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Effect of biochar on seed germination and growth of *Glycine max* L. under induced stress of drought

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Abstract

As a carbon-rich substance made from organic waste by pyrolysis, biochar has demonstrated encouraging promise as a soil supplement to improve soil fertility, water retention, and plant development. Therefore, this study aimed to examine the effects of biochar application on seed germination, growth parameters, moisture content, and biochemical constituents of *Glycine max* L. (hereafter *G. max L.*) under drought stress. The results show that the drought treatment (T1) had a considerably lower germination percentage. In contrast, treatments (T2 and T3) that applied 10 and 20 tons of biochar per hectare had emergence rates of 56.66% and 50.33%, respectively, without significantly changing the germination percentage. Biochar treatments (T4 and T5) exhibited a considerable improvement in germination percentage under drought stress; T4 exhibited an emergence rate of 53.33%. In T2 (17 ± 1.53), the number of roots were maximum, whereas the longest roots were found in T3 and T4, measuring 6.65 cm and 5.87 cm, respectively. Biochar considerably increased shoot length; T2 reached 17.75 ± 0.01 cm. With the use of biochar, the quantity of leaves increased; T3 had the highest count, at 13.00 ± 0.00. Biochar considerably increased the field capacity and soil moisture content, particularly in T3, where the field capacity was 16.92 ± 0.14%, demonstrating the benefit of better soil water retention. In addition, the use of biochar improved the other biochemical substances in soybeans, such as chlorophyll "a" "b", sugar, protein, and proline contents in treatment T2 and T3, respectively. This study demonstrates that biochar treatments may be used as a viable soil amendment to enhance soybean development, growth, stress mitigation, and biochemical composition, with implications for improved agricultural yield and sustainability. Further investigation is essential to reveal the fundamental mechanisms making these benefits under different environmental conditions and to enhance biochar application procedures.

Keywords: Biochar, Biochemical analysis, Drought, Growth, *Glycine max* L.

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Introduction

G. max L. is an annual legume native to East Asia that grows in temperate and tropical areas with moist seasons. It is cultivated for its nourishing beans, which serve as a vital source of protein and oil worldwide. Mahmud et al. (2020) highlight the ability of *G. max* L., an annual plant belonging to the Fabaceae family, to fix nitrogen in the soil through the formation of symbiotic relationships with bacteria. They can be used for so several diverse industrial applications, comprising animal feed, production of food, and biofuel, whereas soybeans have been grown for thousands of years. Based on their high nutritional content, they have a high value for use (Voisin et al., 2014). In agriculture, *G. max* L. is a staple crop, it supplies and contributes to the world's food and induces economic growth due to its adaptability to climate change and various soil situations (Ndakidemi et al., 2006; Dilawari et al., 2022).

Climate and other stressors (Tabassum et al., 2018; Dilawar et al., 2021; Asad et al., 2022; Shahid et al., 2023) including drought (Ali et al., 2017) highly affect the crops and induce threats to food security and productivity (Abbas et al., 2013; Mehmood et al., 2020). Pakistan is an agricultural country, where the economy mainly depends on crops and food supply (Azam & Shafique, 2017). A prolonged drought condition adversely affects the crops, as a result in reduced productivity of agriculture, damaging livestock, and crops productivity (Daryanto et al., 2015; Leng & Hall, 2019; Shafqat et al., 2019). The problem is impaired by drought because irrigation schemes induced stress on the previously delicate Indus River basin (IRB) and induced rivalry for rare water resources. Scarcity of water can source problems with financing and shortages of food since small-holder farmers, who sort up a large portion of Pakistan's agricultural industry, are particularly sensitive to droughts (Ahmad et al., 2022; Hussain et al., 2022). The different drought-resistance crop varieties, the application of viable water-managing practices, and the support of smart-climate agricultural tools are essential to decline the influence of the absence of humidity on crops (Imran et al., 2018; Dilawar et al., 2021; Abbas et al., 2022), enhance resistance and ensure the most vulnerable populations' access to food.

Biochar, a rich carbon substance produced by the pyrolysis of carbon-based biomass, has a great deal of promise to mitigate the effects of drought on crops (Novotny et al., 2015; Bis et al., 2018). Biochar has the potential to improve soil fertility and structure, induce the water-holding capacity, and induce the availability of nutrients to make plants stronger to abiotic stress (Ali et al., 2017; Asad et al., 2018; Yildirim et al., 2021; Feng et al., 2022; Asad et al., 2024). According to Ahluwalia et al. (2021); Chen et al. (2021), the porosity of soil and the retaining capacity of water by biochar helps plant roots absorb more moisture and mitigate drought. Similarly, it also induces microbial activity in the soil which stimulates root formation, nutrient cycling (Prendergast-Miller et al., 2014; Ibrahim et al., 2021), and tolerance of plants to water scarcity. Likewise, biochar influences its full absorbing capacity of carbon and advantages crops to regulate climate variability, particularly drought (Nyambo et al., 2020). Therefore, the objective of the current study is to assess how biochar influences various growth parameters and the biochemical contents of *G. max* L. under the induced stress of drought.

Materials and Methods

Climate of the study area, seeds collection, and biochar preparation

The current study was conducted from 2018 to 20 at the lab of Botany, Bacha Khan University Charsadda. *G. max* L. (variety NARC I) seeds were purchased from the Agriculture Research Centre in Peshawar, and they were sterilized by first being washed for three minutes in a 0.2% mercury chloride ($HgCl_2$) solution, then being washed again with distilled water. Slow pyrolysis of maize cobs produced biochar for three hours. Biochar was produced using a temperature-controlled batch pyrolysis device at 450 °C. Ten seedlings were planted in each pot for the randomized complete block design (RCBD) experiment, which included six treatments and three duplicates. All the treatments, however, were administered as follows: T0 was the control; T1 was the drought; T_2 - biochar 10 tons per hector; T_3 - biochar 20 tons per hector; T_4 – drought + biochar 10 tons per hector and T^5_drought + biochar 20 tons per hector.

Determination of soil-moisture

The samples of soil were weighed and baked at 105 °C for 24 hours to dry them out. After that, it was cooled and weighed at room temperature. The amount of moisture in the soil has caused the weight difference:

$$\text{Percentage (\%)moisture} = \frac{\text{Fresh weight of soil} - \text{Dry weight of soil} \times 100}{\text{Fresh weight of soil}}$$

Percent field-capacity

Using the following technique, the % rhizospheric soil-field capacity was calculated:

$$\text{Soil percent field capacity} = \frac{\text{Wet weight of Soil (g)} - \text{Dry weight of soil (g)} \times 100}{\text{Soil dry weight (g)}}$$

Morphological parameters of *G. max* L.

Once the seeds had germinated, the following morphological and agronomic data were recorded in the field diary: the number and length of roots; the fresh and dry biomass of the roots; the length of the shoots; the number and area of leaves; the fresh and dry biomass of the leaves; and the germination of the seeds:

The germination of seeds (%) was intended utilizing Close & Wilson formula (2002):

$$\text{Germination (\%)} = \frac{\text{No seeds that sprouted during the period} \times 100}{Total\ number\ of\ sown\ seeds}$$

The rate of germination was observed utilizing the formula of Khan and Ungar (1984):

$$\text{Rate of sprouting} = \frac{\text{Seeds germinated} \times 100}{Total\ number\ of\ seeds\ sown}$$

Determination of chlorophyll

The absorbance of the photosynthetic tissue of the soybean plant was measured using a bio-lambda at wavelengths of 663 and 645 nm. For each solution, the absorbance was observed, and the contents of chlorophyll "a" and "b" were taken into consideration. The photosynthetic tissue of the soybean plant was pulverized and stored in a centrifuge with a solution of 1 part standardized Ammonium hydroxide solution in nine percent acetone (v/v). After the supernatant was extracted from the mixture, 10 milliliters of 80% acetone solution were added to it. After that, it was put into a test tube and then kept at 4 °C for a whole day. The following formula was used to determine the total chlorophyll value for the chlorophyll contents "a" and "b."

$$\text{Chlor. "a" (mg/ml)} = 12.80\ \text{A663} - 02.68\ \text{A645}$$

$$\text{Chlor. "b" (mg/ml)} = 22.70\ \text{A645} - 04.69\ \text{A663}$$

Determination of protein contents

Total soluble proteins (TSP) were determined by centrifuging a leaf (1 g) at 10,000 g for 25 minutes after it had been crushed in 4 mL of Na_3PO_4 buffer (50.0 mM, pH-7.8) to extract TSP. After mixing 2.5 mL of Bradford's reagent with the supernatant sample (20 µL). For fifteen minutes, the mixture was incubated. Using a Double Beam UV-visible spectrophotometer, the absorbance was dignified at 595 nm.

Proline and sugar determination

The quantity of proline was strongminded using the previously noticeable methodology by Yousaf et al. (2021), while the sugar level was calculated by following the strategy of Du et al. (2020).

Statistical analysis

With two parameters (treatment and shelf period in days), the experimental design was set up using a fully randomized design (CRD), whereas the mean was compared for significance by ANOVA and using SPSS for window software.

Results and Discussion

Effect of biochar on seeds germination (%)

Results on the proportion of soybeans that emerged showed that treatment T1 considerably reduced emergence, while treatments with 10 and 20 tons of biochar per hectare had no discernible influence on emergence (Table 1). Treatments T4 and T5 significantly outperformed situations with water stress in terms of germination percentage. However, in the absence of water shortage, no seed failures were noted, which would have reduced the germination rates. The study found that biochar did not substantially change the parameters of germination of seeds rate, membrane integrity index, and the proportion of water in the leaves of soybeans under water stress (T1). In comparison, the outcomes of Akhtar et al. (2014) show that the addition of biochar to the soil improves the membrane stability index, leaf relative water content, and water usage efficiency. Based on the data analysis, it appears that there is no substantial impact on emergence when applying more biochar during a drought. Compared to biochar-treated plots, control plots showed 50% less blooming and a delayed flowering period, which is in line with other studies showing more flowers and fruits on biochar-treated plants (Rogovska et al., 2014).

Table 1 Effects of biochar and drought on *G. max* L. blooming emergence percent (%)

Treatment	Number of seeds emerged (out of 10 seeds)			Average (%)
To	5.00	6.00	5.00	53.33
T1	3.00	4.00	4.00	36.66
T2	5.00	6.00	6.00	56.66*
T3	6.00	4.00	5.00	50.33*
T4	4.00	5.00	4.00	53.33*
T5	5.00	4.00	5.00	50.00*

To illustrate the control; T1 = drought; T2 = biochar at 10 tons per hectare; T3 = biochar at 20 tons per hectare; T4 = biochar plus drought at 10 tons per hectare; and T5 = biochar + drought at 20 tons per hectare. * indicates a significant threshold at $p < 0.05$.

Effects of biochar growth parameters of *G. max* L.

Root length and number of roots

Table 2 demonstrates the influence of biochar addition and drought stress on the average root length (cm) of each plant. The *G. max* L. plants with the shortest roots were treatment T5 (20 tons' ha-1 of biochar with drought), which was subjected to drought stress and had a notable reduction in root length. For treatments T3 (20 tons of biochar per hectare) and T4 (10 tons of biochar per hectare with drought), the longest root length was measured. The results indicate that the treatment T2 (biochar at 10 tons per hectare) had the highest root number of *G. max* L., with a mean of 18 ± 1.53; the control treatment T0 had the lowest root number (15 ± 1.00). Although there were no statistically significant changes seen across the treatments, the root number of *G. max* L was more significantly impacted by biochar. Root numbers under submerged pressure circumstances grew considerably. In addition to the number of roots, water-stressed plants in treatment T1 showed longer roots when compared to the control treatment T0; however, in both biochar concentrations, treatments T4 and T5 showed no appreciable variations in root lengths when compared to the control. Plants that are experiencing a drought must draw water up from deeper soil layers, which causes their roots to grow longer (Golzardi et al., 2012). However because biochar increases the soil's ability to retain water, plants don't essential to develop extended roots because water is more effortlessly obtainable to them (Agbna et al., 2017).

Shoot length (cm)

In contrast to the control treatment (T0), the results showed that soybean shoot length decreased under drought stress, while increased shoot length was seen in plants that received regular irrigation. The optimal species of *G. max* L. was measured for shoot length after crop development. Table 2 shows that, in contrast to the control action, the biochar treatment considerably improved the height of *G. max* L.

shoots. T1 (drought treatment) had the shortest shoot length, measuring 14.46±0.00 cm, whereas T2 (biochar at 10 tons per hectare) had the tallest shoot height, measuring 17.75±0.01 cm. These findings demonstrate a strong effect of biochar on *G. max* L. shoot length (cm), which may be explained by a rise in intrinsic biochar content leading to a reduction in shoot length under stress. Taghavimehr (2012) pointed out that biochar can be utilized to promote soil microorganisms and increase soil water-holding capacity, which in turn increases the rate of photosynthesis and encourages plant shoot height. Various concentrations of the soil biochar amendments have varied potentials to promote the height of the plant, mitigating physiochemical. The impressive usage of plants as medicines against illnesses is what holds the ancient relationship between humans and plants together (Ahmad et al. 2024; Asad et al., 2018; Begum et al., 2018; Begum et al., 2021; Bibi et al., 2023), which I induced the growth of the medicinal plant by various stress utilizing biochar.

Number of leaves

Table 2 displays the impact of biochar on *G. max* L. leaf counts. The treatment T3 (biochar @ 20 tons/hectare) had the highest leaf numbers, measuring 13.00±0.00, whereas T1 (dry period) had the lowest base number of leaves of the selected species, measuring 9.33±0.55. This indicates that the application of biochar as a whole sparked the growth of leaves, despite the adverse effects of a dry period on *G. max* L. Similar results were reported by Zwart and Kim (2012), who stated that biochar increased the plant height, leaf quantity, weight, dried plant mass, dry root mass, and volume. The findings showed that the expansion of biochar increased the amounts of *G. max* L. leaves, but no such increase was observed in the dirt containing no such blend.

Table 2 Effects of biochar on the fresh weight, dry mass, length, and total number of roots of *G. max* L. under drought stress

	Glycine max L.			
Treatments	Root length (cm)	Root number	Stem length (cm)	Number of leaves
T0	3.99 ± 00.67	15.00 ± 01.00	16.69 ± 00.04	10.66 ± 00.34
T1	5.33 ± 00.58*	16.00 ± 01.53*	14.46 ± 00.01	09.45 ± 00.55
T2	4.67 ± 00.62	17.00 ± 01.53*	16.75 ± 00.03*	12.17 ± 00.51*
T3	6.65 ± 00.65*	16.00 ± 00.58*	17.48 ± 00.24*	13.00 ± 00.00*
T4	5.87 ± 00.13*	16.00 ± 00.58*	16.08 ± 00.07	12.66 ± 00.05*
T5	4.00 ± 01.00*	16.00 ± 01.53*	17.06 ± 00.13*	11.66 ± 00.00*

Impact of biochar on moisture content and field capacity (%)

The findings show that under drought stress, the moisture content of the soil of soybeans in the T1 treatment was lower than in the treatment. Table 3 illustrates the strong impact of biochar addition on soil moisture content, especially in treatments T2 and T3. Additionally, the soil moisture content of the biochar-treated stressed treatment T4 and T5 showed improvements. A simple procedure involved weighing a known-weight sample of soil to determine the initial moisture content of the soil. After that, the dirt was dried for around 24 hours at 105 °C in an oven. Upon reaching room temperature, the soil samples were subjected to another weight measurement; the disparity in weight indicated the soil's moisture content. The corresponding field capacity levels for the *G. max* L. species are shown in Table 3. It was shown that both the drought conditions and the biochar treatments had a significant effect when comparing the chosen species of *G. max* L. to the treatment T0, which did not receive either biochar treatment or drought treatment. Treatment T3 (biochar at 10 tons/hectare) had the greatest field capacity level, measuring 16.92 ± 0.14, whereas treatment T1 (drought) had the lowest field capacity level, measuring 10.070.01. According to these findings, biochar significantly affected the field capacity level and lessened the negative impacts of the drought on field capacity. The findings show that both biochar application and drought stress had an impact on soybean field capacity. Furthermore, biochar treatments have a main influence on soil.

Table 3 Impact of biochar on the moisture and field capacity of soil under induced drought stress

Treatments	Percent moisture content	Percent field capacity
T0	12.21 ± 00.09	10.07 ± 00.01
T1	11.98 ± 00.84	13.59 ± 00.09*
T2	14.33 ± 01.12*	15.82 ± 00.03*
T3	11.88 ± 00.76*	16.92 ± 00.14*
T4	13.66 ± 00.61*	13.39 ± 00.02*
T5	13.06 ± 00.41*	11.09 ± 00.42

Effect of biochar on biochemical constituents of *G. max* L.

Impact on photosynthetic pigments of *G. max* L.

G. max L.'s chlorophyll "a" concentration (mg/g) was determined before blooming, and plant samples were taken before scheduled examination under drought stress. For *G. max* L, treatment T_3 (biochar at 20 tons/hectare) had the highest reported chlorophyll "a" concentration (Fig. 1a). On the other hand, it was discovered that there was very little chlorophyll "a" in treatments T0 (control) and T_4 – drought + biochar @ 10.00 tons per hectare, T_5 -drought +biochar @ 20.00 tons per hectare. This indicates that while combination treatments performed worse in terms of developing chlorophyll "a" individual application of biochar appeared to have a comparatively greater favorable impact on the concentration of chlorophyll "a". Additionally, compared to Treatment T0, the chlorophyll "b" content (mg/g) of the selected series of *G. max* L. exhibited the highest values in Treatment T3 (Fig. 1b). Chlorophyll "b" contents, on the other hand, differed between treatments; T_0 (control), T_1 (drought), and T4 (drought plus biochar @ 10 tons/hectare) all had lower levels. T3's lower level of chlorophyll "b" suggests that different biochar treatments have different effects on promoting the formation of chlorophyll "b." These results are consistent with earlier research that showed a drop in chlorophyll content during drought stress, which was linked to oxidative stress and chlorophyll deterioration. On the other hand, adding biochar to the soil raised the amount of chlorophyll in both well-watered and stressed situations, indicating that it may be able to lessen the adverse influences of drought stress on soybeans.

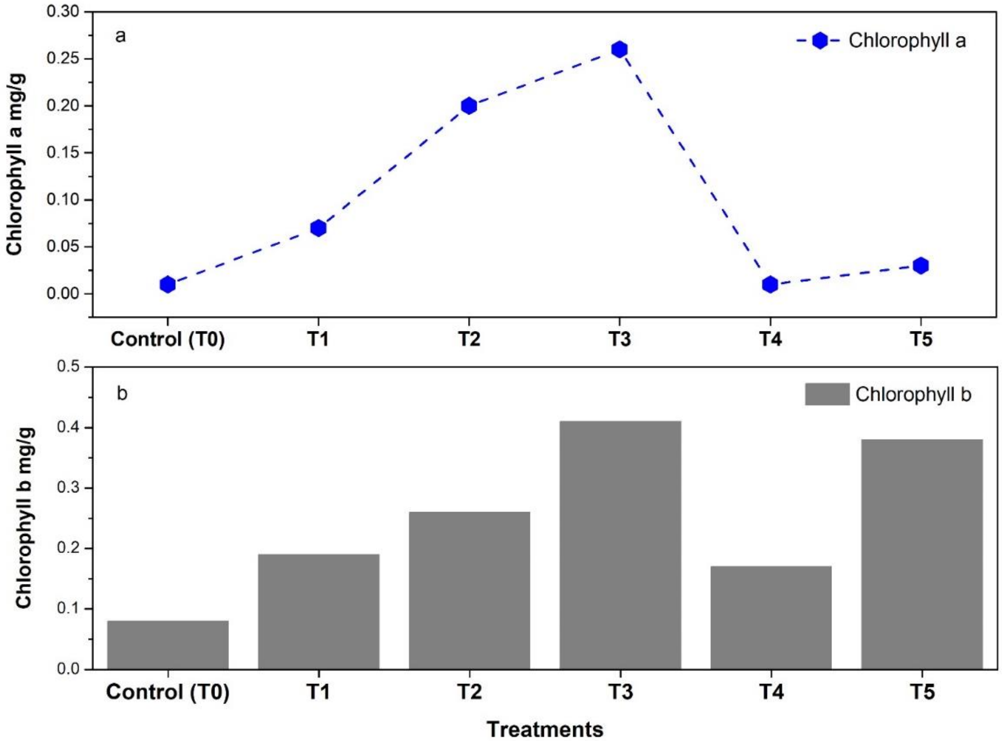

Fig. 1 Impact of biochar on G. max L.'s chlorophyll a and b content during drought-induced stress

Impact of biochar on proline contents (µg/g) of *G. max* L. under drought stress

Fig. 2 shows that in the *G. max* L. species, treatment T3 (biochar at 20 tons/hectare) had the highest proline content, whereas T1 (drought) had the lowest protein content. This implies that, in comparison to the control, a larger quantity of biochar contributed positively to an increase in proline content. In keeping with studies by Abass and Mohamed (2011), who observed a considerable rise in proline and soluble sugar content in typical bean plant stems in drought stress, the quantity of proline in soybean plant leaves increased dramatically under drought circumstances. Furthermore, when various biochar doses were feasible for the soil, the proline concentration in the leaves of soybean plants rose dramatically in comparison to the plants that were not affected by dryness and the control group. There is a correlation between the rise in free proline levels and the reduction in plant water uptake. Proline stabilizes genetic material in plants under stress, contributing to lower reactive oxygen species and maintaining the internal structure of cell constituents. Plants under stress will always have access to water, nutrients, and the ideal level of porosity if biochar is added to the soil. As a result, soil treated with biochar has assured water availability and may not experience plant stress, which would otherwise result in a significant increase in proline content.

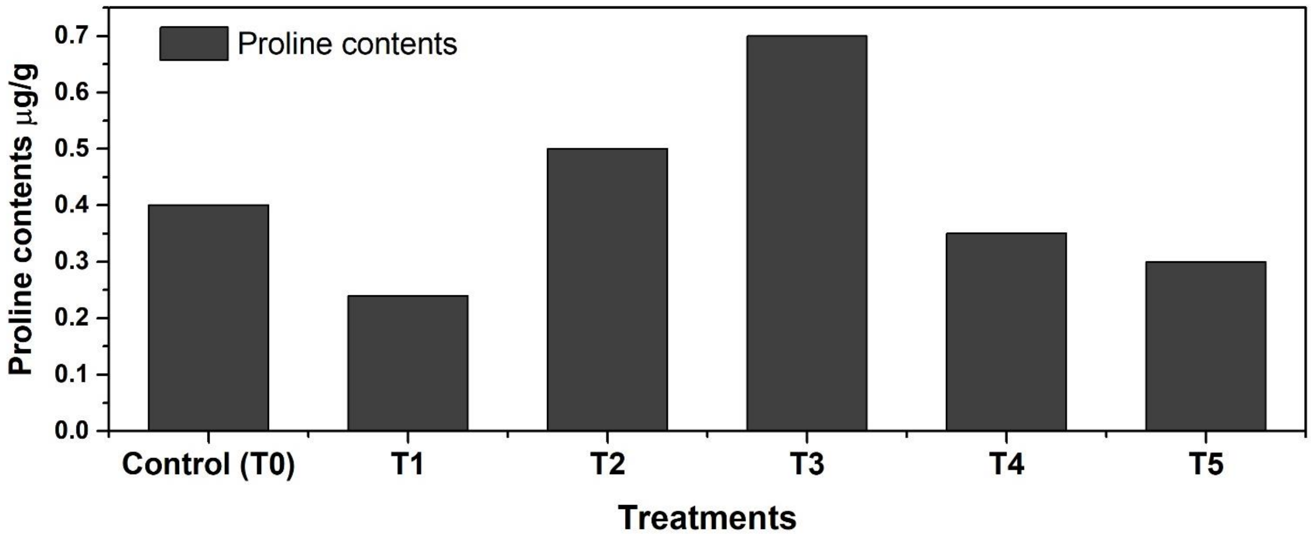

Fig. 2 Impact of biochar on *G. max* L. proline content (µg/g) during drought stress

Impact of drought and biochar on *G. max* L. protein contents

Biochar considerably increased the protein content of soybean leaves, however the drought treatment and the biochar + drought treatment had a negative impact (Fig. 3). For the *G. max* L. species, Treatment T2 (biochar at 10 tons/hectare) had the maximum protein content, whereas Treatment T1 (drought) had the lowest protein content. This implies that, in comparison to both larger amounts of biochar and dry circumstances, the right quantity of biochar contributes positively to boosting protein content. The outcomes of Batool et al. (2015), which presented that heavy metal exposure had a deleterious effect on seed germination, protein content, sugar levels, and hydrolytic enzyme activity, are consistent with the present investigation.

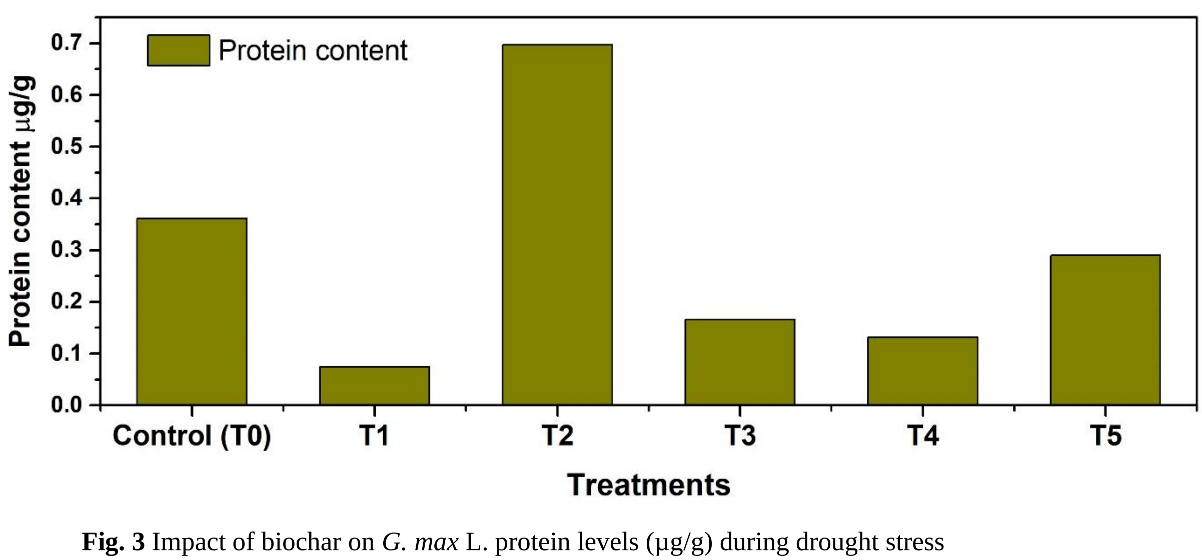

Fig. 3 Impact of biochar on *G. max* L. protein levels (µg/g) during drought stress

Impact of biochar and drought on sugar contents of *G. max* L.

Fig. 4 shows the assessment of sugar content in the selected variety of *G. max* L. under the combined effects of biochar and drought. The findings show that Treatment T2 (biochar at 10 tons/hectare) had the maximum sugar concentration, whereas Treatment T1 (drought) had the lowest sugar content. These results corroborate those of Schmidt and Noack (2000), who found that when biochar was added, plant height, biomass, chlorophyll content, relative water content, photosynthetic content, protein content, and starch increased considerably in comparison to the treatment of control.

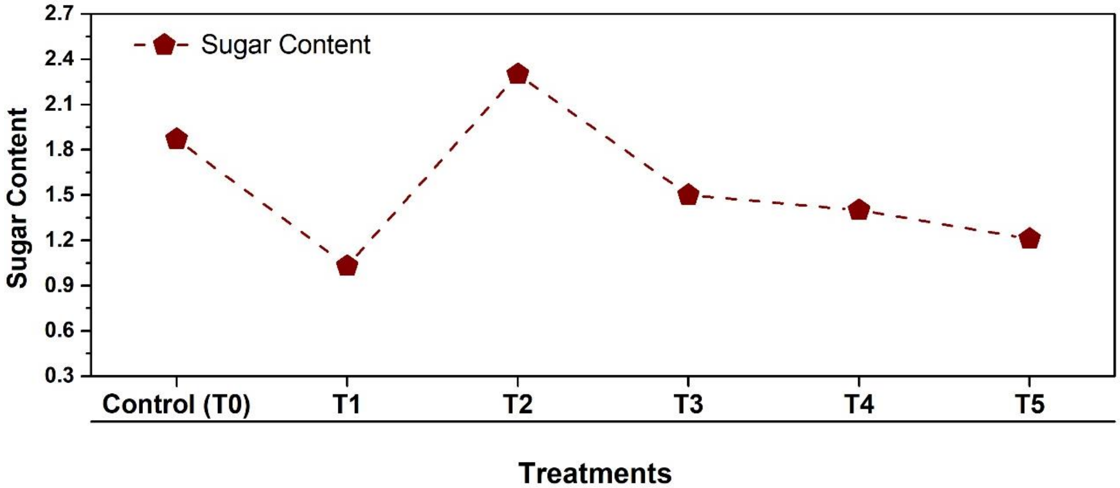

Fig. 4 Influence of biochar on sugar concentration of *G. max* L. under drought stress

Conclusion

This study explored how the use of biochar wedged some aspects of *G. max* L. growth and biochemical contents, particularly in the application of drought stress. The results reveal that though biochar had no significant effect on seed sprouting, it significantly enhanced the growth rates under the induced stress of drought. The utilization of biochar improved the root length, shoot length, number of leaves, and biochemical contents of *G. max* L., and can retain soil moisture. These findings reveal the biochar potential as a defensible soil supplement to improve agricultural crop growth and production and induce the mitigation of climate stress such as drought. However, additional research is needed to increase the techniques of biochar application and reveal the basic mechanisms liable for these experiential benefits in a variety of environmental circumstances.

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