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EFFECT OF BIOCHAR APPLICATION ON SEED GERMINATION AND SEEDLING GROWTH OF *GLYCINE MAX* (L.) MERR. UNDER DROUGHT STRESS

YOUSARA HAFEEZ[1], SUMERA IQBAL[1*], KHAJISTA JABEEN[1], SOBIA SHAHZAD[2], SUMMERA JAHAN[1] AND FAHD RASUL[3]

[1]*Department of Botany, Lahore College for Women University, Lahore, Pakistan*
[2]*Department of Botany, University of Agriculture Faisalabad, Pakistan*
[3]*Department of Agronomy, University of Agriculture Faisalabad, Pakistan*
[*]*Corresponding author's email: sumeraiqbal2@yahoo.com*

Abstract

Drought is one of the most detrimental stresses which limit seed germination, plant growth, development and crop yield. The influence of corn cobbiochar producedat 450°C by slow pyrolysis technique was investigated on seed germination and seedling growth of Soybean (*Glycine max* (L.) Merr. under drought condition. The experiment was sequenced according to Randomized Complete Block Design in three replicates. There were six treatments viz., Control (0 t ha^{-1}biochar and no drought), drought, 10 t ha^{-1}biochar, 20 t ha^{-1}biochar, drought + 10 t ha^{-1}biochar, drought + 20 t ha^{-1}biochar. The results exhibited that under water stress seed vigor, germination percentage, shoot length, membrane stability index, chlorophyll and carotenoid contents of soybean seedlings decreased significantly compared to control. However biochar applied @ 20 t ha^{-1}proved to be more effective in mitigating the drought stress impacts in all these parameters. A significant increase ($p>0.05$) in sugar and proline content was observed while protein content and rate of seed germination were not affected significantly ($p<0.05$) in drought compared to control. Biochar applied @ 20 t ha^{-1}mainly helped to decrease sugar and proline contents but other parameters remained unaffected. So, biochar can be appraised as effective tool to mitigate the impact of water stress on soybean seedlings. Long term effects of biochar on soybean production and soil properties particularly in drought prone areas could be suggested as future thrust.

Key words: Drought, Biochar, Mitigation, Soybean, Seed germination.

Introduction

One of the key challenges of the modern era is to cope with water scarce conditions. Water scarcity had been termed as the most critical disaster among all catastrophes seriously affecting human life (Hewitt, 1997; Wilhite, 2000; Miyan, 2015). During recent times, drought epochs prevail in all parts of Pakistan including major parts of Sindh and Baluchistan (Chandio, 2012). Owed to massive drought episodes there was a massive set back in agricultural production (Singh *et al*., 2000). Soybean has been found sensitive towards water deficit at all the growth stages (Casagrande *et al.,* 2001; Hamayun *et al*., 2010). Water deficiency during vegetative development of soybean plants largely caused its yield reduction (Brevedan & Egli, 2003). As soybean is an economically important oil seed and energy cum pulse crop so some advanced solutions should be developed to get better yield even under water scarce conditions. To deal with water scarce conditions small scale solutions would be more acquiescent and can be easily implemented with high probability of large scale changes (Munang & Nkem, 2011). Biochar could be one of the solutions as it improves soil physical properties and aids in improving soil hydrology. Biochar is a finely divided pyrolysed material prepared for soil improvement (Ernsting, 2011). Due to negatively charged surfaces and high surface area, biochar soil amendments improved water holding capacity of soil and thus protected the crops against drought, while minimized the soil hardening and hence reduced soil bulk density. Application of biochar have some supplementary benefits as it moderated fertilizer requirement through absorbed nutrients and gradually released to the plants, and hence favored the reclamation of degraded soils (Cushion *et al*., 2010). It also aided to control global warming owing to its potential for reducing the emission of greenhouse gases in contrast to open air burning and decay of organic matter. In addition, biochar helped the sustainable management of massive agricultural and industrial waste (Woolf *et al.,* 2010). Inference from these various studies led to target work on analyzing the effect of biochar on germination and early vegetative growth of *Glycine max*. L. Merr (variety NARC II) under drought stress.

Materials and Methods

Plant material and growing conditions: The experiment was conducted in Lahore College for Women University, (31.5450° N, 74.3272° E) Lahore Pakistan. Seeds of Soybean (*Glycine max* L. Merr.) variety NARC II were collected from National Agriculture Research Centre (NARC), Islamabad. Surface sterilization of seeds was done by washing seeds with 0.2% Mercuric chloride ($HgCl_2$) solution for 3 min followed by rinsing with distilled water thrice. For water level treatments pots were watered after two days and pots with non stress conditions were watered daily. Biochar was produced by slow pyrolysis of corn cobs over three hour duration. Batch pyrolysis temperature controlled unit was used to produce biochar at 450°C. Sandy loam soil (pH 7.1 and Ec 2.13ds/m) was used which was mixed with biochar having pH about 8.6.

To check the effects of biochar on seed germination and seedling growth of soybean, biochar was incubated in the soil 10 days prior to sowing. Experiment was carried out in randomized complete block design (RCBD) with six treatments and three replicates, in each treatment18 pots were taken and 10 seeds were sown in each pot and after germination of seeds, thinning was also done to keep 3 plants per pot after germination. Following treatments were applied.

Table 1. Treatments applied.

Sr. No.	Treatments
1.	Control (T_0)
2.	Drought (T_1)
3.	Biochar @ 10 tons/ hectare (T_2)
4.	Biochar @ 20 tons /hectare (T_3)
5.	Drought + Biochar @ 10 tons/hectare (T_4)
6.	Drought + Biochar @20 tons/hectare (T_5)

The parameters studied are as follows:

Germination percentage was determined by formula Close & Wilson (2002):

$$\text{Germination percentage} = \frac{\text{No of seeds germinated during the time interval}}{\text{Total number of seeds sown}} \times 100$$

Rate of germination was determined by (Khan & Ungar, 1984) formula as below:

$$\text{Rate of germination} = \frac{\text{First day germinated seeds}}{\text{Total number of seeds sown}} \times 100$$

Tolerance index was calculated by using following formula (Iqbal & Rahmati, 1992).

$$\text{Tolerance index} = \frac{\text{Mean root length in treatment}}{\text{Mean root length in control}} \times 100$$

Seed vigor of the seedling was estimated by following (Abdul Baki & Anderson, 1973) formula:

Seed vigor = germination percentage × seedling length

RWC was calculated by using following formula (Weatherley, 1950).

$$\text{RWC \%} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Calculated the MSI by method of Premchandra *et al.* (1990) which was modified by Sairam (1994) using following formula:

$$\text{MSI} = [1 - C_1/C_2] \times 100$$

The value of chlorophyll 'a' and'b', total chlorophyll and carotenoid content was calculated by following formulae (Lichtenthaler & Welburn, 1983).

Chlorophyll a = $12.21(A_{663}) - 2.81(A_{645})$
Chlorophyll b = $20.13(A_{645}) - 5.03\ (A_{663})$
Total chlorophyll = $20.4(A_{645}) + 8.02\ (A_{663})$

$$\text{Carotenoid} = \frac{1000(A_{470}) - 3.27\ (\text{chl a}) - 104(\text{chl b})}{227}$$

Protein (Lowry *et al.*, 1951), proline (Bates *et al.,* 1973) and sugar (Dubois *et al.*, 1956) contents of leaves were also calculated.

Statistical analysis: All the data were analyzed by ANOVA (analysis of variance) followed by Duncan's Multiple Range Test using statistical software COSTAT.

Results and Discussion

Water stress caused serious consequences to the seedlings development and the various treatments had shown variable results in response to water availability, the drought treatment (T_1) negatively influenced seed vigor (Fig. 1) of soybean while biochar application (20 tons per hectare) in T_5significantly ($p<0.05$) increased the seed vigor under water deficit conditions in contrast to treatment receiving water stress without biochar. Severe effects of water stress are usually recorded in terms of inhibition in cell enlargement and cell division. This could be due to the disruption of various metabolic and physiological processes in the cell such as disruption in ion uptake, photosynthesis, growth promoters, nutrient metabolism and respiration which consequently interfere with all the stages of plant growth especially seed vigor (Farooq *et al*., 2008; Jaleel *et al*., 2007 & 2008; Kumar *et al.*, 2011). Biochar application is known to enhance the water holding capacity of the soil due to its high surface area (Laird *et al*., 2010) porosity and carbon sequestration (Lehmann, 2007) that may help the seed to attain more vigor. Similarly biochar have positive effects on the most important physiological processes of plants such as increase in the rate and capacity of photosynthesis (Xu *et al*., 2015), it retained and improved nutrient uptake by the plants such as N, K, P, Ca and Mg in the plants which were primarily associated with growth responses of plants especially K is involved in regulating osmotic potential inside the plants (Walter & Rao, 2015; Uchida, 2000).

Results regarding germination percentage of soybean (Fig. 2) showed that it is inhibited significantly ($p<0.05$) under water stress in treatment T_1 than control T_0 and biochar treatments under both rates (10 & 20 tons per hectare) had no significant effects on the germination percentage in treatments T_4 and T_5as compared to water stress. In water deficit seed imbibition does not occur and germination percentage is adversely affected (Golzardi *et al*., 2012).

The rate of seed germination (Fig. 3), membrane stability index (Fig. 6) and relative water content (Fig. 7)were not significantly affected in soybean leaves due to water stress (T_1) and none of the biochar treatment (T_2, T_3, T_4 and T_5) had shown significantly different effects. Whereas in contrast to our results Akhtar *et al.* (2014) reported that addition of biochar in soil increases water use efficiency, leaf relative water content, membrane stability index. Infect, biochar is an organic amendment and it takes long time to completely interact with the soil structure and brings more constructive changes in the soil physical properties in the long run only after its weathering (Sohi *et al*., 2009).

Fig. 4 depicts an increase in root length in water stressed plants in treatment T_1 than control treatment T_0 and there were no significant differences in root lengths in treatment T_4 and T_5 under both biochar concentrations in comparison to control treatment T_0.Since under drought condition plant has to absorb water from deep soil so, it develops long roots (Nahar & Gretzmacher, 2011). However biochar improves the water holding capacity of soil so, roots do not need to grow longer, as water is easily available to the plant (Yu *et al*., 2013).

A significant ($p<0.05$) reduction in shoot length (Fig. 5) of soybean had been observed under drought stress in T_1 in contrast to control (T_0) treatment while increase in shoot length was observed for the seedling growing in the biochar treated soil under stress in treatments T_4 and T_5. The reason might be increase in endogenous ABA causes reduction in shoot length during stress conditions (Creelman *et al.,* 1990). Different rates of biochar amendments in soil had shown the different potential to increase plant height alleviating the physiochemical stresses particularly water and nutrient scarcity (Mustafa *et al.,* 2010; Batool *et al.,* 2015). These findings are in agreement with the findings of Ellen *et al.* (2010) who reported that tomato plant show significant increase in height when grown in soil amended with biochar in stress conditions.

Fig. 8 showed that in $T_5$20 tons per hectare biochar application seedling had highest value of tolerance index and lowest value was found for water stressed plants in T_1. Biochar immediately after its amendment enhances soil nutrient status and biochar elements composition usually includes major growth promoting nutrients and they might help the plants to cope with water stress (Zhang *et al*., 2015).

Chlorophyll a, b (Figs. 9, 10) and total chlorophyll content of soybean under water stress in T_1 had been declined to a significant level ($p>0.05$) as compare to control treatment T_0. The soil treated with biochar had shown significant impact to mitigate the consequence of water scarcity. The decrease in chlorophyll under drought stress is mainly the result of damage to chloroplasts caused by active oxygen species (Smirnoff, 1995). Biochar positively affects chlorophyll content and related parameters such as increased activity of PS II and facilitates electron transport, which boost the total Photosynthetic performance index (Lyu *et al*., 2016).

Under water stress in T_1, there was found a significant decline in carotenoid content (Fig. 12) and increased concentration of carotenoid content was traced with 20 tons per hectare biochar under drought condition in T_5. This is in agreement with Younis *et al.* (2015) who reported that the increasing percentages of cotton straw biochar from 3% to 5% under water stress caused a higher production of chlorophyll, carotenoid, amino acids and protein in plants because when water is available in the soil, it is provided to the leaves through xylem, only then metabolic activities are properly regulated by plant leading to consequent increase in these essential molecules.

A significant ($p<0.05$) increase in sugar content in soybean under water stress (T_1) was observed whereas low sugar content was found under biochar application in treatments T_0, T_2, T_4 and T_5than alone drought stress in T_1(Fig. 13). Under drought stress, degradation of starch results in consequent increase in soluble sugar content in the cells (Fisher & Holl, 1991).

Protein content of soybean leaves (Fig. 14) was not significantly ($p<0.05$) effected under drought T_1 or biochar application T_4 and T_5, But slight increase in protein content had been observed with the application of biochar in the soil. Since the presence of organic amendments (like biochar) in the soil help the soil to retain more nutrients and water (due to negatively charged surface area and small particle size) increasing its fertility status. And these nutrients and water is ultimately extracted easily by plant roots and are used to regulate its metabolism (Karhu *et al*., 2011and Scott *et al*., 2014).

Data regarding proline content (Fig. 15) showed that proline content increased in water stressed plants than control. However biochar application in T_5 (20 tons per hectare) application had significantly reduced proline content. Proline accumulation was higher in drought stress condition in T_1. The increase in free proline occurs with decrease in water supply to plants (Zhang *et al.,* 2006; Bano & Yasmeen, 2010; Mafakheri *et al.,* 2010). Proline reduces the reactive oxygen species, helping stabilize the DNA in plants under stress conditions thus maintain a stable internal composition of cell constituents (Szabados & Savoure, 2010). When biochar is applied to the soil it ensures the availability of water, proper porosity and nutrients to the plant under water stress conditions (Shao *et al.,* 2005). Thus in biochar amended soil water is available and stress might not be developed for the plant thus there is no increase in the proline content.

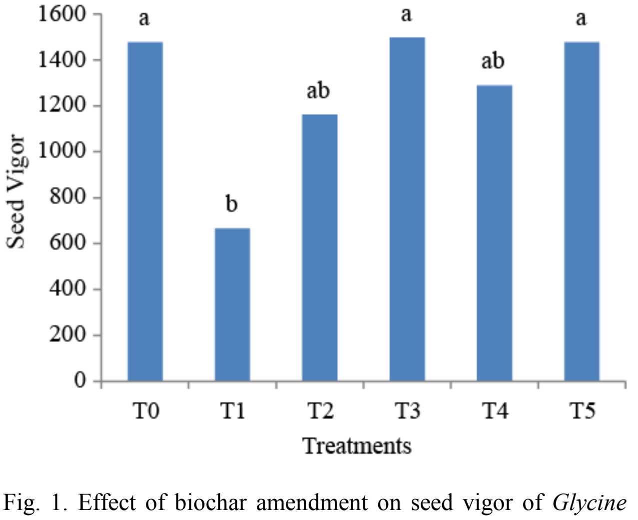

Fig. 1. Effect of biochar amendment on seed vigor of *Glycine max.*(L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought +10 Tons/hectare biochar, T5 = 20 Tons/hectare Biocha

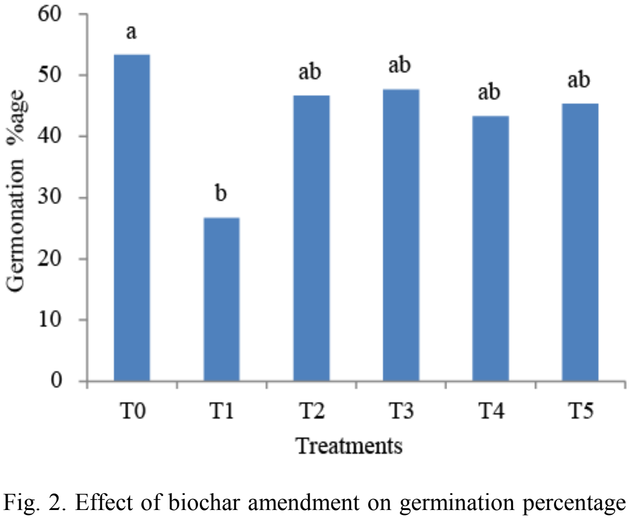

Fig. 2. Effect of biochar amendment on germination percentage of *Glycine max* (L.) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

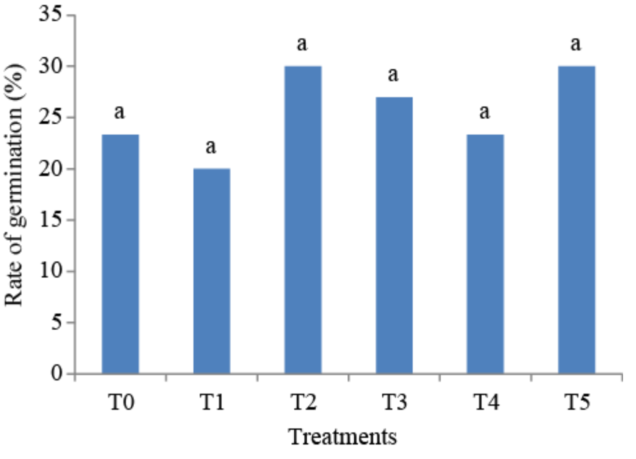

Fig. 3. Effect of biochar amendment on rate of seed germination of *Glycine max.* (L.) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biocha

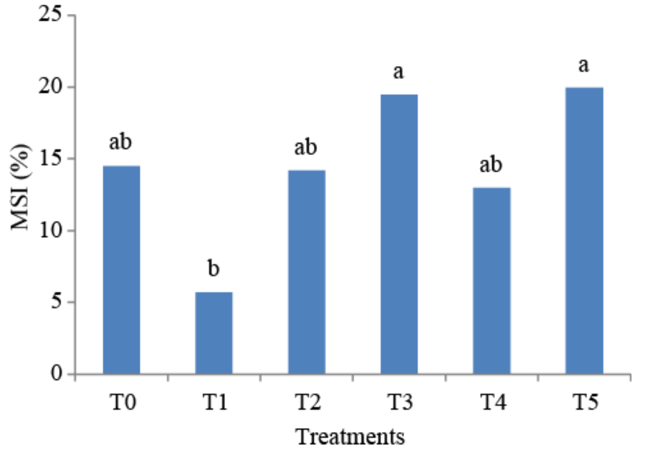

Fig. 6. Effect of biochar amendment on membrane stability index of leaf of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biocha

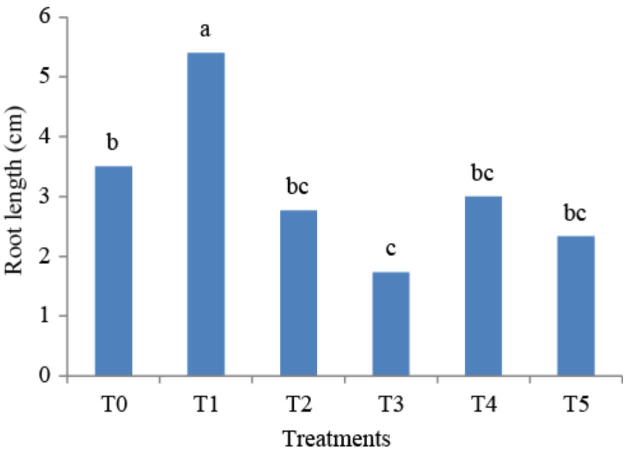

Fig. 4. Effect of biochar amendment on root length of *Glycine max.* (L.) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

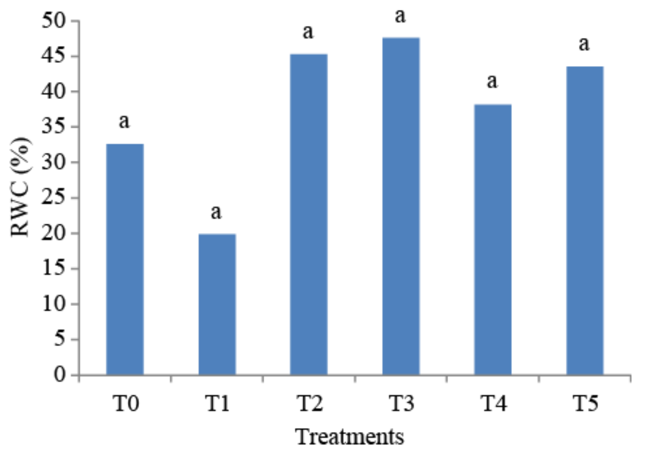

Fig. 7. Effect of biochar amendment on relative water content of leaves of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

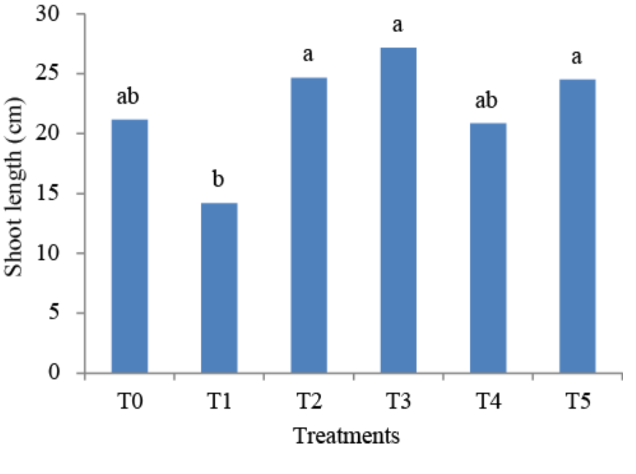

Fig. 5. Effect of biochar amendment on shoot length of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biocha

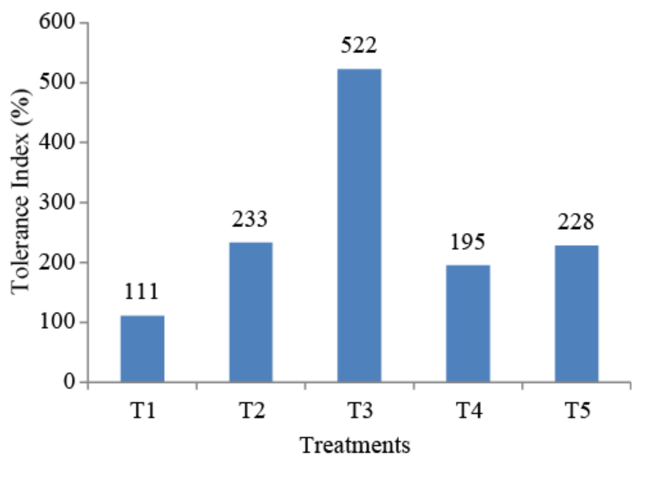

Fig. 8. Effect of biochar amendment on tolerance index of *Glycine max.* (L) Merr. under water stress.
T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

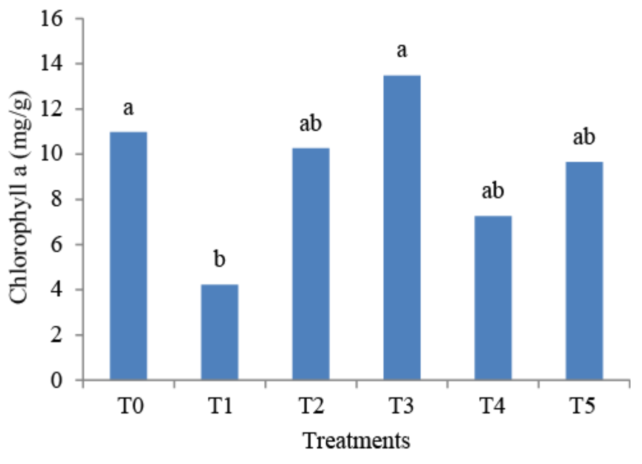

Fig. 9. Effect of biochar amendment on chlorophyll a content of leaves of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biocha

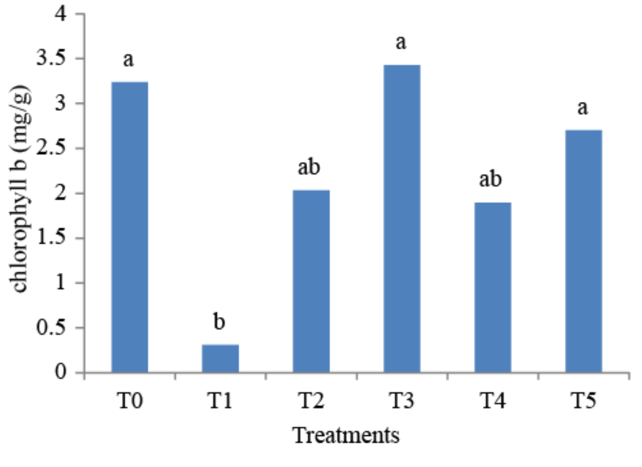

Fig. 10. Effect of biochar amendment on chlorophyll b content of leaves of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

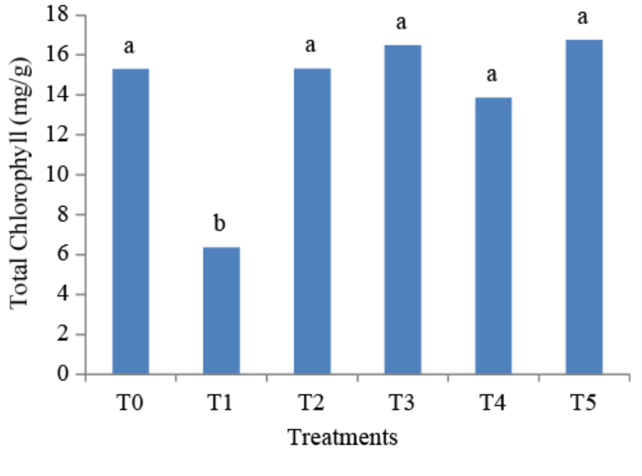

Fig. 11. Effect of biochar amendment on total chlorophyll content of leaves of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

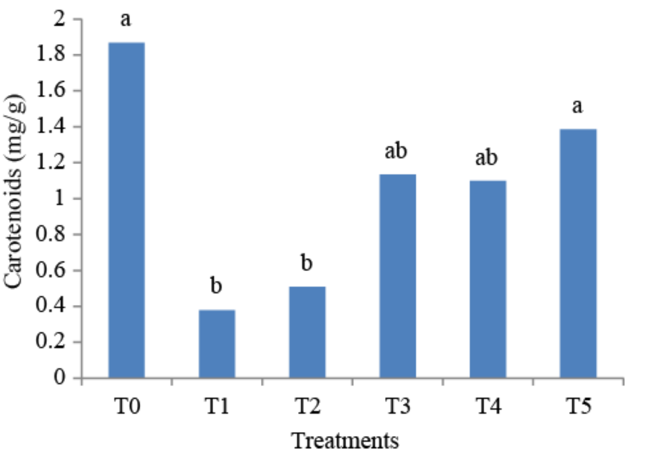

Fig. 12. Effect of biochar amendment on carotenoid content of leaves of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

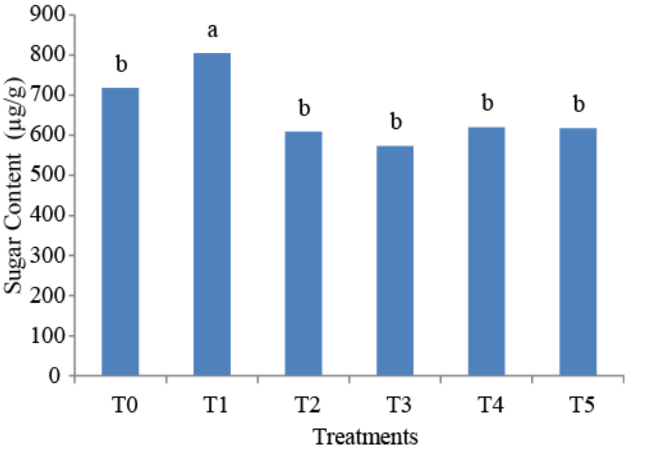

Fig. 13. Effect of biochar amendment on sugar content of seedling of *Glycine max*. (L.) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

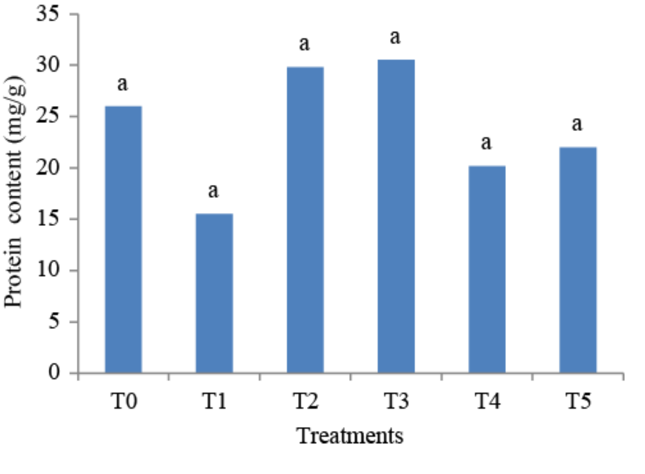

Fig. 14. Effect of biochar amendment on protein content of leaves of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

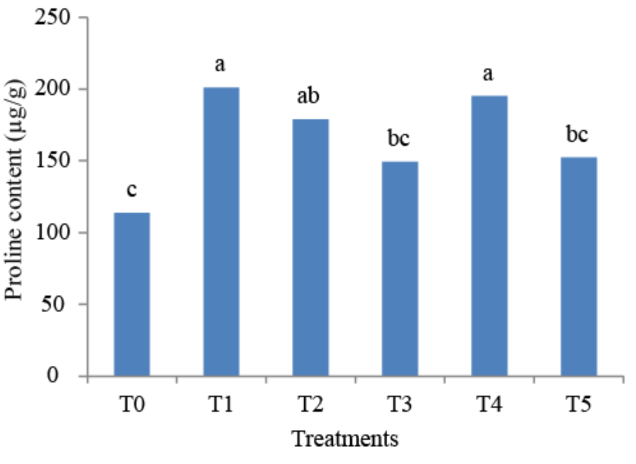

Fig. 15. Effect of biochar amendment on proline content of leaves of *Glycine max.* (L) Merr. under water stress.
T0 = Control, T1 = Drought, T2 = 5 Tons/hectare Biochar, T3 = 10 Tons/hectare Biochar, T4 = Drought + 10 Tons/hectare biochar, T5 = 20 Tons/hectare Biochar

Conclusion

This preliminary research work exhibited that Soybean *(Glycine max.* (L.) Merr.) var. NARC IIis sensitive towards water stress at germination and seedling growth stage and both the concentrations of biochar @10 tons/ hectareand 20 tons/ hectare have proven effective in mitigating the effects of water stress on soybean seedling but biochar @ 20 tons/ hectare was found to be more effective in this respect. Moreover Under normal or without stress condition sbiochar has enhanced seedling growth, but to understand the role of biochar completely, long term impacts of biochar on soil properties and plant productivity should also be investigated.

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