Ecotoxicology and Environmental Safety 137 (2017) 64–70

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress

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ARTICLE INFO

Keywords:
Phaseolus vulgaris L.
Salt stress
Biochar
Hydrogen peroxide
Malondialdehyde
Antioxidant activities

ABSTRACT

Salinity damaged cellular membranes through overproduction of reactive oxygen species (ROS), while osmolytes and antioxidant capacities play a vital role in protecting plants from salinity caused oxidative damages. Biochar also could alleviate the negative impacts of salt stress in crops. The pot experiment was conducted to investigate the effects of biochar on some antioxidant enzyme activities and osmolyte adjustments of common bean (*Phaseolus vulgaris* L. cv. Derakhshan) under salinity stress. Bean plants were subjected to three salinity levels (non-saline, 6 and 12 dSm^{-1} of NaCl) and biochar treatments (non-biochar, 10% and 20% total pot mass). Shoot and root dry weights of bean were decreased at two salt stress treatments. Salinity increased the activity of catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), polyphenol oxidase (PPO) and superoxide dismutase (SOD), and the content of malondialdehyde (MDA), oxygen radicals ($O^{2\bullet-}$), and hydrogen peroxide (H_2O_2) in leaf and root compared to control. Additionally, increased magnitudes of proline, glycine betaine, soluble sugar and soluble protein contents were more pronounced under 12 dSm^{-1} NaCl than those under 6 dSm^{-1} NaCl. In contrast, biochar applied to soil enhanced the shoot and root dry weight in comparison with the non-biochar treatment. Furthermore, all of the antioxidant activities of seedlings in soil treated with biochar, particularly at 20% biochar, declined. With the addition of biochar, the contents of MDA, $O^{2\bullet-}$ and H_2O_2 displayed remarkable decrease, and the osmotic substances accumulation in leaves and roots also reduced. The presented results supported the view that biochar can contribute to protect common bean seedlings against NaCl stress by alleviating the oxidative stress.

1. Introduction

Salt stress takes place in the presence of excessive accumulation of soluble salts in the soil. It suppresses plant growth and productivity due to its negative effects on ion homeostasis and osmotic balance (Parida and Das, 2005). In addition to ionic and osmotic components, salt stress, like other abiotic stresses, leads to oxidative stress through an increase in reactive oxygen species (ROS) (Jaleel et al., 2007). Accumulation of ROS is a major cause of loss of crop productivity worldwide. ROS affect many cellular functions by damaging nucleic acids, oxidizing proteins, and causing lipid peroxidation (Foyer and Noctor, 2005). Plants possess efficient systems for scavenging ROS that protect them from destructive oxidative reactions (Foyer et al., 1994). As part of this system, the most important key elements in the defense mechanisms are antioxidative enzymes. Stress-induced ROS accumulation is neutralized by enzymatic antioxidant systems such as superoxide dismutase (SOD), ascorbate peroxidise (APX), peroxidase (POX), polyphenol oxidase (PPO) and catalase (CAT) (Mittler et al., 2004). SOD that metabolizes oxygen radicals ($O^{2\bullet-}$) to hydrogen peroxide (H_2O_2), thus protecting cells from damage. CAT, APX, and a variety of POX catalyze the subsequent breakdown of H_2O_2 to water and oxygen (Garratt et al., 2002). High levels of antioxidants in plants have been reported to lead to greater resistance to this oxidative damage (Spychalla and Desborough, 1990).

The ability of plant cells to adjust osmotically and to accumulate organic solutes is another main factor of salt tolerance mechanisms. The accumulation of key osmolytes such as free proline, endogenous glycine betaine, soluble sugar and protein in plant tissue can provide an symptom of the degree of tolerance to stress induced by osmoregulation (Hare et al., 1998). Proline is one of the major non-enzymatic antioxidants that microbes, animals, and plants need to counteract the inhibitory effects of ROS (Chen and Dickman, 2005). The accumulation of soluble sugar in plants under salinity has been reported by Murakeozy et al. (2003). These osmotic solutes make osmotic balance, control water influx (reduce efflux) and enable turgor maintenance. It is well known that plants as a response to saline condition maintain their

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http://dx.doi.org/10.1016/j.ecoenv.2016.11.029
Received 3 September 2016; Received in revised form 28 November 2016; Accepted 29 November 2016
Available online 19 December 2016

turgor by osmotic adjustment (Chaparzadeh et al., 2003).

Currently, biochar as a product of thermal degradation (pyrolysis, more than 250 °C) of organic materials in the absence of oxygen is defined, and it is privileged from traditional charcoal by the use as a soil amendment (Lehmann et al., 2011). Due to the specific conditions and methodology of biochar production about 50% of the initial carbon can be sequestered as compared to open-air burning and biological decomposition (Joseph et al., 2007). Irrespective of environmental benefits, biochars substantially influence soil physicochemical properties and fertility status (Verheijen et al., 2014). In addition, studies have also shown that biochar can enhance plant growth either by its direct or indirect mechanism of actions. The direct growth promotion under biochar amendment related to supply mineral nutrients, i.e. Ca, Mg, P, K and S etc., to the plant, whereas, indirect mechanism involves improving soil physical, chemical and biological characteristics (Cheng et al., 2012; Enders et al., 2012). Despite numerous observations in both field and controlled experiments showing that plant yield increases due to biochar application (Graber et al., 2010; Van Zwieten et al., 2010; Jeffery et al., 2011), the exact mechanistic background of biochar effects is not known. Lashari et al. (2015) used a biochar manure compost, i.e. a mixture of pyrolysed carbon and fresh organic matter, which mitigated salt stress, but the mechanisms behind this remained uncertain. Thomas et al. (2013) added salt over a top dressing of biochar to forb pot cultures and concluded that biochar ameliorated salt stress through salt absorption showed strongly improved conductivity following salt addition. Akhtar et al. (2015) reported that incorporation of biochar into salt-affected soil could diminish salinity stress in potatoes largely due to its high salt adsorption potential. Up to date, there has been no study concerning the biochar effect on the antioxidant activities and osmolytes of *Phaseolus vulgaris* L. seedlings under salt stress. Therefore, the study was conducted to assess the effects of biochar derived from maple residues on antioxidant enzyme activities (CAT, APX, POD, PPO and SOD), MDA, H_2O_2, and $O_2^{\bullet -}$contents, proline, glycine betaine, soluble sugar and soluble protein contents of common bean under salinity stress.

2. Materials and methods

2.1. Preparation of biochar

The procedure of preparing the biochar is in accordance with an adapted method (Qian and Chen, 2013). Biochar used in this experiment was produced with maple residues (*Acer pseudoplatanus* L.) at 560 °C for 6 h under anaerobic condition. The heating rate was controlled at 5 °C min^{-1}. The carbon (C), hydrogen (H), nitrogen (N) and oxygen (O) contents of biochar were measured by using an elemental analyser (Elementar, Germany). The basic biochar properties are shown in Table 1.

Table 1
Some physical and chemical characteristics of the experimental soil and biochar.

Soil		Biochar	
Texture	Silty loam	N (%)	0.75
pH	8.1	C (%)	32.96
EC (dSm^{-1})	1.23	H (%)	1.7
Organic carbon (g kg^{-1})	14.1	O (%)	28.43
Total N (%)	0.05	Na (mg kg^{-1})	8.3
P (mg kg^{-1})	33	K (mg kg^{-1})	3210
K (mg kg^{-1})	165	Ca (mg kg^{-1})	3470
Cation exchange capacity (cmol kg^{-1})	17.4	Mg (mg kg^{-1})	960
		Cation exchange capacity (cmol kg^{-1})	20.8
		pH	7.8

2.2. Experimental conditions

This experiment was conducted in a greenhouse at the Faculty of Agriculture, University of Tabriz, Iran, in 2016 with a factorial arrangement on the basis of randomized complete block design with four replications. Common bean plants (*Phaseolus vulgaris* L. cv. Derakhshan) were tested under three levels of salinity (non-saline, 6 and 12 dSm^{-1} of NaCl) and three biochar treatments (non-biochar, 10% and 20% total pot mass). Salinity levels were chosen according to the range of the salinity tolerance of bean plants (Grieve et al., 2012) and biochar values were selected for testing physiological responses of bean seedlings under different levels of this organic matter. Five seeds of bean were sown in each plastic pot (7 cm radius and 20 cm height) containing 2.5 kg soil. Before filling into the pots, soil was mixed well with biochar and sieved by passing through 2 mm mesh. Some physical and chemical characteristics of the experimental soil are presented in Table 1. The plants were kept under controlled conditions of greenhouse with 25/20 °C day/night temperature, and 65–70% relative humidity. Plants were irrigated daily with tab water during the period of emergence and seedling establishment to keep the soil water content near field capacity. After emergence of the first trifoliate leaf, salt was added to irrigation water supplied to saline treatments.

2.3. Plant performance

Four weeks after planting, shoot and root dry weights measured. The dry weights of all seedling samples were determined after oven drying at 70 °C for 72 h.

2.4. Measurement of proline, glycine betaine, soluble sugar and soluble protein contents

0.5*g* of plant sample was homogenized in 5 mL of sulphosalycylic acid (3%) and afterwards 2 mL of the extract was taken into a plastic tube and 2 mL of glacial acetic acid and 2 mL of ninhydrin were added to this tube. The resulting mixture was heated at 100 °C for 1 h in water bath. The mixture was extracted with toluene, and the absorbance of fraction with toluene aspired from upper liquid phase was read at 520 nm, using a spectrophotometer (Model Camspec M330 UV/Vis). Proline content was determined by the calibration curve and expressed as mg g^{-1} dry weight (DW) (Bates et al., 1973). Glycine betaine was estimated according to Grieve and Grattan (1983) method and presented as mg g^{-1} fresh weight (FW). The Plant sample extract was prepared in 20 mL test tubes by chopping 0.5*g* leaves in 5 mL of toluene-water mixture (0.05% toluene). All the tubes were shaken for 24 h at 25 °C. After filtration 0.5 mL of extract was mixed with 1 mL of 2 N HCl solution then and 0.1 mL of potassium tri-iodide solution (having 7.5*g* iodine and 10*g* potassium iodide in 100 mL of 1 N HCl) was added and shaken in an ice cold water bath for 90 min and then 2 mL of ice-cooled water was added after gentle shaking 10 mL of 1,2 dichloroethane (Chilled at −10 °C) was pour in it. Upper aqueous layer was discarded and optical density of organic layer was recorded at 365 nm. The content of soluble sugar was estimated according to phenol sulphuric acid method (Kochert, 1978). Glucose was used as a standard. Soluble sugar content was determined as mg g^{-1} FW, using a calibration curve. The soluble protein contents were defined by using the method of Bradford (1976). Plant samples (1*g*) were homogenized with 4 mL Na- Phosphate buffer (pH 7.2) and then centrifuged at 4 °C. Supernatants and dye were pipetting in spectrophotometer cuvettes and absorbances were measured using a Uv–vis spectrophotometer at 595 nm.

2.5. Measurement of antioxidant enzymes

One unit (U) of superoxide dismutase (SOD) activity was defined as the amount of enzyme affecting 50% of the maximum inhibition of