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Research article

Residual effects of biochar on growth, photosynthesis and cadmium uptake in rice (*Oryza sativa* L.) under Cd stress with different water conditions

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ABSTRACT

Soil cadmium (Cd) contamination and drought stress are among the main issues hindering global food security. Biochar has been used to reduce metal uptake by plants and water stress mitigation, but long-term residual effects of biochar under Cd stress at different moisture levels needs to be investigated. A following rice (*Oryza sativa* L.) was grown after wheat on Cd-contaminated soil amended with different levels of biochar (0, 3.0, and 5.0%, w/w). Thirty five days old plants were irrigated with three moisture levels including zero drought as a control (1–2 cm water layer on soil), mild drought (MD, 50% of soil water holding capacity, WHC), and severe drought (SD, 35% of soil WHC) for an accompanying 35 days. Plant height, biomass and photosynthesis were reduced whereas oxidative stress increased under MD and SD than control in un-amended soil while opposite trends were observed in plants grown in biochar amended soil. At the same biochar addition, Cd concentrations in seedlings were lower in continuous flooding than MD and SD treatments. The biochar supply reduced the bioavailable Cd in the soil whereas increased the soil EC and pH than the control treatment. In conclusion, continuous flooding plus residual biochar can be strategized in mitigating Cd-contamination in paddy soils and decreased Cd concentrations in rice which may reduce the potential risks to humans.

1. Introduction

In the present era, contamination of agricultural soils with cadmium (Cd) is a serious problem for global food health. The main sources of Cd entry to agricultural soils are anthropogenic activities and wreathing of Cd-enriched rocks (Nagajyoti et al., 2010). Cadmium is well known as a non-essential toxic metal for both plants and humans (Gallego et al., 2012). It is well documented that Cd caused toxicity in plants at morphological, physiological and molecular levels with varying extents (Shi et al., 2015; Rizwan et al., 2016a). Drought stress is another major abiotic stress limiting plant growth and yield, especially in semiarid areas worldwide (Farooq et al., 2014; Rizwan et al., 2015). Recently, it has been reported that Cd uptake significantly reduced under drought stress in *Brassica juncea* (Bauddh and Singh, 2012) and *Ricinus communis* (Shi et al., 2015). Furthermore, Cd uptake by plants depends upon the growth stage and exposure time of the drought stress. The Cd uptake reduced at pre-flowering stage and increased at maturity stage in drought-stressed peanut (*Arachis hypogaea*) but response varied with cultivars studied (Xia et al., 2015). Contrarily, the drought stress decreased Cd concentrations in peanut at seedling stage whereas its contents increased in shoots at the pod-filling and pod-ripened stages (Liu et al., 2017). Thus, drought stress affected Cd accumulation in plants but response varied with species and growth stages. However, little information is available regarding Cd accumulation in cereals under drought despite the fact that reasonable work has been done on accumulation of Cd in cereals when grown on Cd-contaminated soils.

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Rice (*Oryza sativa* L.) is the main grain crop grown worldwide and is consumed by more than half of world population as a staple food (Rizwan et al., 2016b). It is well documented that rice has a higher ability to accumulate Cd than other cereals when grown in Cd-contaminated soils (Gao et al., 2016; Rehman et al., 2017).

Biochar, a stable organic material prepared under limited oxygen supply, has been recognized as an amendment used for the improvement of soil fertility and reduction of abiotic and biotic stresses such as heavy metals and drought stress (Rizwan et al., 2016c; Ali et al., 2017). Biochar has been shown to enhance soil water holding capacity and plant production under limited water supply (Cornelissen et al., 2013; de Melo Carvalho et al., 2014; Zong et al., 2016). Furthermore, biochar supply reduced trace element toxicity in plants and increased metal immobilization in the soil (Younis et al., 2016; Abbas et al., 2017a). However, little is known about the residual effects of biochar under combined abiotic stresses. In a previous study, biochar application reduced Cd concentration in drought-stressed wheat by decreasing soil bioavailable Cd and increasing plant growth and biomass (Abbas et al., 2017b). However, the fate of this reduced Cd in the soil is very important before use of biochar for plant growth in metal-contaminated soils under drought stress. Furthermore, residual effects of biochar on latter crops may provide more clear information about its sustainable use in contaminated soils aiming to reduce Cd uptake in plants with different moisture levels.

The objectives of the current experiment were to investigate the residual effects of biochar on plant growth, physiology and Cd uptake in rice under Cd stress and its bioavailability in the soil with different water levels. Rice was chosen as a following crop because wheat-rice rotation is the main cropping pattern in many areas worldwide including Pakistan (Rehman et al., 2017). It was anticipated that this research will help to formulate strategies regarding the management of metal-contaminated soils.

2. Materials and methods

2.1. Soil properties and biochar treatments

In this study, we used the soil that was previously used for wheat cultivation and detailed physicochemical properties of soil are reported there (Abbas et al., 2017b) and other previous studies (Rehman et al., 2015). In brief, a field was finalized in the suburb of Multan city which has been irrigated with raw effluent for about 30 years and is used for the growth of food crops. Soil sampling was done from 0 to 20 cm depth and samples were air-dried under shade and sieved through 2 mm. Soil was texturally classified as clay loam with sand 44%, silt 25%, and clay 31%. Furthermore, soil pH was 7.33, EC_e 2.97 dS m^{-1}, and CEC of 4.96 $cmol_c$ kg^{-1} that were measured by using standard procedures described by US Salinity Lab. Staff, 1954 (Page et al., 1982). Total as well as bioavailable Cd and other metals were estimated according to the standard procedures (Soltanpour, 1985; Amacher, 1996). Three biochar levels (0, 3.0, and 5.0% w/w) were applied before wheat sowing (Abbas et al., 2017b) and after the harvest of wheat along with its roots, the soil was saturated for rice crop without any amendment for the current study. Biochar was prepared from rice straw as a feedstock at 450 °C for two hours and having pH 10.0, ash content 22.5%, volatile matter 24%, EC 2.4 dS m^{-1}, C 42.3%, N 1.5%, K 2.54%, P 0.3%, and Na 1.1% (Qayyum et al., 2015).

2.2. Experimental setup and water management

The study was performed in a botanical garden of Government College, University, Faisalabad, Pakistan, under ambient conditions. Thirty days old rice nursery (cv. Kainat) was transferred to the pots containing about 3.0 kg of soil from an earlier experiment. Initially, six seedlings were grown in each plastic pot with four replicates following CRD (completely randomized design) and finally plants were maintained after ten days of sowing and remaining were crushed and mixed in to same pots. To avoid nutrient deficiency, fertilization was done with urea, diammonium phosphate, and potassium sulfate at a rate of 120-50-25 NPK kg ha^{-1} respectively. Half N and full PK doses were applied to the plants after 15 days and remaining N was applied after 30 days of sowing. All pots were irrigated with tap water by maintaining 1–2 cm layer of water on the soil for 35 days. After this, three water treatments were applied including control (1–2 cm layer of water on the soil), mild drought (MD) (soil water content at 50% of soil water holding capacity, WHC), and severe drought (SD) (soil water content at 35% of WHC) for an additional 35 days. Water management in MS and SD was done by weighing method by considering the soil WHC of 31.2%. Control was selected as this was the major practice of the local farmers in the area for rice growth, especially during early growth period (Rehman et al., 2015). There were total 9 treatments (3 × 3) and 36 pots (4 replicates of each treatment). MD and SD meant mild drought stress and severe drought stress later in the text.

2.3. Plant harvesting and measurement of growth parameters

Plant harvesting was done after 70 days of transplanting in the pots and data related to height of plants and tillers per plant were recorded. Root samples were extracted from each pot, washed with dilute hydrochloric acid (1.0%) and followed by washing with distilled water. A part of fresh samples was used for analysis and some fresh samples were stored in liquid N_2 for the estimation of antioxidant enzymes. Then both samples were oven dried (70 °C) after carefully wiping and air drying of the samples in green house. Air dried samples were placed in the oven till constant weight has been achieved to record oven dry weights. Samples were grounded to small size and stored in air tight plastic jars for analysis.

2.4. Physiological measurements

Chlorophyll *a* and chlorophyll *b* contents and gas exchange attributes (photosynthetic rate (Pn), stomatal conductance (Gs), water use efficiency (WUE), and transpiration rate (Tr)) were measured from the upper leaves before harvesting (70 days of sowing). Chlorophyll contents were measured after extracting the leaves with acetone (85% v/v) and using the standard equations (Lichtenthaler, 1987). A portable Infra-Red Gas Analyzer (IRGA) (Analytical Development Company, Hoddesdon, England) was used for the measurement of gas exchange attributes between 10:00 and 12:00 h with maximum day light intensity.

Before harvesting the plants, the fresh leaves were sampled for the estimation of electrolyte leakage (EL) and malondialdehyde (MDA) levels as well as hydrogen peroxide (H_2O_2) contents in samples as detailed previously (Abbas et al., 2017b). In brief, EL of the leaves was estimated by measuring the initial and the final EC of the samples after incubating the samples in distilled water at 32 °C for 2 h termed as EC_1 and then at 121 °C for 20 min termed as EC_2 and then putting these values in equation (Dionisio-Sese and Tobita, 1998).

$$\text{Electrolyte leakage (\%)} = \left(\frac{EC1}{EC2}\right) \times 100 \quad (1)$$

Heath and Packer (1968) method with minor changes later (Dhindsa et al., 1981; Zhang and Kirkham, 1994) was used to determine MDA contents in leaves by thiobarbituric acid (TBA) reaction. For H_2O_2 level estimation in leaves, 3.0 mL of phosphate