et al. 2020; Ababaf et al. 2021). Here, SOD, POD and CAT activity showed no significant differences among the full irrigation and reduced irrigation regimes (Fig. 5; Table 2) under both Ferralsol and Anthrosol (Fig. 9); yet PRD plants tended to have higher SOD activity than DI plants (Fig. 5a). PRD induced the greater ABA could stimulate ROS generation by membrane-bound equivalent (NADPH) oxidase, followed by activating plant antioxidant defense system (e.g., SOD) against oxidative damage (Jiang and Zhang 2002). In turn, plant antioxidant systems are also involved in hormonal regulation for enhancing plant tolerance to abiotic stresses (Awan et al. 2020; Begum et al. 2020). These results imply that PRD treatment may mitigate oxidative damage to plants caused by drought stress, thus optimizing plant growth and enhancing WUE under water stressed conditions (Hu et al. 2010).

Maintaining normal cellular metabolic function under stressful conditions designed to minimize oxidative damage requires ROS generation-degradation balance. Recent researches reported that biochar addition relieved the abiotic (e.g., water) stress-induced oxidative damage by up-regulating the enzymatic antioxidant system (Farhangi-Abriz and Torabian 2017; Hafez et al. 2020; Khan et al. 2021). In this study, the application of biochar especially with WSBC significantly increased SOD and POD activities under reduced irrigation regimes, despite CAT activity was depressed (Fig. 5; Table 2), in line with results by Abideen et al. (2020). The natures of porous structure and enriched oxygen functional groups of biochar (Suliman et al., 2017) might have enhanced soil water-holding capacity (Liu et al. 2021a) to normalize turgor pressure and stabilize the activity of plasma membrane of plant to reduce oxidative damage (Khan et al. 2021). Moreover, biochar addition elevated soil pH (Liu et al. 2021c), which may influence the rhizosphere-biochemistry and alter the acid-alkalinity balance within the plant, thereby affecting enzyme activity in leaves (Gul and Whalen 2016; Guo et al. 2021). Hasanuzzaman et al. (2017) and Awan et al. (2020) have indicated that the plant antioxidant systems and phytohormonal regulation are inter-linked and interact with each other under stressed conditions. Consistent with this, here, the significant enhancements of [ABA], [GA3] and [IAA] in biochar treatments (Fig. 4a-c) might

have co-evoked the up-regulation of SOP and POD activity (Jiang and Zhang 2002; Hu et al. 2010), as shown in the PCA plots (Fig. 9). SOD establishes the first line of defense versus toxic radicals, abolishing superoxide from cells and thus lowering the damage to metabolic pathways (Choudhury et al. 2017; Ahanger et al. 2021). POD is mainly located in the apoplastic space and vacuole and performs an important role in catalyzing $H_2O_2$ to $H_2O$ and $O_2$ (Gratao et al. 2005). Thus, enhanced SOD and POD activities with biochar addition could prevent drought stress-induced reduction in photosynthesis by sustaining membrane stability due to the rapid elimination of ROS (Yang et al. 2014; Abideen et al. 2020). Early studies have shown that biochar addition can enhance plant productivity by reducing $O_2\bullet^-$, MDA (malonyldialdehyde) and $H_2O_2$ concentration by strengthening antioxidant enzyme activities (Farhangi-Abriz and Torabian 2017; Abideen et al. 2020). Notably, Ferralsol-plants possessed lower SOD activity than Anthrosol (Fig. 5a; Table 2), which may be related to the difference in soil properties. Ferralsols, typically with spreading stratigraphic boundaries, low activity clays-dominated clay assemblages, low pH, and high sesquioxides of Fe and Cu (IUSS 2015). Such natures ensure hydrogen peroxide reacts with $Fe^{2+}$ and $Cu^+$ to form toxic uncharged $OH^\bullet$ which can penetrate the cell membrane and leave the chloroplast (Gill and Tuteja 2010). This further leads to the generation of cytotoxic lipid aldehydes, alkenes and hydroxyalkenals, such as MDA (Abideen et al. 2020), thus restrained the antioxidant enzyme activities.

## Conclusion

Globally, drought stress has led to considerable drops in crop yield by disrupting plant growth and physiology processes, thus tapping efficient agricultural practices to mitigate the adverse effects of drought stress on plants and its mechanisms are very urgent. Our findings indicate that reduced irrigation regimes including DI and PRD adversely affects tobacco morphophysiological traits as indicated by the decreased plant height, stem diameter, leaf area and leaf biomass as well as restricted leaf gas exchange induced by depressed leaf relative water content. Nevertheless, compared to DI, PRD enhanced crop tolerance to water stress by regulating the drying-rewetting

cycle-mediated endogenous phytohormonal levels and enzymatic antioxidant systems, efficiently reducing $g_s$ and maintaining $A_n$, thereby increasing $WUE_i$. Furthermore, biochar, especially wheat-straw biochar, ameliorated the water stress-mediated growth decline in tobacco plants by increasing superoxide dismutase (SOD) and peroxidase (POD) and decreasing catalase (CAT) activities, as well as up-regulating phytohormone levels (such as ABA) and altering phytohormones homeostasis (such as the ration of ABA to GA3), which enables plants to maintain functional stability, thereby improving biomass production. Therefore, in agricultural practices, combined biochar/PRD application may have outstanding efficacy in maintaining high yield output while reducing water usage, particularly under water scarcity conditions.

**Data Availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Declarations**

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