Pak. J. Bot., 52(4): 1159-1168, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-4(19)

AMELIORATION POTENTIAL OF BIOCHAR FOR CHROMIUM STRESS IN WHEAT

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Abstract

Chromium is one of the hazardous heavy metals used in tanning industries. Wheat is highly vulnerable to chromium stress. In present study 5ppm-500ppm concentrations of chromium were applied in solution form to elucidate chromium toxicity on wheat seed germination. Two wheat varities (NARC 2009 and NARC 2011) and 1% biochar was used during the experiment. Wheat seeds continued to germinate till 200 ppm and complete inhibition was observed at 500 ppm. However, the application of biochar improved the germination and growth attributes of wheat seedlings in presence of chromium. Biochar significantly improved the germination percentage upto 100 ppm by 16.8%. In the similar way, seedling root and shoot length was improved significantly by 10%-30% in the presence of 1% biochar. Furthermore, biochar amendment improved the chlorophyll and proline contents upto (9.5%-10%). The superoxide dismutase activity effected greatly the chromium stress and after, application of biochar, resulted in a considerable increase of (13%) at 100ppm as compared to control. The total chromium contents in dry biomass of wheat seedlings was 0.06 ± 0.1 and in presence of 1% biochar the total chromium contents was reduced upto 0.29 ± 1.01. Hence, biochar proved to be an effective amendment for amelioration of chromium effect and improvement of wheat attributes such as germination, physiological and growth condition.

Key words: Chromium, Wheat, Germination, Biochar, Superoxide dismutase.

Introduction

Heavy metals have significantly polluted the cultivated areas throughout the world and are well known for their perseverance in the soil for an indefinite time period (Mench *et al*., 2010). As these toxic metals persist in the soil, the usage of food from these contaminated soils may result in their bioaccumulation. Industrial activities, fertilizers, pesticides, sewage sludge, and other anthropogenic activities are a continuous source of toxic heavy metals. The accumulation of these metals in the soil is transferred to plants, ultimately causing health hazards and food security threats.

Chromium is severely toxic to plants and drastically suppresses plant growth and development furthermore, hexavalent chromium is highly carcinogenic and mutagenic for various crops (Messer *et al*., 2006; Ahmad *et al*., 2012).

Wheat is cultivated approximately on one sixth of the total arable land in the world (Maccaferri *et al*., 2009). Furthermore, wheat is grown as staple crop in Pakistan and approximately 8,0669,00 ha area is under cultivation of wheat. Wheat is highly sensitive to various biotic and abiotic stresses such as heavy metal stress (Khan & Ashraf, 2008; Rahaie *et al*., 2013). Furthermore, the wheat is highly sensitive towards chromium stress (Diwan *et al*., 2012). Seed germination is fundamental step in proper growth and establishment of a healthy plant. As germination is a profound indicator of stress tolerance of seeds towards a specific metal stress. Accumulation of heavy metals inside seeds results in chlorosis, delayed germination, inhibition of photosynthesis and respiration (Liu *et al*., 2008).

Biochar is a cost effective solid product, derived from organic material via oxygen-limited pyrolysis (Wang *et al*., 2013; Xu *et al*., 2015). Biochar acts as a useful absorbent for various heavy metals. It has strong ability to absorb pollutants from soil and solutions because of high porosity, surface area and cation exchange capacity (Yuan *et al*., 2011). Biochar can interact and sorbs various heavy metals due to specific surface ligands and charged particles which form complexes with the heavy metals (Wang *et al*., 2014; Ahmad *et al*., 2012b; Awad *et al*., 2013; Uchimiya *et al*., 2011 a,b; Chan *et al*., 2008). A study was designed to assess the toxic effect of chromium on germination attributes, biochemical status and growth of wheat seeds. Furthermore, the amelioration potential of biochar against chromium stress on wheat attributes was assessed under chromium stress.

Material and Methods

Germination analysis: Experiment was designed to test the chromium toxic effect on seed germination and growth attributes of wheat in controlled conditions. Two wheat varieties (NARC 2009) written as (V1) and NARC 2011 written as (V2) were collected from NARC Islamabad Pakistan. Surface sterilization was done with 0.1% NaOCl (Abdul Baki & Andreson, 1973; Mazhar *et al*., 2016). For germination analysis, seeds were placed in a set of petriplates containing a double layer of Watman's filter paper. Each set of petriplates was provided with 10 ml of distilled water. Different concentrations of $K_2Cr_2O_7$ (Sigma Aldrich Quality Grade) were prepared from stock solution of 1000ppm in sterile MiliQ distilled water. Ten ml of each concentration was poured into petriplates to impose metal stress. Biochar was used in 1% formulation. Seeds were monitored daily for germination. Each seed was considered germinated when radical emerged up to 5mm length.

Experimental layout for germination experiment.

To	Control
T1	1% Biochar
T2	5ppm chromium
T3	5ppm chromium + 1% Biochar
T4	10 ppm chromium
T5	10ppm chromium + 1% Biochar
T6	15ppm chromium
T7	15ppm chromium + 1% Biochar
T8	25ppm chromium
T9	25ppm chromium + 1% Biochar
T10	50ppm chromium
T11	50ppm chromium + 1% Biochar
T12	100ppm chromium
T13	100ppm chromium + 1% Biochar
T14	200ppm chromium
T15	200ppm chromium + 1% Biochar
T16	500ppm chromium
T17	500ppm chromium + 1% Biochar

Biochar production: Fallen pine needles and dried leaves of different trees were used for Biochar production. The biomass (BM) was pyrolysed at a temprature range of 300-400°C for 12 hours. After cooling, biochar was passed from 60-Mesh sieve. Pyrolysed material was further grounded and sieved with mesh size 0.05mm.

Biochar characteristics analysis: Biochar was analysed for physical and chemical analysis such as pH, moisture contents, ash contents and macronutrients such as Carbon and Nitrogen (Yuan *et al.*, 2011) (Table 1). Following germination attributes were analysed.

Germination percentage: Germination percentage and following germination parameters were calculated.

Promptness index: Method of Noreen *et al.*, (2007) was adopted for the calculation of promptness.

PI= (1.00) nd2+ (0.75) nd4 + (0.50)nd6 + (0.25)nd8 Where nd is the number of seeds germinated on the respective days.

Table 1. Biochar characteristics analysis.

Sr. No	Carbon %	Nitrogen %	Potassium % K	Phosphorus % P	pH	Ec	Yield %	Ash content
1.	74.35	2.46	1815	6034	7.9	134	40.7%	15%

Seedling vigour index: For the analysis of seedling vigour index method of Abdul Baki & Anderson (1973) was used accordingly.

Morphological Parameters: Morphological parameters for wheat seedling after germination were measured which included seedling fresh weight, dry weight, root and shoot length in (cm).

Pigment analysis: The chlorophyll contents of wheat leaves were measured by using the UV spectrophotometer after grinding the leaves in the acetone by adopting the method of (Bruinsma, 1963).

Membrane stability index: Membrane stability index was measured according to the method of (Sairam *et al*., 1994). Discs of equal size from flag leaves were used to analyze the MSI. Discs were made and heated for one hour at 60°C to measure M1 and EC1. M2 was measured by placing the discs at 100°C for 15 minutes and EC2 was calculated.

Proline: Ninhydrin method was used for the proline quantification in chromium stress and controlled conditions (Bates *et al*., 1973). Glacial acetic acid and Ninhydrin were added to leaf material and boiled for an hour and after boiling extract was placed in the ice for cooling. The absorbance was recorded at 520 nm. Total proline: Absorbance of sample x K value /Weight of fresh tissue.

Sugar analysis: Sugar content was measured by applying phenol sulphuric acid method (Dubois *et al*.,1951). 0.5g of fresh plant tissue was taken and ground by using pestle and mortar. Extract from each treatment was taken in a set of test tubes and 10ml of 80% ethanol was mixed with the extract. Then the test tubes containing mixture were placed in water bath for 1 hour at 80ºC. After boiling 0.5ml extract from these test tubes was transferred to another set of test tubes. 1ml phenol (18%) and 0.5ml of unionized water was added in test tubes containing extract and shaked thoroughly after mixing extract was left for 1 hour at 25ºC. After that 2.5 ml of concentrated sulphuric acid was added carefully. Absorbance of sample was checked at 490nm with the help of UV spectrophotometer and calculation was done by applying following formula:

$$\text{Sugar content } (\mu g/ml) = \frac{\text{Absorbance value} \times \text{K value} \times \text{dilution factor}}{\text{Plant tissue weight (g)}}$$

Super oxide dismutase assay: The method of Giannopolistis & Ries (1977) was used to estimate the activity of SOD enzyme in the wheat seedlings according to following formula:

$$\text{SOD (unit protein per minute)} = \frac{\text{Absorbance of sample} \times \text{K value} \times \text{dilution factor}}{\text{Weight of sample(g)}}$$

Chromium analysis in wheat: The accumulation of heavy metals was measured by using the method of (Ouzounidou, *et al*., 1992). Heavy metals in each sample were estimated individually using atomic absorption spectrophotometer.

Statistical analysis

For statistical analysis SPSS.20 version was applied to analyse the data. Analysis of variance method was used and each treatment was with three replicates. The duncan's multiple range test (DMRT) was used to analyze the difference between mean values at the level of significance ≤ 0.05. Furthermore PCA analysis was conducted as shown in Figure 12(A, B, C, D).

Results

Seed germination is considered to be a very important step for the establishment of plants and is affected by various stress conditions. A study was designed to investigate chromium stress on wheat seeds germination and to evaluate the mitigation effect of biochar. Concentrations of chromium ranging from 5, 10, 15, 25, 50, 100, 200 and 500ppm were applied. Both wheat varieties showed a considerable reduction in germination with a subsequent increase in Cr concentration. The maximum limit of wheat seeds germination was 200 ppm. After 200 ppm continuous decline in the germination was observed. A gradual decrease of 10, 20, 25, 40, 50, 70, 90 and 95% in germination percentage was observed at 5ppm till 500 ppm respectively. Furthermore, at 500 ppm germination was completely inhibited. Significant improvement was observed in treatment with biochar particularly upto100ppm chromium concentration. A significant increase of 16.67% in germination percentage was recorded at 100 ppm chromium concentration with 1% biochar as compared to control (Fig. 1).

Data regarding to promptness index showed that chromium concentrations from 5ppm to 500ppm reduced the promptness index from 22% to 98% as compared to respective control. However, significant increase of 14.7% in promptness index was recorded with 1% biochar at 100 ppm concentration as compared to control (Fig. 2). While biochar significantly increased the seedling vigor index upto 100ppm chromium. The maximum increase of 20.41% and 16.67% in seedling vigor index was observed at 25ppm and 50ppm chromium concentration with the application of 1% biochar as compared to respective control (Fig. 3).

Shoot length showed a remarkable reduction from 5ppm to 200ppm chromium concentration when compared to control. Shoot length of seedlings was significantly improved with biochar treatment upto 10% (Fig. 4). In the similar way, root length was decreased from 5ppm to 500ppm and the same trend was observed for dry weight in response to different treatments of chromium (Fig. 5,6). However, by 1% biochar application significant results were obtained as increase in the dry weight of wheat seedlings at 5ppm till 100 ppm was observed.

Chlorophyll contents of wheat seedling were decreased upto 98% at 500ppm as compared to control. However, biochar improved the total chlorophyll contents and the maximum increase was observed at 100ppm with 9.5% (Fig. 7). Membrane stability index was significantly reduced from 4.8 till 99% from 5ppm upto 500 ppm, however, biochar has shown a strong effect on maintaining the membranes stability index uptill 100ppm with a maximum increase of 10% at 50ppm (Fig. 8).

Proline and soluble sugar contents were increased with the gradual increase of chromium stress, however, after the 100 ppm due to the inhibitory effect of chromium no germination so results remained nonsignificant after 200ppm. However, Biochar improved the proline and soluble sugar contents upto 10%-14.3% uptill 100ppm chromium concentration (Figs. 9, 10). Chromium stress increased the superoxide dismutase activity in chromium treated plants. However, the SOD activity showed decline upto 11-20% with a successive increase in chromium concentration from 5 ppm upto 500 ppm. However, the biochar significantly improved the SOD activity upto 4-13% at 10 ppm to 100 ppm chromium concentration with their respective control (Fig. 11). Higher amount of Cr upto 0.06±0.1 mg/kg was detected in the wheat plants when exposed to 200ppm $K_2Cr_2O_7$. However the concentration of Cr decreased significantly in plant dry weight when biochar was present. Furthermore, the application of biochar significantly reduced the heavy metal concentration upto 0.29±1.01mg/kg and ameliorated the stress effect (Table 2).

Table 2. Heavy metal uptake by wheat plants under Cr stress in the presence of Biochar (concentration in milligram per kilogram).

S. No.	Control mg/kg	$K_2Cr_2O_7$ mg/kg	PGBiochar+ $K_2Cr_2O_7$ mg/kg
1.	0.06 ± 0.1	0.41 ± 0.09	0.29 ± 1.01

Discussion

According to the present findings the effect of chromium stress on wheat seed germination could be ameliorated by application of biochar under chromium stress. The growth of many crop plants, including wheat is effected greatly due to various heavy metals including the chromium, as chromium disturbs the normal growth processes of plants, including germination, root and shoot length, stomatal conductance, chlorophyll contents and net photosynthesis (Dey *et al*., 2009). Higher levels of chromium, such as 200-500ppm affected the total seed germination as shown in (Fig. 1, 2 and 3). However, the results of the study showed that the biochar significantly improved the germination of wheat upto (16.4%) in the presence of chromium. Our results are also similar to the findings of Jain *et al*., 2000 where chromium stress reduced the seed germination of Sugar cane and Phaseolus. The reduction in germination percentage might be due to a decrease in amylase activity in germinating seeds. As reduction in amylase activity is responsible for a decline in sugar breakdown and transport resulting in reduced seed germination. Reduction in seed germination can also be due to the strong effect of protease enzyme which increases with the subsequent increase in chromium concentration (Zeid, 2001).

However, biochar ameliorated the chromium stress effect and improved the seed germination, as overall results analysis revealed that biochar had a strong effect on wheat seeds germination and growth uptill 100ppm chromium concentration. Biochar plays an important role for heavy metal immobilization, by reducing the phytotoxicity and bioavailability of metals towards plants (Choppala *et al*., 2012). Biochar has the ability to absorb pollutants from soil and solutions due the high cation exchange capacity and high pH (Yuan, *et al*., 2011). Moreover, biochar adsorb different charged species such as positive ions AL^+, K^+, Na^+? due to electrostatic attraction. Furthermore, biochar can assist the germinating seedlings, required for growth by providing various nutrients due to its rich nutrient properties. Similar results were also documented by (Lehmann, 2003; Laird et al., 2010). Furthermore, seedling vigor index was higher upto 20.41% at 25ppm $K_2Cr_2O_7$ when amended with 1% biochar as indicated in (Fig. 4).

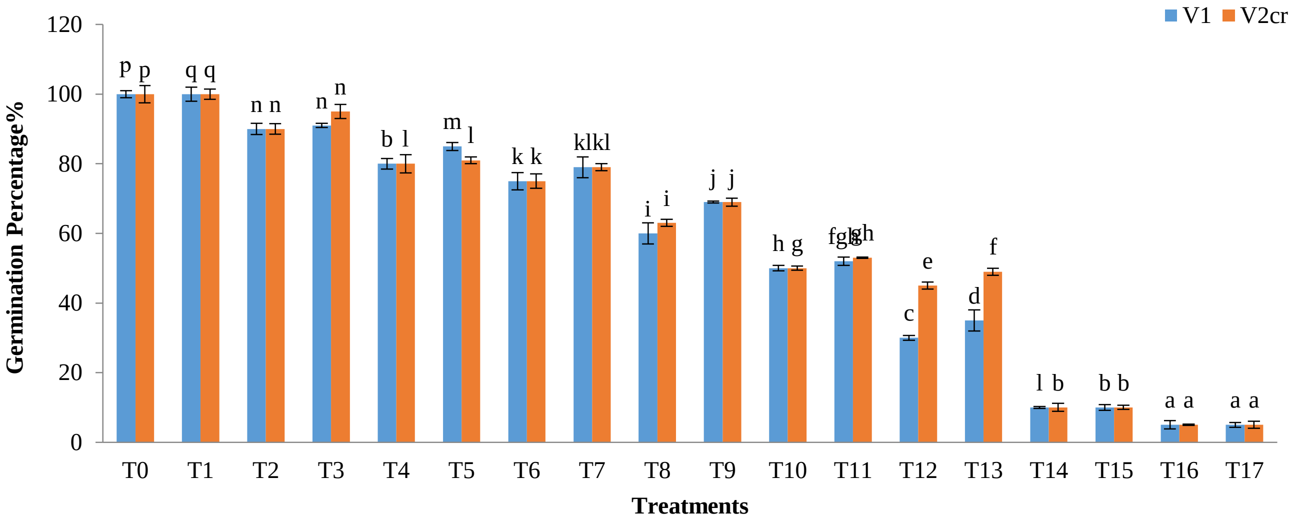

Fig. 1. Chromium stress effect and its amelioration by biochar on germination percentage of selected wheat varieties (V1 and V2). Values with same letter are not significantly different at $p\leq0.05$.

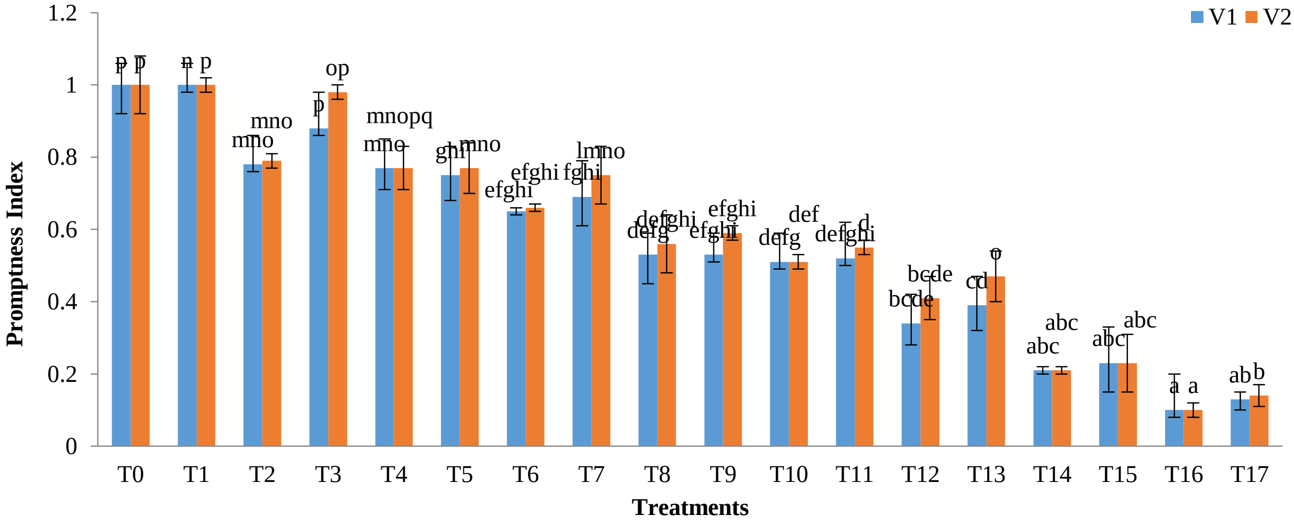

Fig. 2. Chromium stress effect and its amelioration by biochar on promptness index of selected wheat varieties. Values with same letter are not significantly different at $p\leq0.05$.

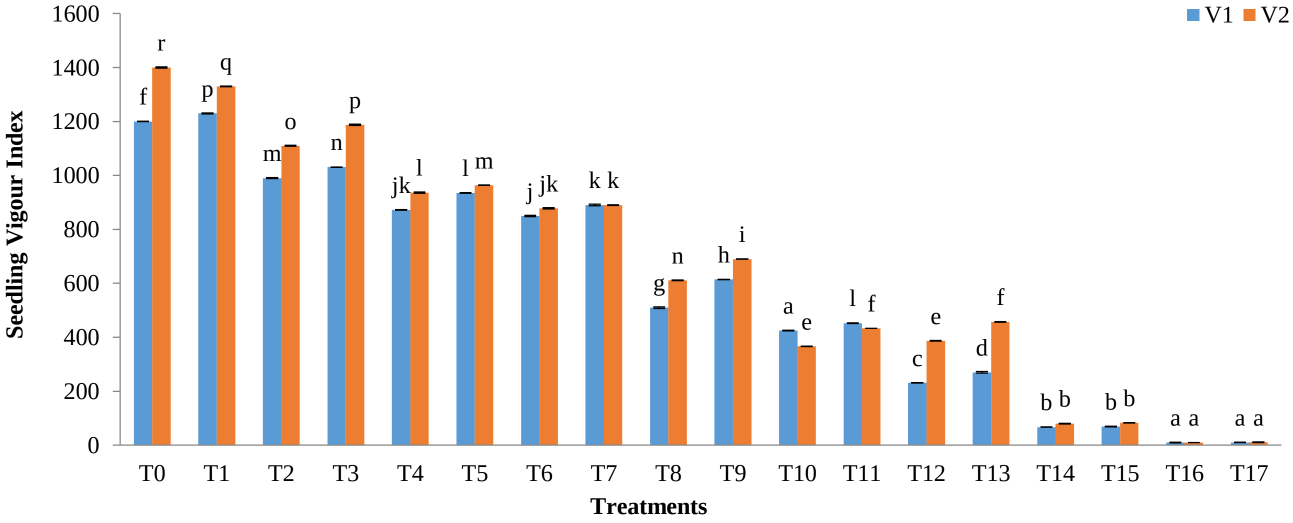

Fig. 3. Chromium stress effect and its amelioration by biochar on seedling vigour index percentage of selected wheat varieties. Values with same letter are not significantly different at $p\leq0.05$.

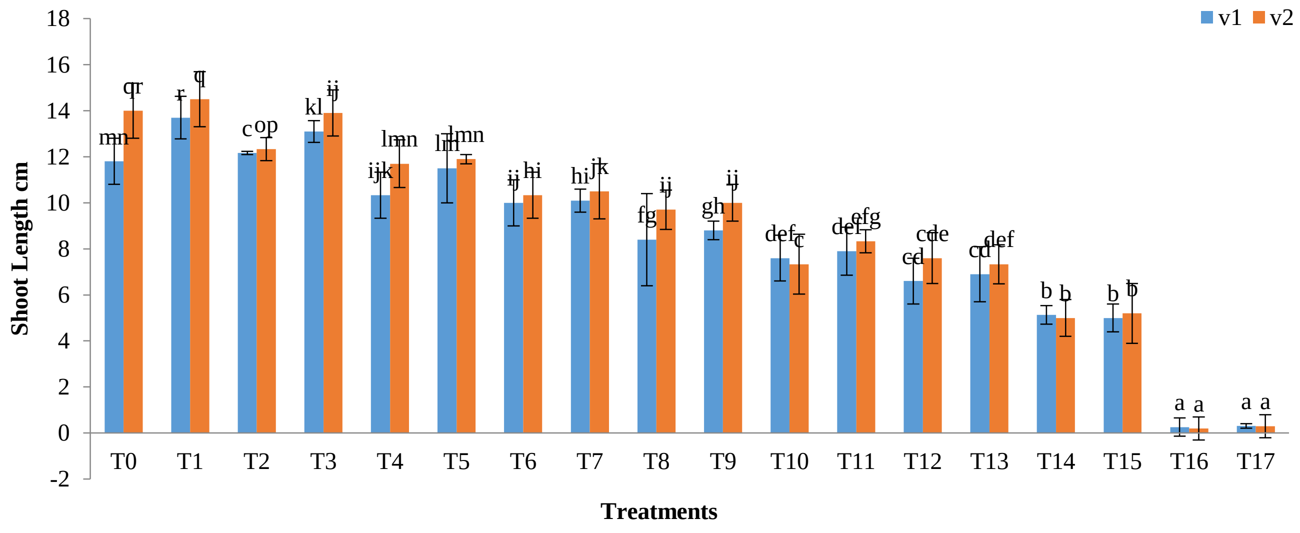

Fig. 4. Chromium stress effect and its amelioration by biochar on shoot length of selected wheat varieties. Values with same letter are not significantly different at $p \leq 0.05$.

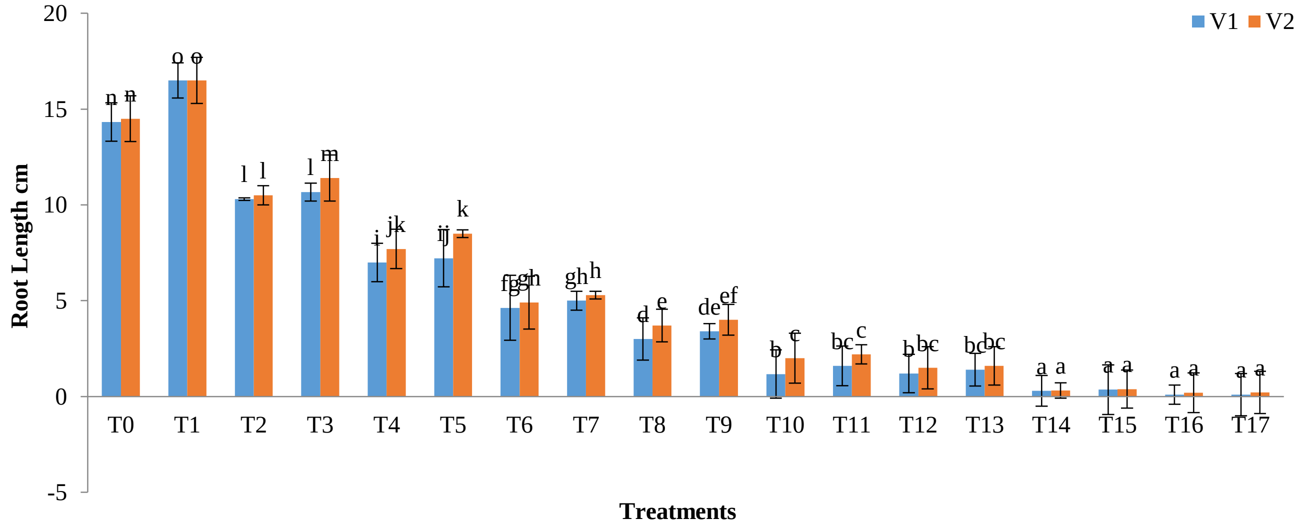

Fig. 5. Chromium stress effect and its amelioration by biochar on root length of selected wheat varieties. Values with same letter are not significantly different at $p \leq 0.05$.

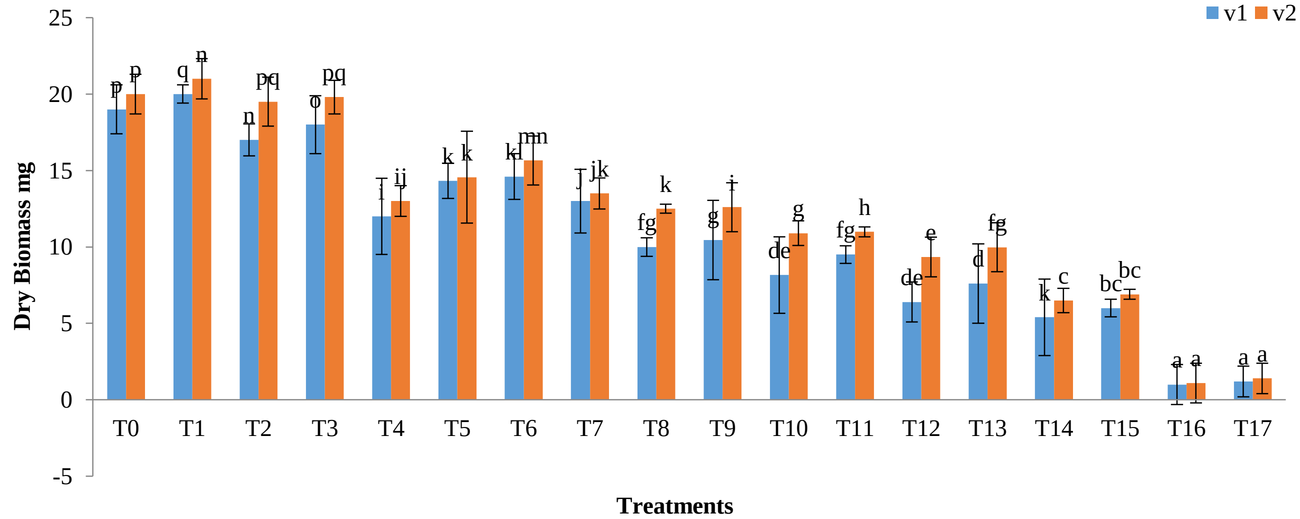

Fig. 6. Chromium stress effect and its amelioration by biochar on dry biomass of selected wheat varieties. Values with same letter are not significantly different at $p \leq 0.05$.

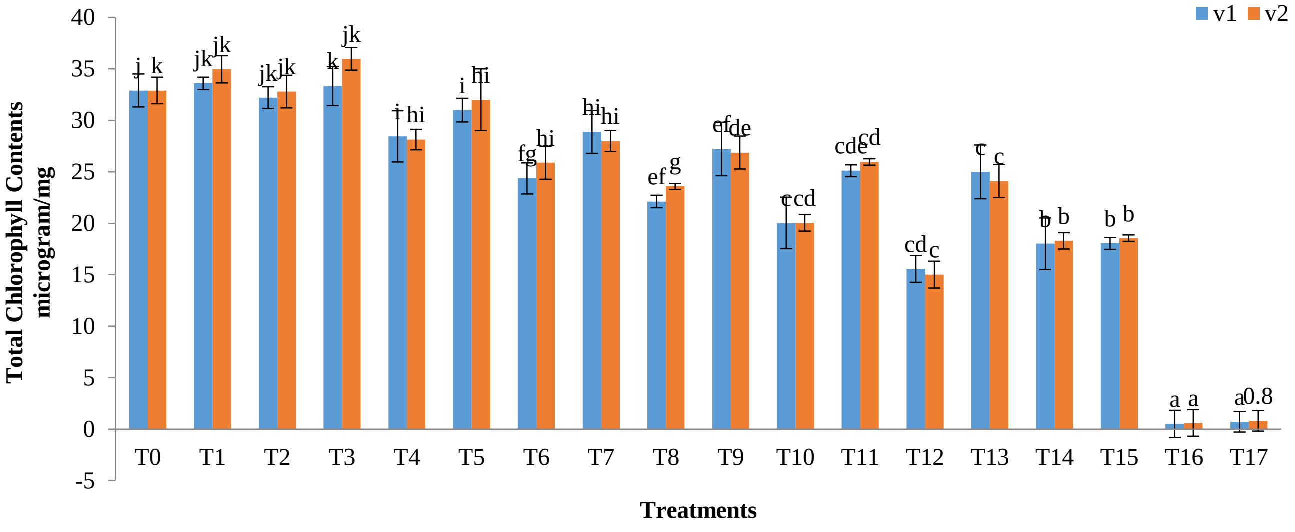

Fig. 7. Chromium stress effect and its amelioration by biochar on chlorophyll contents of selected wheat varieties. Values with same letter are not significantly different at $p \leq 0.05$.

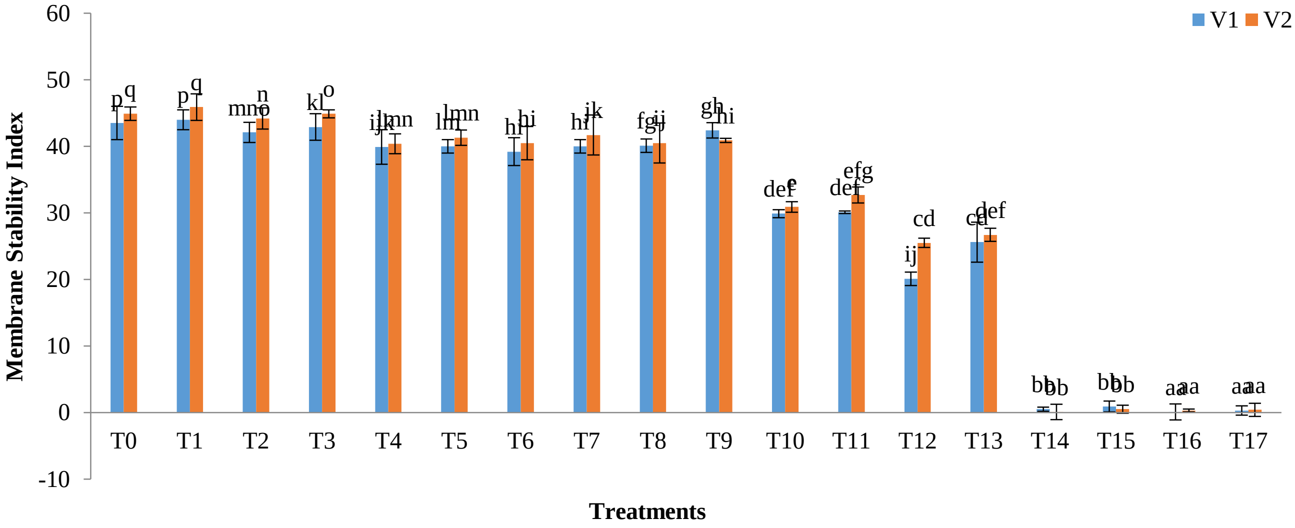

Fig. 8. Chromium stress effect and its amelioration by biochar on membrane stability index of selected wheat varieties. Values with same letter are not significantly different at $p \leq 0.05$.

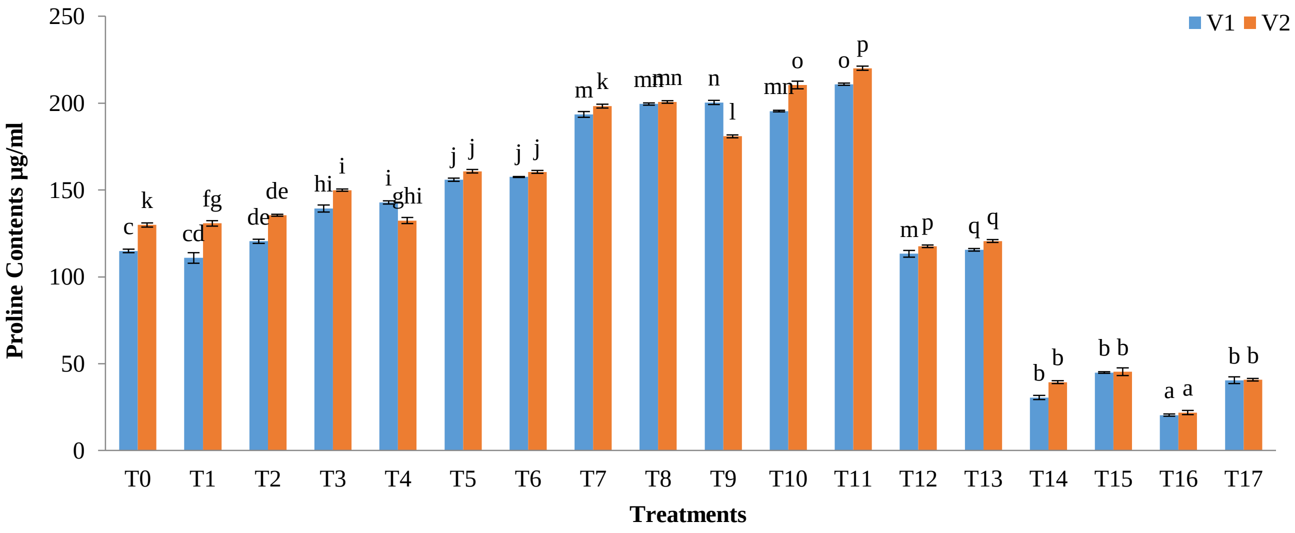

Fig. 9. Chromium stress effect and its amelioration by biochar on proline contents of selected wheat varieties. Values with same letter are not significantly different at $p \leq 0.05$.

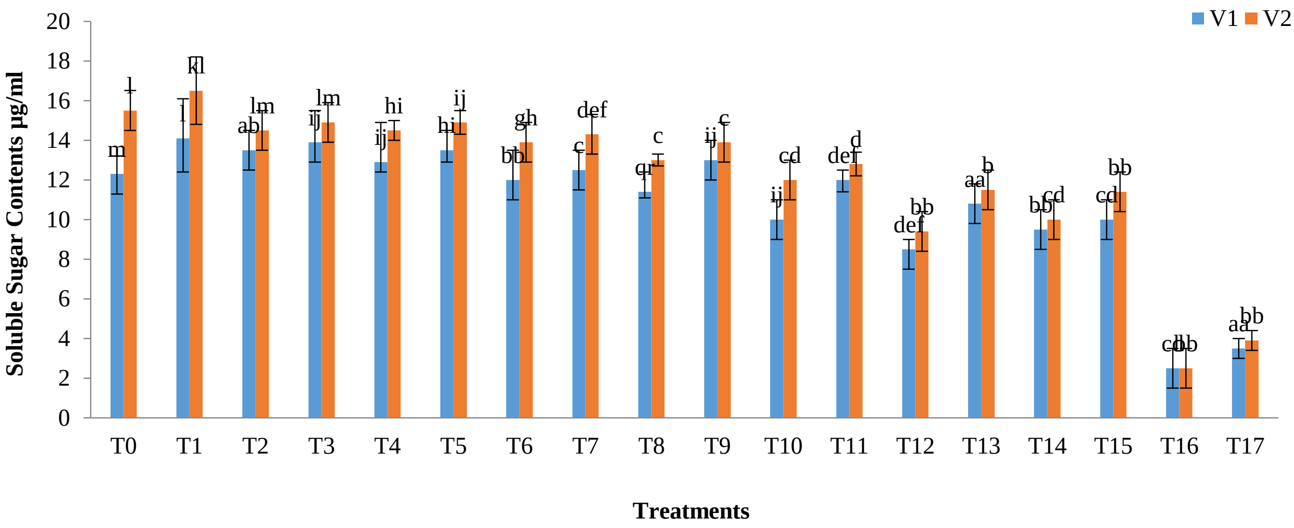

Fig. 10. Chromium stress effect and its amelioration by biochar on soluble Sugar contents of selected wheat seedling. Values with same letter are not significantly different at $p \leq 0.05$.

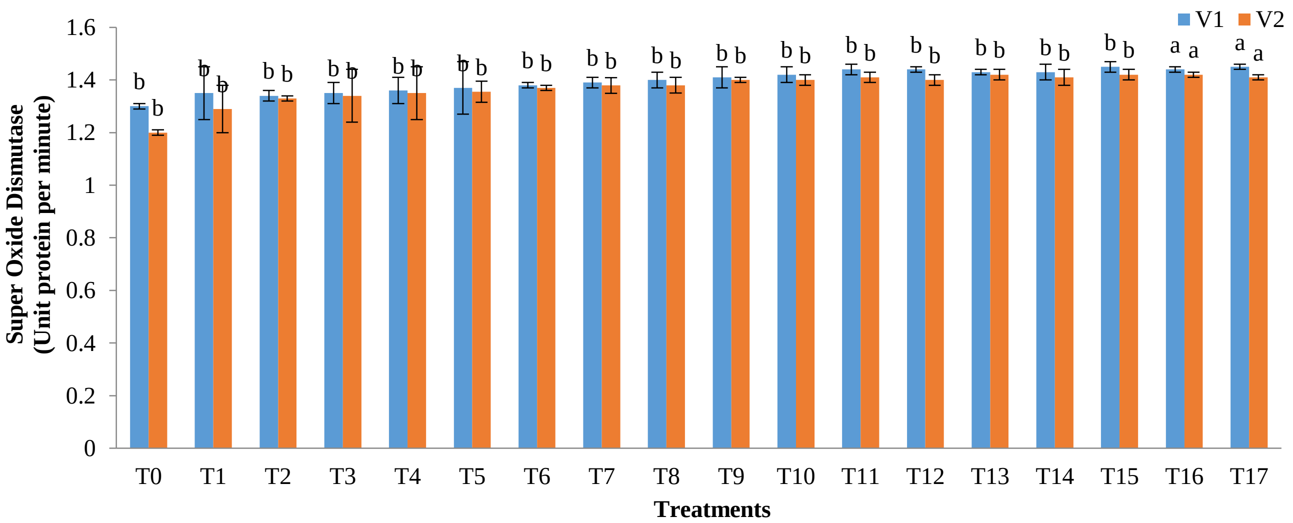

Fig. 11. Chromium stress effect and its amelioration by biochar on superoxide dismutase activity of selected wheat varieties (unit protein per minute) . Values with same letter are not significantly different at $p \leq 0.05$.

Toxic effect of chromium was more pronounced at root length as compared to shoot. The results revealed that seedling length was reduced drastically with the gradual increase of chromium in solution uptill 500ppm (Fig. 4,5). Metallic ions are responsible for inhibition in cell division and reduction in the supply of nutrients and water transport which might be an ultimate reason for the reduction in shoot length. Accumulation of metals inside the plants resulted in the reduction of plant height (Shanker *et al.*, 2005). However, biochar treatment was found effective in mitigating the chromium stress and improved the root and shoot length upto 30 and 10% (Fig. 4,5). Since it helps the germinating seedlings to tolerate toxic effect and significantly reduce the metal effect. Biochar has a strong effect on growth, for example, a cation exchange mechanism for metal binding and removal from solutions. High nutrient retention such as iron and phosphate, which are the prime nutrients for better plant growth and biomass production (Chan *et al.*, 2008; Chan & Xu, 2009). Several studies have indicated the strong effect of biochar on plant height and greater biomass (Mustafa *et al.*, 2010; Hossain *et al.*, 2012) which is also shown in our results that biochar improved the dry biomass significantly our results are also in accordance with the Major *et al.*, 2010.

Chlorophyll contents were reduced at 500ppm chromium concentration up to 98% (Fig. 7). The decrease in chlorophyll contents could be due to the inhibition of the activities of enzymes that play important role in the synthesis of these pigments. Chromium can reduce the protein contents and pigment production in plants (Shankar *et al.*, 2005; Dey *et al.*, 2009). Improvement in chlorophyll contents under stress could be an indication of tolerance towards metal stress. According to the results biochar improved the pigment concentration with the maximum increased by 9.5% at 100ppm by limiting the bioavailability of heavy metals and made it less available to roots directly Furthermore, biochar can stabilize heavy metals by absorption and cation exchange mechanism (Ippolito *et al.*, 2011; Park *et al.*, 2011). In the similar way biochar can improve the photosynthetic rate by stabilization of various pigments such as chlorophyll (Xu *et al*., 2015).

According to our results, a decrease of 99% in MSI was observed at 500 ppm under chromium stress (Fig. 8). Oxidative stress could occur due to metal accumulation inside the plant cells and as a result of the production of reactive oxygen species, biochemical and molecular disturbances may occur (Hossain *et al*., 2012). However, the biochemical and physiological attributes were improved by biochar upto 100ppm, such as MSI and relative water contents. The results further revealed the increase in proline content. As proline is a multifunctional osmoprotectant accumulated in plant cells in response to various stresses. However, the toxic effect of chromium was ameliorated by application of 1% biochar uptill 100ppm and significant improvement in proline contents upto 10% observed (Fig. 10). According to Kanwal *et al*., (2017) the application of biochar under salt stress mitigates the toxic ionic effect and improved the wheat growth. To survive with the toxic effect of chromium there was an increase in the superoxide dismutase activity in wheat seedlings, but at a higher level of chromium a decline in sod activity was observed (Fig. 11). Plants have developed various antioxidant mechanisms to cope up with stress conditions, but when exposed to high concentration of contaminants such as heavy metal they are unable to maintain homeostasis inside their cells (Wang *et al*., 2013; 2014). The decrease in SOD activity might be due to denaturation of the protein structure of various enzyme which leads to the reduction in their activity. However, according to the results the toxic effect was significantly reduced under chromium stress after biochar application, as leaching of metals from soil towards plants could be minimized by biochar application due to its various redox reaction and metal binding properties (Choppala *et al*., 2012; Li *et al*., 2015). In last few years several studies documented the role of biochar addition to phytoremediation and including soil biological activity enhancement (Lu *et al*., 2014). According to the results 1% biochar application significantly reduced the chromium metal uptake in wheat seedlings (Table 2). The decreased uptake might be due to high surface area and high aromaticity of biochar (Tong, *et al*., 2011).

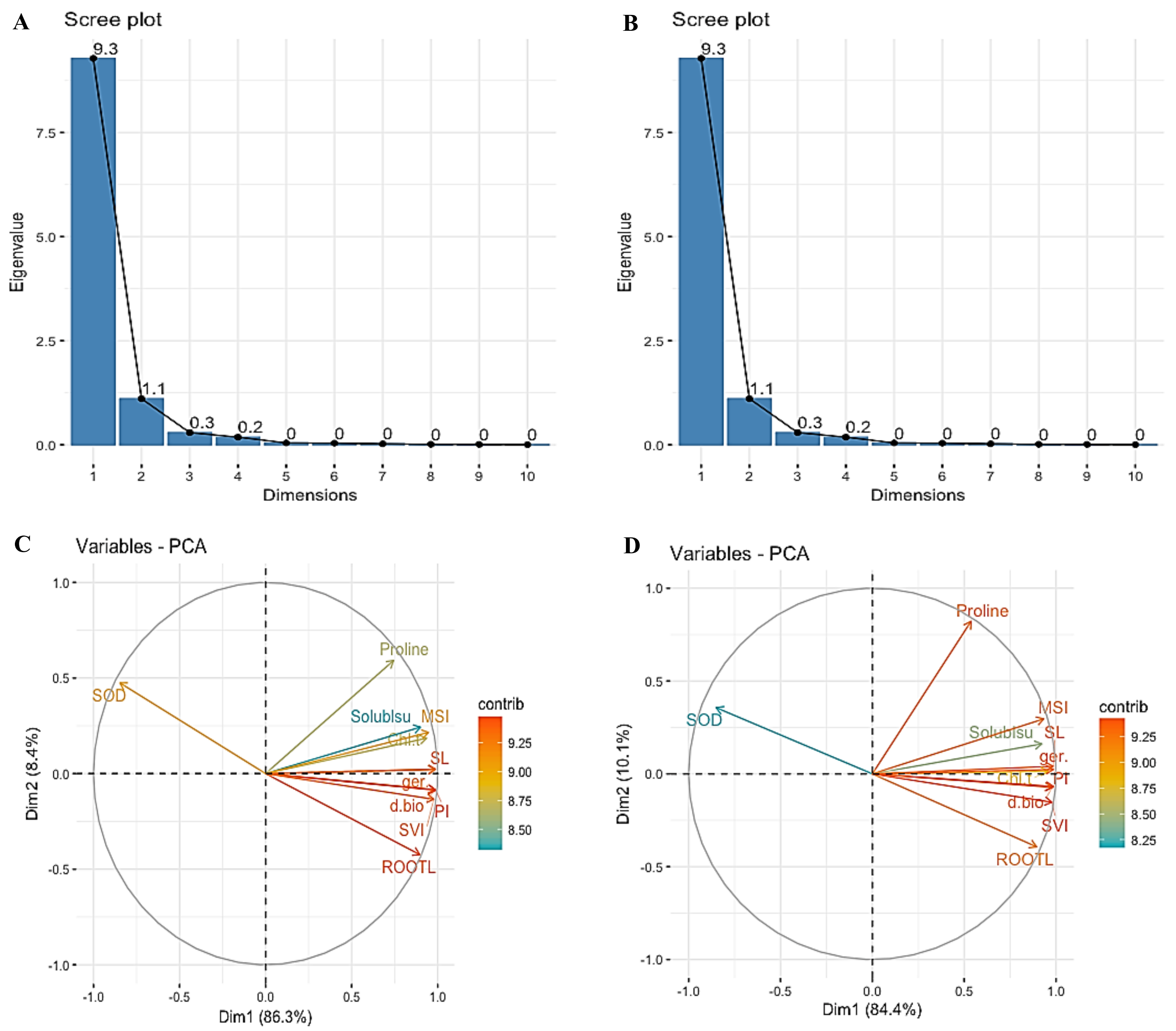

Fig. 12. A-B,C-D: PCA Analysis for germination and growth attributes of wheat seedlings under chromium stress.

Conclusion

Present study investigates the chromium stress effect on the wheat seeds germination. According to the findings higher chromium concentration inhibited the wheat seed germination e.g. 200-500ppm reduced the growth of emerging seedlings. Biochar improved all the germination attributes and growth conditions such as root, shoot length, MSI, chlorophyll contents and SOD activity of wheat seeds upto 100ppm chromium concentration. Further evaluation and a different formulation of biochar are needed for future optimization. This information can be a useful factor in finding the tolerance and the uptake limit of wheat against chromium stress.

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(Received for publication 5 December 2018)