



## Impacts of Acidified Biochar on Wheat growth under Deficit Irrigation systems

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**D**ROUGHT is an abiotic stress that threatens sustainable food production worldwide. The current study investigates the feasibility of using acidified-biochar to increase wheat productivity in arid soils under deficit irrigation systems. To attain this aim, acidified-biochar was prepared from maize-stover at 550°C, then mixed with 0.1M H<sub>2</sub>SO<sub>4</sub>. A pot experiment was then conducted following a complete randomized-block-design comprising 2 factors: (1) maintaining soil moisture at 60, 80 and 100% of soil field capacity (FC<sub>60</sub>, FC<sub>80</sub> and FC<sub>100</sub>, respectively) and (2) biochar application dose 0 (B<sub>0</sub>), 5 (B<sub>5</sub>) and 10 g kg<sup>-1</sup> (B<sub>10</sub>). All pots received recommended N and P doses. Results reveal that deficit irrigations lessened available N and K concentrations, while in case of available-P, its concentration followed the sequence of: FC<sub>80</sub>>FC<sub>100</sub>>FC<sub>60</sub>. Likewise, NPK uptake and proline content followed the same sequence of available-P. On the other hand, application of biochar elevated NPK uptake by plants, and also raised proline contents in shoots, especially with increasing its dose. Generally, there were significant positive correlations among values of NPK uptake by wheat plants and, in particular the increases in biomasses of different plant parts were highly correlated with P uptake by plants. Concerning the combination between the two factors, the highest shoot and grain yields were detected for FC<sub>100</sub>+B<sub>5</sub>. This treatment also recorded the highest NPK uptake. In conclusion, acidified biochar boosted wheat growth and productivity while rationalizing 20% of water inputs. Further investigations are needed to be carried out under field conditions for at least 2 successive years.

**Keywords:** deficit irrigations; acidified biochar; arid soils; NPK uptake; proline.

### 1. Introduction

Drought is an abiotic stress (Nyaupane *et al.* 2024) that threatens sustainable food production worldwide (Sári *et al.* 2024, Dianatmanesh *et al.* 2022), particularly within the arid and semi-arid regions (Yildirim *et al.* 2021). This stress impedes many vital processes within plants; accordingly it destructively

affects plant growth and production (Emami Bistgani *et al.* 2024). In particular, stressed plants become less capable of utilizing soil nutrients (Yildirim *et al.* 2021), in addition to the adverse effects of drought on plant respiration and photosynthesis (Emami Bistgani *et al.* 2024). Using biochar may improve water and mineral uptake by plants (Mansoor *et al.* 2021), because it forms soluble organic complexes with the immobile nutrients in soil (Lalarukh *et al.* 2022a, Mohamed *et al.* 2024a, Elshony *et al.* 2019,

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Mohamed *et al.* 2024b), beside of its high porosity and hydrophilic characteristics (Li and Tan 2021) that enable it to retain nutrients on its surface against loss by leaching (Laird *et al.* 2010). Moreover, biochar enhances many physiological and biochemical attributes (Yildirim *et al.* 2021, Abdelhafez *et al.* 2024) such as increasing activities of antioxidants and osmoprotectants (Khan *et al.* 2021, Lalarukh *et al.* 2022a). Overall, biochar application is guaranteed to lessen the impacts of drought stress on plants (Bassouny and Abbas 2019, Zhang *et al.* 2023).

Soils of Egypt are characterized by their high pH values (Abd El-Mageed *et al.* 2021), and at the same time, biochar is of alkaline nature (Fidel *et al.* 2017) which may reduce the availability of most soil macro and micronutrients upon its application (Khalil *et al.* 2023). The acidified biochar may alternatively be used to overcome this problem (Khalil *et al.* 2023, Abd El-Mageed *et al.* 2021, Abuzaid *et al.* 2025). This additive is characterized by its relatively rapid degradation rate which enriches soils with nutrients needed for proper plant growth (Ramzani *et al.* 2017).

Wheat is a strategic crop (Lalarukh *et al.* 2022b, Parveen *et al.* 2024) that plays an important role in insuring food security (Langridge *et al.* 2022, Parveen *et al.* 2024). It is probably the most important winter crop in Egypt (Farid *et al.* 2023a, Farid *et al.* 2023b, . Ali *et al.* 2024); in spite of that Egypt has become the largest wheat importer worldwide (Abdalla *et al.* 2023). To increase wheat

production, many strategies could be followed to cope with stress conditions (Bapela *et al.* 2022, Mwadzingeni *et al.* 2016), such as amending soils with acidified biochar (Farid *et al.* 2025) and/or following deficit irrigations (Saad *et al.* 2023).

The current study investigates the feasibility of using acidified biochar to alleviate drought stress and increase productivity of wheat grown on an arid soil while following the deficit irrigation techniques, i.e. irrigation at 60 and 80% of soil field capacity. A reference treatment was also considered where wheat plants were irrigated at 100% of FC while their rhizosphere did not receive any organic addition. The main hypothesis of this study is that the application of acidified biochar may alleviate the drought stress, and also enriches soils with nutrients in available forms to improve plant growth and productivity. Distribution of NPK nutrients within different plant parts and their possible modes of action to cope with drought stress were further aims of the study

## 2. Materials and Methods

A surface soil sample was collected for the experimental farm of the Faculty of Agriculture, Benha University, Egypt (31° 13' 24.4" E. and 30° 21' 22.2" N). This sample was air dried, crushed and sieved via a 2 mm sieve then analyzed for its physical and chemical properties according to Klute (1986) and Sparks *et al.* (2020), respectively. The obtained results are shown in Table 1.

**Table 1. Soil physical and chemical characteristics**

Character	Value	Character	Value
Particle size distribution (%)		Field capacity (%)	32
Fine sand	1.05	Soil Chemical analyses	
Coarse sand	1.3	Soil pH	7.97
Silt	19.25	Soil EC (dS m <sup>-1</sup> )	1.49
clay	46.72	Calcium carbonate content (g kg <sup>-1</sup> )	17.31
Textural class	Clay	Soil organic matter (g kg <sup>-1</sup> )	14.23

Soil pH was determined in soil:water suspension prepared at a ratio of 1:2.5, while EC was determined in soil paste extract

Maize stover was collected from the nearby farms, oven-dried at 65°C overnight, and pyrolyzed under limited aeration conditions via a muffle (Magma Term Laboratory Furnace- Model SNOL 8,2/1100) at 550°C for 2 h. This product was acidified with 0.1 M H<sub>2</sub>SO<sub>4</sub> (1:100 w/v), shaken for 4

h at 150 rpm, filtered and rinsed in tap water then distilled water to remove chemicals (El-Sharkawy *et al.* 2022). Afterwards, biochar was oven dried at 70 °C. It exhibited the following characteristics: pH=4.23, EC= 2.86 dS m<sup>-1</sup>, and its contents of C, N, H, O and S were 68.45, 0.41, 3.25, 12.28 and 1.05%, respectively. Water holding capacity was determined

gravimetrically according to Hien *et al.* (2021) after being soaked in distilled water and the obtained value was 3.61 g water g<sup>-1</sup> dry biochar. Further characterizations of biochar were inspected at the Atomic Energy Authority, Cairo, Egypt, i.e. (1) FTIR spectroscopic analysis by Nicolet iS10 over the range of 400-4000 cm<sup>-1</sup>, (2) X-Ray Diffraction by Pw 1730 Powder X Ray Diffractometer, (3) Scanning electron microscopy (SEM) by JEOLJSM-5400 scanning microscope (Japan) and elemental analysis using energy dispersive X-ray spectroscopy (JEOL-JSM 5300, Japan).

### 2.1. Experimental procedures

Twenty seven plastic pots (25 cm diameter × 19 cm depth) were packed uniformly with about 4 kg of soil, then planted with 10 seeds of wheat (Giza 171) on the 25<sup>th</sup> of November 2023. These pots were arranged under the greenhouse conditions in a complete randomized design comprising 2 factors: (1) deficit irrigations at 60 and 80% of the field capacity (FC<sub>60</sub> and FC<sub>80</sub>, respectively) as well as 100% of the field capacity (FC<sub>100</sub>). (2) biochar application at three rates 0 (B<sub>0</sub>), 5 g kg<sup>-1</sup> soil (B<sub>5</sub>) and 10 g kg<sup>-1</sup> soil (B<sub>10</sub>). The combinations between these two factors brought a total number of 9 treatments with three replicates each.

All pots received the recommended N and P doses, i.e. 75.6 g N kg<sup>-1</sup> soil in the form of urea (its content was 460 g N kg<sup>-1</sup>) and 15 g P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> in the form of calcium super phosphate (its content was 156 g P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup>). Soil moisture was monitored periodically by a tensiometer (model Theta-θ-Probe ML2x) and well water (EC=0.59 dS m<sup>-1</sup>) was added to compensate water loss and keep soil moisture at the abovementioned regimes. At physiological maturity stage whole plants were collected, placed on sieves, washed with tap water then distilled water. Moreover, soils were sampled from the rhizosphere of each pot.

### 2.2. Plant and Soil analyses

Plant materials were separated into roots, shoots and grains then oven dried at 65 °C for 48 h. Portions of these materials (equivalent to 0.5 g) were wet digested using perchloric and sulfuric acids on a sandy hot plate at 250 °C according to Cottenie *et al.* (1982), where nitrogen was determined in plant digests by micro Kjeldahl, phosphorus was assessed following molybdate-ascorbic acid method, then

photometrically by JENWAY 6405 UV/VIS. Potassium was quantified by flame photometer (model JENWAY PFP7). Proline was determined in shoots after 50 days of cultivation according to Bates *et al.* (1973).

Available contents of soil nutrients were extracted according to Page *et al.* (2020) as follows: available N by K<sub>2</sub>SO<sub>4</sub> (1%), then determined by Kjeldahl in presence of Devarda's alloy and MgO, (2) available K by ammonium acetate solution (pH=7) then indicated on flame photometer and (3) Olsen P was estimated photometrically. Soil pH was determined in soil:water suspension prepared at a ratio of 1:2.5 using pH meter (model Consory C3210) while EC was measured in soil paste extract using EC meter (model WTW inolab Cond 720).

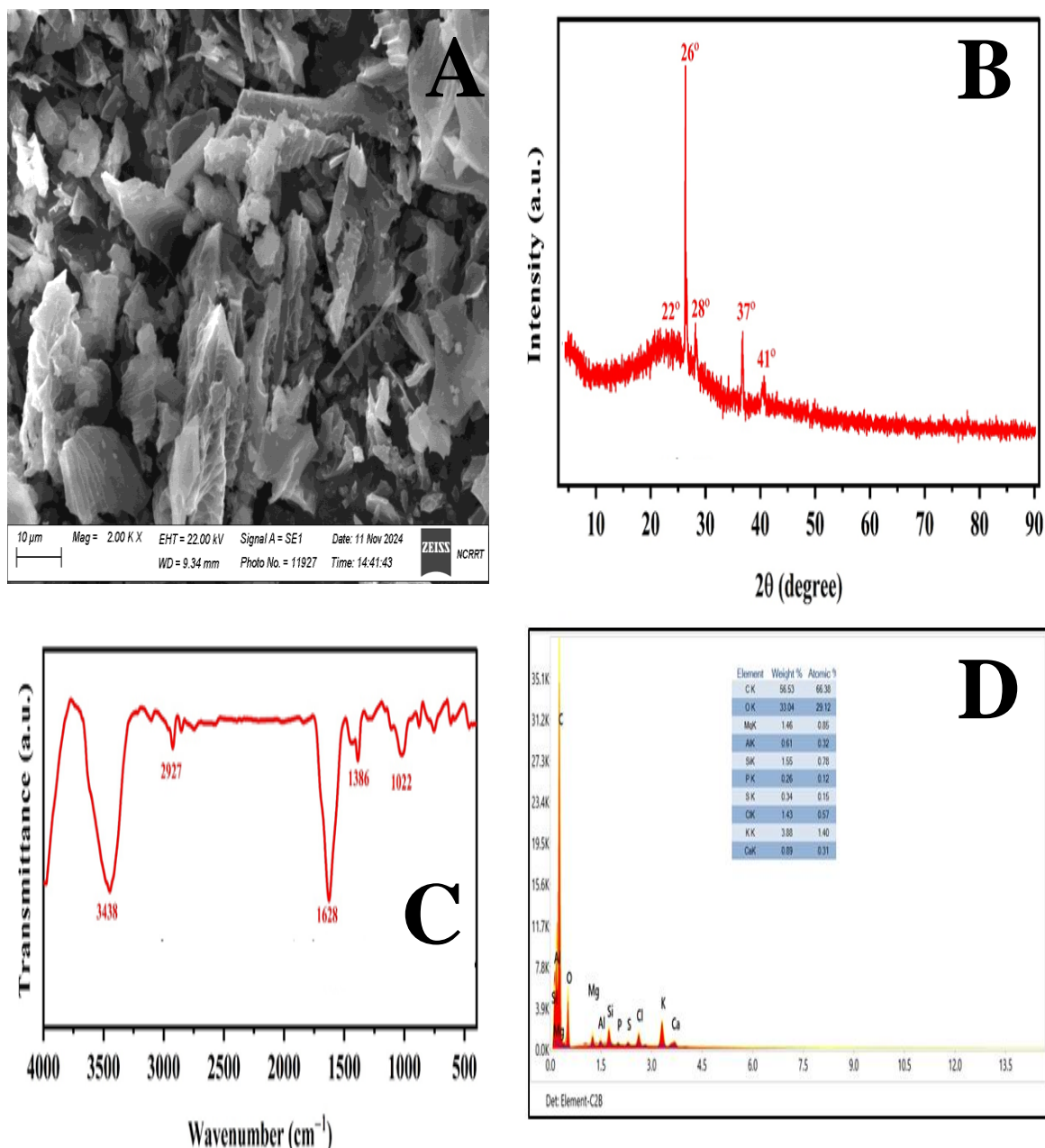
### 2.3. Data processing

All chemicals were of analytical grade. Obtained data were analyzed statistically using two-way ANOVA and Duncan's test through SPSS statistical software version 18. Graphs were plotted using Sigma Plot software version 10.

## 3. Results

### 3.1. Characteristics of the used acidified biochar

Acidified biochar shows aggregation with large, dense clusters (≈ mean particle diameter of 4998 nm, Fig 1A). The XRD pattern exhibited 5 peaks at 2θ values at 22°, 26°, 28°, 37° and 41°, characterizing the presence of amorphous carbon structures which guarantee its mechanical stability and durability in soil (Fig 1B). The FTIR spectra displayed a broad peak at 3400 cm<sup>-1</sup>, which refers to O–H stretching indicating biochar's ability to retain moisture and nutrients. The peak at 2956 cm<sup>-1</sup> corresponds to C–H stretching vibrations, suggesting the presence of aliphatic compounds, which contribute to biochar's hydrophobic regions. The 1623 cm<sup>-1</sup> peak represents C=C stretching in aromatic rings, indicating the presence of stable carbon structures that can resist microbial degradation. Peaks at 1346 cm<sup>-1</sup> (C–O or C–H bending) and 1022 cm<sup>-1</sup> (C–O stretching) are associated with ether or ester linkages, which may enhance biochar's adsorption sites for potentially toxic elements or organic pollutants.

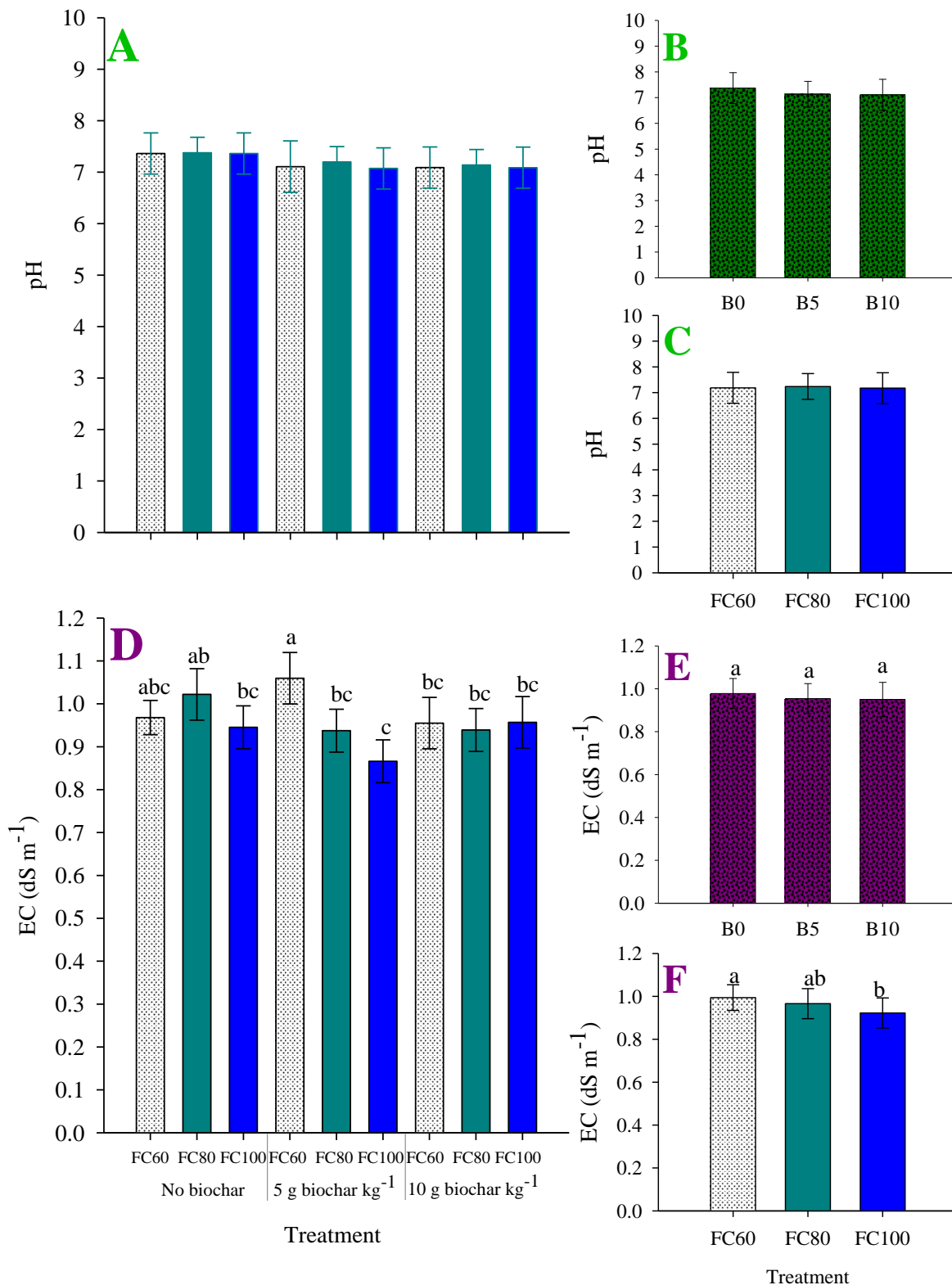


**Fig 1. SEM (A), XRD (B), FTIR spectrum (C) and X-ray Spectroscopy (EDX) (D) of the used acidified biochar**

### 3.2. Effect of biochar on soil pH and EC under deficit irrigations

No significant effects were deduced for using deficit irrigations on soil pH (Fig 2A). Likewise, acidified biochar application and its interaction with irrigation levels recorded no further significant impacts on this parameter. On the other hand, soil EC varied significantly under the drought conditions; yet the effect of acidified biochar addition was of no significant impact in this concern (Fig 2B). Generally, soil EC increased in soils that received

60% of the field capacity ( $\text{FC}_{60}$ ), followed by the ones that received 80% of the field capacity (FC) ( $\text{FC}_{80}$ ) then 100% of FC ( $\text{FC}_{100}$ ). Interactions between irrigation levels and acidified biochar application were also of significant effect on soil EC. In this aspect, the least EC value was detected in the treatment that received  $\text{FC}_{100} + \text{B}_5$ ; while the highest increases were found in the soil that was treated with  $\text{FC}_{60} + \text{B}_5$



**Fig 2.** Soil pH and EC (means ± standard deviations) as affected by application of acidified biochar under deficit irrigation systems. Note: B<sub>0</sub>: no added biochar, B<sub>5</sub>: acidified biochar applied at a rate of 5 g kg<sup>-1</sup>, B<sub>10</sub>: biochar added at a rate of 10 g kg<sup>-1</sup>. Similar Duncan’s letters indicate no significant variations among treatments

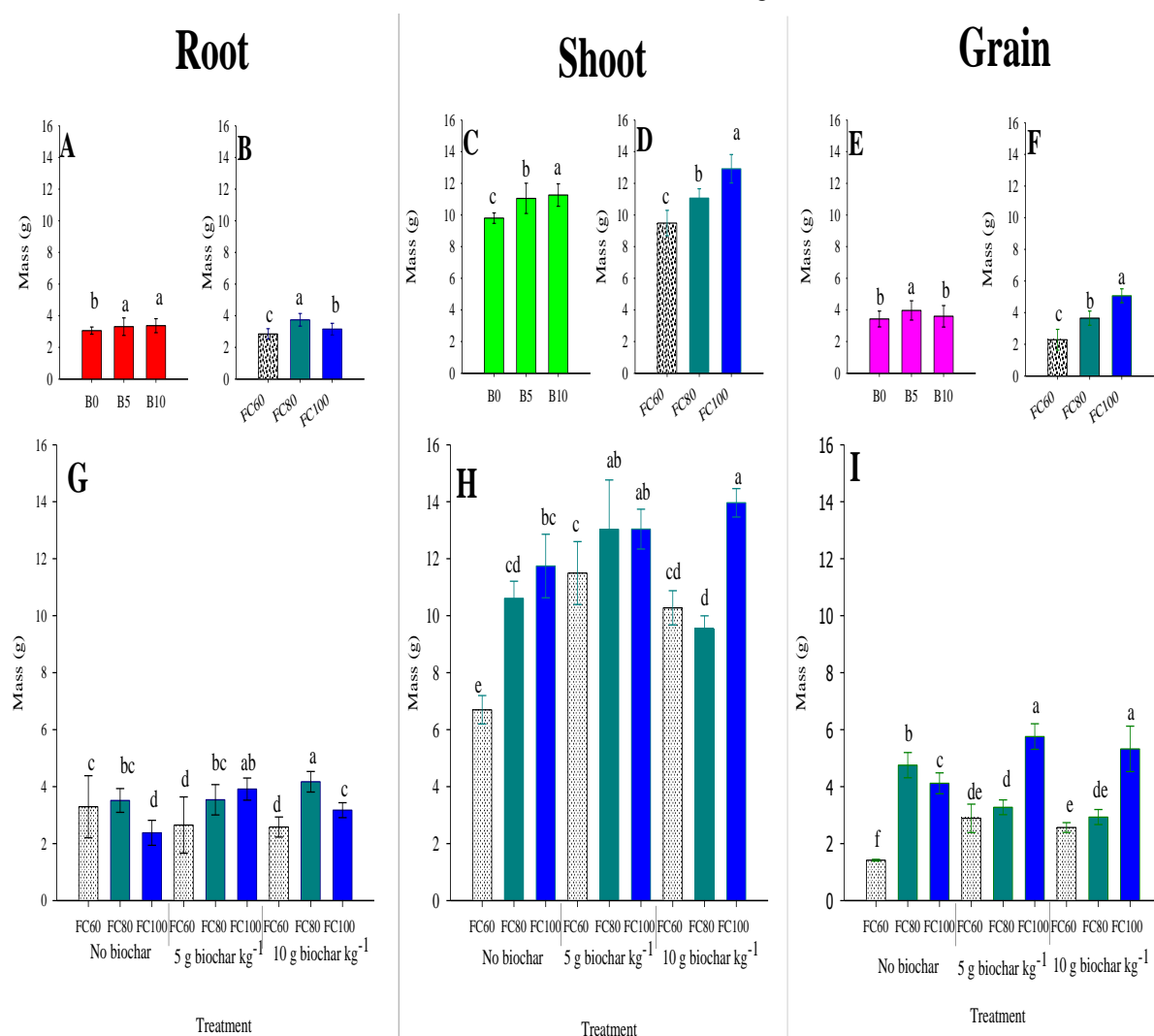
### 3.3. Effect of biochar on the dry weights of wheat roots, shoots and grains

Drought stress lessened the biomasses of wheat shoots and grains whose values followed the descending order: FC<sub>100</sub>>FC<sub>80</sub>>FC<sub>60</sub> (Figs 3 D & F). Likewise, this stress lessened root biomass; nevertheless, the obtained sequence was somehow different (FC<sub>80</sub>>FC<sub>100</sub>>FC<sub>60</sub>) (Fig 3B).

Application of the acidified biochar minimized the salinity stress on plants when being applied at a rate of 5 g kg<sup>-1</sup> as this rate enhanced significantly wheat root, shoot and grain yields (Figs 3A, C & E). Higher dose of biochar (10 g kg<sup>-1</sup>) boosted shoot growth, while decreased grain productivity versus the

lower biochar dose (5 g kg<sup>-1</sup>). Despite that, this higher dose of biochar (10 g kg<sup>-1</sup>), generally increased root and shoot biomasses over B<sub>0</sub>, while recorded no significant variations in grain yields.

Interactions between biochar doses and the soil moisture levels were also significant (Figs 3G, H & I). It was found that the least root biomasses were detected in plants treated with either of FC<sub>100</sub>+B<sub>0</sub>, FC<sub>60</sub>+B<sub>5</sub> or FC<sub>60</sub>+B<sub>10</sub> with no significant variations between these two treatments (Fig 3G). The former treatment recorded the highest increase in shoot biomass (Fig 3H). Regarding the grain yield, the highest increases were attained for both FC<sub>100</sub>+B<sub>5</sub> and FC<sub>100</sub>+B<sub>10</sub> (Fig 3I).

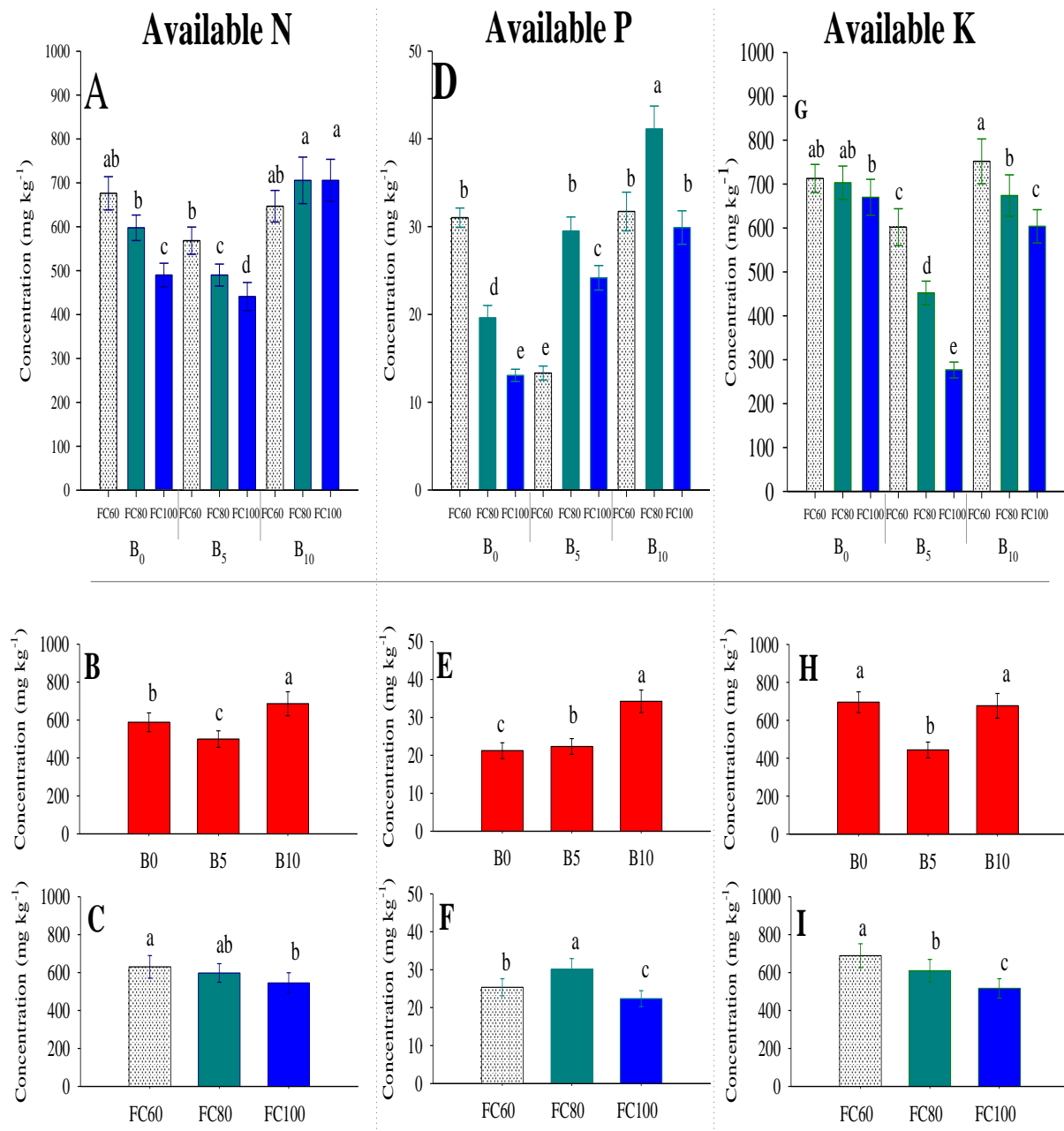


**Fig 3.** Dry weight values of wheat roots, shoots and grains (means  $\pm$  standard deviations) as affected by application of acidified biochar under deficit irrigation systems. Note: B<sub>0</sub>: no added biochar, B<sub>5</sub>: acidified biochar applied at a rate of 5 g kg<sup>-1</sup>, B<sub>10</sub>: biochar added at a rate of 10 g kg<sup>-1</sup>. Similar Duncan's letters indicate no significant variations among treatments

3.4. Effect of biochar on NPK available contents in soil under deficit irrigations

Available N and K decreased significantly in soils with increasing soil moisture content (Figs 4 C & I), while in case of P, its available content increased at FC<sub>80</sub> and then dropped again at FC<sub>100</sub> (Fig 4 F).

Biochar application at a rate of 5 g kg<sup>-1</sup> diminished the available concentrations of N and K in soil (Figs 4 B & H) while raised P-available content (Fig 4E). Though, the available contents of these three nutrients increased in the soil that received the higher dose of biochar.



**Fig 4.** Available NPK (means ± standard deviations) in soil as affected by application of acidified biochar under deficit irrigation systems. Note: B<sub>0</sub>: no added biochar, B<sub>5</sub>: acidified biochar applied at a rate of 5 g kg<sup>-1</sup>, B<sub>10</sub>: biochar added at a rate of 10 g kg<sup>-1</sup>. Similar Duncan’s letters indicate no significant variations among treatments

Interactions between the irrigation level and biochar were also significant on NPK available contents in soil (Figs 4A, D & G). Generally, the highest

increases in available P was found for FC<sub>80</sub> +B<sub>10</sub>(Fig 4B) while the highest available K was found for FC<sub>60</sub> +B<sub>10</sub> (Fig 4C). In case of nitrogen, no significant

variations in N-available contents were noticed among these three irrigation levels when the soil received 10 g biochar kg<sup>-1</sup> (Fig 4A).

### 3.5. Effect of biochar on nutrient concentrations within different wheat parts and their uptake by plants under deficit irrigations

Uptake and bioaccumulation of N, P and K within different parts of wheat plants are presented in Table 2.

**Table 2. Concentrations of NPK (means ± standard deviations) and their uptake by wheat plants as affected by deficit irrigation and application of acidified biochar**

	Nitrogen (g kg <sup>-1</sup> )				Phosphorus (g kg <sup>-1</sup> )			
	B <sub>0</sub>	B <sub>5</sub>	B <sub>10</sub>	Mean	B <sub>0</sub>	B <sub>5</sub>	B <sub>10</sub>	Mean
	<b>Concentration in roots (mg g<sup>-1</sup>)</b>							
FC <sub>60</sub>	11.20 de	12.60 d	9.90 e	11.23 C	19.62 f	33.33 4b	30.17 cd	27.71 C
FC <sub>80</sub>	13.83 c	16.50 b	14.33 bc	14.89 B	26.33 e	33.50 b	29.00 d	29.61 B
FC <sub>100</sub>	13.83 c	14.07 bc	21.25 a	16.38 A	43.50 a	31.50 a	32.83 bc	35.94 A
Mean	12.96 B	14.39 A	15.16 A		29.82 C	32.78A	30.67B	
	<b>Concentration in shoots (mg g<sup>-1</sup>)</b>							
FC <sub>60</sub>	0.21 d	0.21 d	0.19 d	0.20 C	0.33 a	0.23 b	0.13 e	0.23A
FC <sub>80</sub>	0.34 a	0.28 b	0.24 c	0.29 A	0.16 cd	0.16 cd	0.14 de	0.15B
FC <sub>100</sub>	0.21 d	0.24 c	0.35 a	0.27 B	0.17 c	0.15 1 d	0.16 cd	0.16B
Mean	0.25 A	0.24 A	0.26 A		0.22 A	0.18B	0.14C	
	<b>Concentration in grains (mg g<sup>-1</sup>)</b>							
FC <sub>60</sub>	8.27 bc	8.42 ab	7.49 c	8.06 C	1.38 de	1.35 e	1.16 f	1.30C
FC <sub>80</sub>	9.35 ab	9.59 a	7.76 c	8.90 A	1.52 bc	1.65 b	1.43 c	1.53B
FC <sub>100</sub>	8.83 ab	8.37 b	7.89 c	8.36 B	1.38 de	1.41 cd	2.13 a	1.64A
Mean	8.82 A	8.79 A	7.71 B		1.43 C	1.47 B	1.57A	
	<b>Total uptake per pot (mg pot<sup>-1</sup>)</b>							
FC <sub>60</sub>	50.72 g	60.37 f	46.04 g	52.39 C	68.94 f	95.57 d	81.69 e	82.07C
FC <sub>80</sub>	96.31 c	93.44 c	85.01 d	91.59 B	101.30 c	96.93 d	79.96 e	118.14B
FC <sub>100</sub>	72.20 e	106.32 b	113.53 a	97.35 A	111.49 b	133.29 a	117.35 b	120.71A
Mean	73.08 C	86.71 A	81.53 B		94.25C	118.26A	108.52B	
	<b>Potassium (g kg<sup>-1</sup>)</b>							
	<b>Concentration in roots (mg g<sup>-1</sup>)</b>							
FC <sub>60</sub>	4.52 c	4.91 ab	4.31 bcd	4.58 B				
FC <sub>80</sub>	4.22 cd	5.77 a	4.17 d	4.72 A				
FC <sub>100</sub>	4.41 bc	4.74 ab	4.23 cd	4.46 C				
Mean	4.39 B	5.14 A	4.24 B					
	<b>Concentration in shoots (mg g<sup>-1</sup>)</b>							
FC <sub>60</sub>	0.22 a	0.19 ab	0.18 b	0.20A				
FC <sub>80</sub>	0.18 b	0.17 bc	0.18 b	0.17B				
FC <sub>100</sub>	0.20 ab	0.15 c	0.13 c	0.16B				
Mean	0.20 A	0.17 B	0.16 B					
	<b>Concentration in grains (mg g<sup>-1</sup>)</b>							
FC <sub>60</sub>	10.00 ab	9.17 ab	8.33 b	9.17C				
FC <sub>80</sub>	8.33 b	9.17 ab	10.67 a	9.39A				
FC <sub>100</sub>	10.00 ab	9.00 ab	8.83 b	9.28B				
Mean	9.44A	9.11 C	9.28 AB					
	<b>Total uptake per pot (mg pot<sup>-1</sup>)</b>							
FC <sub>60</sub>	31.32 f	41.75 e	33.67 f	35.58 C				
FC <sub>80</sub>	56.13 c	52.62 cd	50.55 d	53.10 B				
FC <sub>100</sub>	54.45 cd	72.33 a	62.01 b	62.93 A				
Mean	47.30B	55.56A	48.74B					



Note: B<sub>0</sub>: no added biochar, B<sub>5</sub>: acidified biochar applied at a rate of 5 g kg<sup>-1</sup>, B<sub>10</sub>: biochar added at a rate of 10 g kg<sup>-1</sup>. Similar Duncan's letters indicate no significant variations among treatments

### 3.5.1. Nitrogen uptake and concentrations within wheat parts

Nitrogen uptake and its content within wheat roots augmented significantly with increasing the irrigation level, following this sequence: FC<sub>100</sub>>FC<sub>80</sub>>FC<sub>60</sub>. Regarding its accumulation within shoots and grains, this nutrient exhibited lower concentration at FC<sub>60</sub> then increased at FC<sub>80</sub> while again decreased in FC<sub>100</sub> treatments.

Biochar application at a rate of 5 g kg<sup>-1</sup> upgraded nitrogen contents within different wheat parts as well as its total uptake by plants. Increasing biochar application level from 5 to 10 g biochar kg<sup>-1</sup> recorded significant reductions in N- content within grains as well as its total uptake by plants, while no significant differences were detected in its content in either roots or shoots.

Interaction results between irrigation levels and biochar doses revealed that FC<sub>100</sub>+B<sub>10</sub> recorded the highest increases in N-uptake, which was accumulated mainly in roots and shoots, while the treatment FC<sub>80</sub>+B<sub>5</sub> recorded the highest N content in grains.

### 3.5.2. Phosphorus uptake and concentrations within wheat parts

Deficit irrigations led to significant reductions in P contents within the different plant parts (except in shoots) and also lessened its total uptake by plants, following generally the descending order : FC<sub>100</sub>>FC<sub>80</sub>>FC<sub>60</sub>. In particular, the highest P concentration was found in shoots of the stressed plants which were irrigated with FC<sub>60</sub> as if these plants enhanced P translocation to shoots to initiate sooner the reproductive growth.

Application of biochar at a rate of 5 g kg<sup>-1</sup> raised P uptake by plants and also upraised its accumulation within different plant parts. Nevertheless, a higher rate of biochar lessened considerably P uptake and concentrations within plant parts. Interactions between soil moisture levels and biochar dose showed that the highest P-uptake was for FC<sub>80</sub>+B<sub>5</sub>, while the highest increase in P content within grains was for FC<sub>100</sub>+ B<sub>10</sub>.

### 3.5.3. Potassium uptake and concentrations within wheat parts

Increasing soil moisture raised significantly the uptake of K by plants; though, its highest concentrations in both roots and grains were found in

plants irrigated with FC<sub>80</sub>, while the highest concentration in shoot was at FC<sub>60</sub>. Concerning biochar applications, uptake of this nutrient increased in plants that received 5 g biochar kg<sup>-1</sup> while decreased at the higher application dose (10 g biochar kg<sup>-1</sup>). Generally, K concentrations decreased in wheat parts as a result of biochar application which is mostly the consequences of the dilution effect due to increasing plant growth.

### 3.6. Effect of biochar on proline content within wheat shoots under deficit irrigations

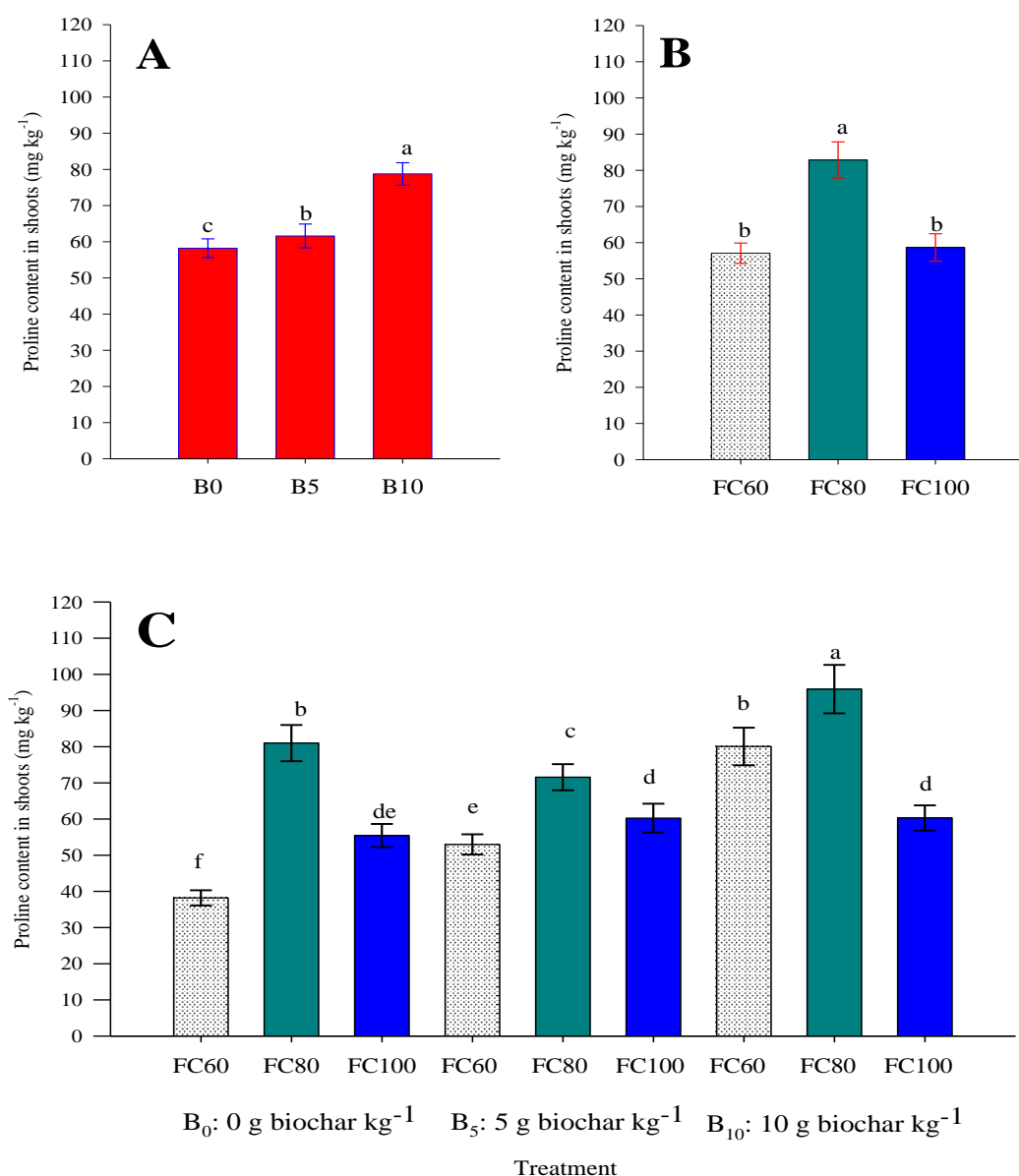
Proline content increased significantly in shoots of FC<sub>80</sub> stressed plants versus those grown at FC<sub>100</sub>, yet the plants subjected to FC<sub>60</sub> contained comparable proline content with those of FC<sub>100</sub>. On the other hand, application of biochar upraised significantly proline concentrations in wheat shoots; and such increase became noticeable upon application of B<sub>10</sub>. Regarding the interactions between deficit irrigations and biochar, soil moisture content seemed to have superior effect over biochar as FC<sub>80</sub> treatments (-/+biochar) recorded the highest increases in proline content within shoots. In plants subjected to either FC<sub>60</sub> or FC<sub>100</sub>, increasing biochar dose led to concurrent increases in proline content within shoots, while in case of plants growing at FC<sub>100</sub>, no significant variations were deduced for the application of biochar on proline content.

### 3.7. Correlations between wheat growth parameters. NPK uptake by wheat and their contents within different plant parts

Wheat grain yield was significantly correlated with P content in grains ( $P<0.05$ ), but not with the corresponding N and K contents (Table 2); nevertheless the grain yield was significantly and positively correlated with these three nutrients in shoots. Probably, plants utilize P in shoots to initiate and increase the reproductive growth ( $r^2=-0.556$ ), while N and K played more major roles in the coping mechanisms of plants against drought stress, especially N which recorded a higher significant positive correlation with the grain yield.

Generally, the uptakes of NPK nutrients were significantly correlated with each other and subsequently affected the grain yield. On the other hand, only P and K uptake values were significantly correlated with straw dry weights, while P-uptake

was the only nutrient that recorded significant correlations with root dry weight.



**Fig 5. Proline concentrations (means  $\pm$  standard deviations) in wheat shoots as affected by application of acidified biochar under deficit irrigation systems. Note: B<sub>0</sub>: no added biochar, B<sub>5</sub>: acidified biochar applied at a rate of 5 g kg<sup>-1</sup>, B<sub>10</sub>: biochar added at a rate of 10 g kg<sup>-1</sup>. Similar Duncan's letters indicate no significant variations among treatments**

These results provide more evidences that N and K were incorporated directly and indirectly in increasing plant resistance against drought stress. In this concern, a negative correlation was found between proline content in shoots and N content in roots. The non-significant correlations between N-uptake and each of root and shoot biomasses indicate that N as an osmoregulator was circulated between

roots and shoots. The mechanism of K in increasing plant tolerance towards drought was somehow different. It increased the osmotic potential in roots thus recorded no significant correlations with root dry weights, while in shoots, this nutrient was mainly incorporated in increasing plant productivity i.e., straw dry weight was significantly correlated with the K-uptake.

Root biomasses were significantly correlated with proline in plants as if plants with more proline became more capable of utilizing soil moisture. Such increases in roots were to find out more water and nutrients from soil; accordingly root biomasses

recorded no significant correlations with either shoot or grain yields. On the other hand, shoot and grain biomasses were significantly correlated with each other.

**Table 2. Pearson’s correlation among concentrations of NPK in wheat parts and their impacts on plant biomass**

	Plant dry weight		Proline content	Concentrations in grains			Concentrations in straw			Concentrations in roots			Nutrients uptake per pot			
	Shoot	Grains		N	P	K	N	P	K	N	P	K	N	P	K	
	<b>Plant dry weight</b>															
Root	0.077	0.125	0.789**	-0.149	-0.212	-0.463*	-0.060	0.035	-0.095	-0.314	-.311	-0.026	0.221	0.577**	0.175	
Straw		0.398*	0.030	0.470*	0.190	-0.096	-0.006	0.024	-0.072	0.241	0.634**	0.554**	0.379	0.551**	0.476*	
Grain			-0.290	0.248	0.518**	-0.267	0.620**	-0.556**	-0.677**	0.617**	0.430*	-0.052	0.941**	0.556**	0.979**	
Proline				-0.525**	-0.592**	-0.241	-0.541	0.179	0.018	-0.680**	-0.358	-0.028	-0.197	0.365	-0.194	
	<b>Concentrations in grains</b>															
N					0.313	-0.192	-0.206	0.135	-0.223	0.315	0.326	0.024	0.262	-0.026	0.253	
P						-0.784**	-0.782**	0.045	-0.806**	0.973**	0.727*	-0.724*	0.684	0.009	0.441	
K							0.973**	-0.062	0.976**	-0.681**	-0.808**	0.920**	-0.379	-0.446*	-0.220	
	<b>Concentrations in straw</b>															
N								-0.149	0.999**	-0.681**	-0.844**	0.932**	0.755**	0.003	0.500**	
P									-0.135	-0.106	-0.121	-0.080	-0.438*	-0.447*	-0.520**	
K										-0.707**	-0.845**	0.933**	-0.749**	-0.425*	-0.643**	
	<b>Concentrations in roots</b>															
N												0.721**	-0.618**	0.723**	0.092	0.557**
P													-0.741**	0.269	0.545**	0.469*
K														0.049	0.114	0.049
	<b>Nutrients uptake per pot</b>															
N															0.533**	0.915**
P																0.631**
K																

Note: \*  $P < 0.05$ ; \*\*  $P < 0.01$

**4. Discussion**

*4.1. Impacts of deficit irrigations and acidified biochar on plant growth parameters*

Drought stress played significant roles in diminishing wheat growth and grain yield. In this concern, plants that were irrigated with FC<sub>60</sub> recorded the least root, shoot and grain biomasses. Probably, this abiotic stress altered the structure of chloroplasts, mitochondria, and chlorophyll content, while raised radical free oxygen which, in turn, causes peroxidation of the lipids of cell membrane (Ahmad *et al.* 2018; Shemi *et al.*, 2021). Also, drought limited the supply of nutrients to plant roots via either mass flow or diffusion (Zeng and Brown, 2000; Marschner and Rengel, 2023). Increasing soil moisture from FC<sub>60</sub> to FC<sub>80</sub>, led to concurrent increases in plant growth and grain yield. Nevertheless, further increases in soil moisture content up to FC<sub>100</sub> decreased root biomass versus FC<sub>80</sub> while augmented shoot and grain yields.

Increasing soil moisture from FC<sub>60</sub> to FC<sub>80</sub> led to concurrent absorption of more nutrients that improved the aboveground plant growth (Saad *et al.*,

2023); nevertheless the grown plants at FC<sub>80</sub> still suffered from drought stress; accordingly, plants stimulated further root growth in search for more water and nutrients (Farooq *et al.*, 2009). When plants receive the adequate soil moisture content (FC<sub>100</sub>), root elongation decreased significantly versus FC<sub>80</sub>; while more metabolites were directed towards shoot growth and grain productivity (Yadav *et al.*, 2021).

Application of biochar at a rate of B<sub>5</sub> probably reduced drought stress; accordingly significant increases occurred in root, shoot and grain dry weights versus B<sub>0</sub>. This additive not only decreased lipid peroxidation within plant tissues (Yildirim *et al.* 2021), but also increased retention of soil moisture and nutrients soluble in it within the top soil (Bassouny and Abbas, 2019; Li and Tan 2021). Additionally, biochar is considered a slow release fertilizer (Elshony *et al.*, 2019). Besides, its application improves considerably soil physical and chemical characteristics (Bassouny and Abbas, 2019). These results indicate the feasibility of biochar for increasing wheat growth and productivity under stress condition and were supported by many

researchers (Hafez *et al.*, 2020; Haider *et al.*, 2020; Zulfiqar *et al.*, 2022). On the other hand, the higher dose of biochar ( $B_{10}$ ) increased shoot growth while decreased grain yield productivity and root growth versus  $B_5$ .

From an economic prospective,  $B_5$  was enough to increase soil productivity under stress conditions. The higher dose of biochar retained more soil moisture for longer time periods and therefore decreased the aeration of the soil rhizosphere needed for health root respiration. This negatively affected shoot growth and grain productivity. Overall, the highest root, shoot and grain weights were recorded for the treatment that received  $B_5+FC_{100}$

#### 4.2. Impacts of deficit irrigations and acidified biochar on available NPK contents in soil

Increasing soil moisture lessened considerably N and K available contents in soil (dilution of salts in soil), while the corresponding effect on available P was not the same. Generally, increasing soil moisture from  $FC_{60}$  to  $FC_{80}$ , raised P-available concentrations in soil, then its concentration decreased with increasing soil moisture from  $FC_{80}$  to  $FC_{100}$ . Probably, two processes affect P availability in soil, i.e. dilution effect which was remarkable from  $FC_{80}$  to  $FC_{100}$ , and, on the other hand, increasing the solubility of several phosphorus compounds which was obviously seen from  $FC_{60}$  to  $FC_{80}$  (Barrow *et al.* 2021, Barrow 2021). Application of biochar at a rate of  $5 \text{ g kg}^{-1}$  lessened considerably N and K available concentrations in soil. This was acceptable because of the concurrent increases that took place in the uptake of these two nutrients by plants at this application level. Further increases in biochar dose, i.e.  $10 \text{ g kg}^{-1}$  re-raised their available levels again in soil, as more N and K salts were set free from the degradation of biochar (Bilias *et al.* 2023, Mohamed *et al.* 2024b), which exceed plant needs. In case of available P, its contents increased significantly with increasing the dose of biochar. Maybe, plants needed and absorbed less P comparable to their needs for both N and K (Saad *et al.* 2023)

#### 4.3. Impacts of deficit irrigations and acidified biochar on the biochemical changes in plants (NPK uptake and concentrations within wheat parts as well as proline content in shoots)

Elevated uptake of NPK was noticed according to the sequence of  $FC_{100} > FC_{80} > FC_{60}$ . In particular, N flows to plant roots via water movement

(mass slow) (McMurtrie and Näsholm 2018) while P and K transfer to roots by diffusion (Lambers 2022, Yadav and Sidhu 2016, Nieves-Cordones *et al.* 2016). At sufficient soil moisture, water increases the solubility of low soluble P and K bearing minerals; thus P and K diffusions to plant roots became faster (Yadav and Sidhu 2016, Suriyagoda *et al.* 2014).

At  $FC_{80}$ , high accumulation of N occurred in shoots and grains versus plants at  $FC_{100}$  as if stressed plants escape drought by earliness and short duration of vegetative growth (Itam *et al.* 2020).

Application of biochar at a rate of  $5 \text{ g kg}^{-1}$  raised significantly NPK uptake by wheat plant; nevertheless, a higher dose of applied biochar led to noteworthy reductions in NPK uptake by wheat plants. The highest concentrations of N were found in roots and shoots of plants that received  $FC_{100}+B_{10}$ . This is not the case in grains because the highest N records were found in plants received  $FC_{80}+B_5$ . The latter treatment ( $FC_{80}+B_5$ ) exhibited also the highest concentrations of P within different wheat parts. It is worth noting that this additive is a source of NPK nutrients for plant growth (Ullah *et al.* 2023, Elshony *et al.* 2019). However, high doses of applied soil nutrients such as N (via increasing biochar dose) increased the utilization of metabolites mainly in increasing the total aboveground biomasses (Meng *et al.* 2013), rather than accelerating the transition reproductive growth or increasing grain productivity (Ghimire *et al.* 2021). Additionally, the high dose of P may precipitate partially with soil micronutrients hence lessen their availability (Farid *et al.* 2023c, Abd El- Aziz *et al.* 2020) and uptake by plants (Farid *et al.* 2023d); nevertheless this assumption was not supported in our results because the highest increases in P content within grains was recorded for  $FC_{100}+B_{10}$

Potassium (K) is an important nutrient to mitigate drought stress (Munsif *et al.* 2022); and its concentrations increased in roots and grains of wheat plants subjected to stress ( $FC_{80}$ ), beside of its impacts on various plant functions (Sardans and Peñuelas 2021). To cope with drought stress, plants ( $B_0+FC_{60}$ ) preserve more potassium in their roots, shoots and consequently grains while continuously consume P and N and retained less concentrations in their tissues.

Proline is an  $\alpha$ -amino acid derivative compound (Dutta *et al.* 2018) which results mostly from the breakdown of proteins under stress conditions (Krasavina *et al.* 2014) to be stored in high concentrations in plant tissue (Krasavina *et al.* 2014). This stress marker is of high solubility and therefore can be used as an osmolyte and also as a ROS scavenger (Krasavina *et al.* 2014). Our results indicate that this stress marker increased considerably under drought stress; and biochar application can further increase this amino acid only in plants subjected to drought. In non-stressed plants, biochar applications did not raise its content within plant tissues, but effectively did in stressed plants (FC<sub>60</sub>), yet in highly stressed ones plant metabolites might be low (Kapoor *et al.* 2020) for biosynthesis of high concentrations of proline. This is because proline functions as the storage sink for cellular carbon and nitrogen (Dutta *et al.* 2018). However, under less severe stress conditions (FC<sub>80</sub>), this marker increased significantly in shoots of plants versus the ones that were not subjected to stress to increase plant tolerance to drought stress conditions.

#### 4.4. Impacts of deficit irrigations and acidified biochar on soil EC and pH

No significant impacts were deduced for the level of soil moisture on soil pH by the end of the growing season. Also, application of acidified biochar was also of no significant impacts in this concern. The alkalinity of biochar could therefore be detectable within the short period after application (Chen *et al.* 2021, Liu and Chen 2022); thereafter this organic product could itself be a buffer for soil pH (Shi *et al.* 2017).

Regarding soil salinity (EC), its values increased significantly with decreasing soil moisture, following the sequence of FC<sub>100</sub>>FC<sub>80</sub>>FC<sub>60</sub>, while remained statistically unchangeable owing to application of biochars. In spite of that the interaction results revealed that EC values significantly decreased in the soil that received 5 g biochar kg<sup>-1</sup> owing to increasing soil moisture.

It is then thought that increasing soil moisture content led to significant reductions in soil EC (dilution of salts), while biochar added more soluble salts to the soil during its degradation (Karimi *et al.* 2020, Khadem *et al.* 2021). Thus, the highest increases in soil EC was found for FC<sub>60</sub>+B<sub>5</sub>. After applying a higher dose of biochar (i.e. 10 g kg<sup>-1</sup>), some of the soluble salts in soil may be retained (Yuan *et al.*

2023), because of increasing the surface negative charge of biochar (Mia *et al.* 2017). Thus, reductions in soil EC were noticeable. This effect was probably comparable to the effect of salt dilution by increasing the irrigation level because non-significant differences were detected in soil EC among FC<sub>100</sub>+B<sub>10</sub>, FC<sub>80</sub>+B<sub>10</sub> and FC<sub>60</sub>+B<sub>10</sub>.

## Conclusions

Application of biochar at a rate of 5 g kg<sup>-1</sup> soil elevated NPK uptake by wheat plants and at the same time recorded the highest increases in grain productivity at FC<sub>80</sub> while decreasing irrigation water inputs by about 20%. This is a good point deems towards rationalizing irrigation water in arid soils. In particular, P inputs played the most significant role in increasing shoot and grain yields under such conditions as well as N. Probably, this nutrient (P) is essential in ATP dependent processes that increase drought resistance in wheat. On the other hand, N and K were almost incorporated in plant coping mechanisms against drought.

## 5. Conflicts of interest

There are no conflicts to declare.

## 6. Formatting of funding sources

List funding sources in a standard way to facilitate compliance to funder's requirements

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