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Short communication

Gypsum alleviated hydroxyl radical-mediated oxidative damages caused by alkaline bauxite residue in leaves of *Atriplex canescens*

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ABSTRACT

The present study investigated pH, electrical conductivity (EC), Al level, and exchangeable sodium percentage (ESP) in a range of alkaline bauxite residue (ABR) treatments. Also investigated was the content change of reactive oxygen species (ROS), malondialdehyde (MDA), and protein carbonyl (PCO) in leaves of *Atriplex canescens* [L.] challenged by ABR in the presence/absence of gypsum (Gy). Local cinnamon soil (CS) was used to improve the nutrient status and physical condition of ABR. Analysis revealed that CS/ABR (2:1, w/w) induced much more hydroxyl radical ($OH^{\bullet}$) accumulation, lipid peroxidation (LPO), and protein oxidation (PO) than CS/ABR/Gy (6:3:1, w/w) did. While superoxide radical ($O_2^{\bullet}-$) and hydrogen peroxide (H_2O_2) accumulation showed little difference between CS/ABR and CS/ABR/Gy treatments. Further, exogenous $OH^{\bullet}$ aggravated LPO and PO in a way similar to CS/ABR treatment, which was impaired by $OH^{\bullet}$ scavengers such as proline and mannitol. Taken together, the data indicate that $OH^{\bullet}$ is more connected with severe oxidative damages than $O_2^{\bullet}-$ and H_2O_2 in this case. The caustic properties of ABR can be ameliorated by gypsum to support vegetation.

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1. Introduction

Alkaline bauxite residue (ABR), a fine-textured, alkaline residue, is the major waste product of bauxite digestion with caustic soda to remove alumina (Xenidis et al., 2005). The primary constraints to vegetation establishment on ABR include high alkalinity, sodicity and salinity coupled with a lack of organic matter and nutrients (Banning et al., 2014; Goloran et al., 2014). Gypsum (Gy) is a commonly used amendment for sodic soil reclamation and can be used as an ameliorant for alkaline bauxite residue remediation (Gräfe and Klauber, 2011; Gräfe et al., 2009). Previous studies suggest that gypsum amendment followed by leaching can provide suitable conditions for alkaline bauxite residue revegetation due to the reduced pH, electrical conductivity, and Na and Al content (Wong and Ho, 1993; Courtney and Kirwan, 2012; Woodard et al., 2008; Xenidis et al., 2005). In addition, gypsum could increase Mn and K concentration in herbage for revegetation of ABR (Courtney and Timpson, 2005). Therefore, as a mineral or industrial by-product, gypsum may serve as a low-cost ameliorant for alumina alkaline bauxite residue reclamation.

Halophytes are plants that are adapted to live in soils containing high concentrations of salt. Factors in association with halophyte viability under salt stress include ion transportation and compartmentation (Shabala and Mackay, 2011; Shabala and Pottosin, 2014; Yuan et al., 2015), antioxidant system (Miller et al., 2010), aldehyde dehydrogenases (Hou and Bartels, 2014), salt gland (Reef and Lovelock, 2015), etc. To survive under salt stress, some halophytes are endowed with great capability to actively excrete Na^+ (Shabala et al., 2014), to sequester it in vacuoles (Apse and Blumwald, 2007) or to pump it into salt bladder (Shabala et al., 2014). In manipulating water transport to reduce the net uptake of salts to the shoots, halophytes operate with reduced stomatal opening and with consequences of generating more reactive oxygen species (ROS) (Flowers and Colmer, 2015) comprising singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\bullet}-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), etc. When the level of ROS goes beyond the control of defense system, a cell is in a state of "oxidative stress". The enhanced production of ROS can cause oxidative damage to proteins, DNA, and lipids (Miller et al., 2010). Plants have evolved complex antioxidant defense mechanisms that consist of enzymatic and non-enzymatic pathways to eliminate excessive ROS accumulation while maintaining an

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optimum level of ROS for signaling (Bose et al., 2014). ROS-scavenging enzymes include superoxide dismutase, catalase, ascorbate peroxidase, monodehydroascorbate reductase, glutathione reductase, glutathione peroxidase, glutathione S-transferases, peroxiredoxin, etc. (Miller et al., 2010). Non-enzymatic antioxidants include ascorbate, glutathione, glycine-betaine, proline, polyamines, tocopherols, polyols, etc. (Bose et al., 2014). $O_2^{\bullet}-$ and H_2O_2 can be eliminated by enzymatic and non-enzymatic antioxidants, while $^1O_2$ and $OH\cdot$ can be scavenged by non-enzymatic antioxidants only (Sharma et al., 2012).

Atriplex species are effective ion absorbers in saline-alkali environment (Glenn et al., 2012; Benzarti et al., 2013). It is well known that *Atriplex* species actively accumulates soluble salts in leaves in association with a tolerance mechanism (Salem et al., 2005). For this reason, they are also considered as excellent species for reducing soil salinity in drylands, if cut and collected. In the present study, the aim is to investigate the different roles of $O_2^{\bullet}-$, H_2O_2, and $OH^{\bullet}$ in oxidative damages caused by ABR in the presence/absence of gypsum, which helps to elucidate how gypsum amendment changes the oxidative response of *Atriplex canescens* [L.] in ABR.

2. Materials and methods

2.1. Sample characterization

Alkaline bauxite residue samples were collected from the red mud storage facilities of Zhongzhou Branch China Aluminium Limited. The samples provided were dewatered and had a moisture content of 5% (w/w). Alkaline bauxite residue (ABR), single mixture (1:2, w/w ABR and cinnamon soil), and composite mixture (1:3:6, w/w gypsum, ABR, and cinnamon soil) were prepared and tested. The samples were thoroughly blended manually and hydrated with deionized water to get pastes with a water-to-solids ratio (WSR) equal to 2.5 L/kg. The pastes were left to equilibrate overnight and vacuum-filtered (SHB-III, Voshin, Wuxi, China) with filter paper (Whatman No. 42). Ca, Na, Mg, and Al concentration as well as the pH and electrical conductivity (EC) of the filtrates were determined. The pH and EC in filtrates were determined by a calibrated pH meter (PHS-3C, Sanxin, Shanghai, China) and a EC meter (ECTestr, LI-COR, Lincoln, Nebraska, USA), respectively. Chemical analyses of the filtrates were performed by atomic absorption spectrometry (AA6000, Techcomp, Shanghai, China). Exchangeable sodium percentage (ESP) was determined using the method of Gräfe and Klauber (2011).

2.2. Plant materials and treatments

Atriplex canescens [L.] was used in the present study. Plant studies were established in jars with dimensions of 0.54 m wide, 0.51 m deep for the analysis of *A. canescens* under ABR stress. Jars were placed in a greenhouse and filled with cinnamon soil, single mixture (1:9, w/w gypsum and cinnamon soil), single mixture (1:2, w/w ABR and cinnamon soil), or composite mixture (1:3:6, w/w gypsum, ABR, and cinnamon soil). Hoagland nutrient solution (Hoagland and Arnon, 1950) was uniformly surface-applied to the material in all jars to supplement the average precipitation of 220 mm that the region normally received monthly. The jars were watered for 1 month before 60 *A. canescens* species (168-D-old, 42–55 cm) were transplanted in. After transplantation, the leaves were sampled at different time points for further analysis. To study the effects of exogenous $OH^{\bullet}$, *A. canescens* leaves were foliar-applied with a freshly prepared mixture of 1 mM $CuCl_2$ and 1 mM L-ascorbic acid to generate $OH^{\bullet}$ (Gutteridge and Halliwell, 1999). To study the effects of mannitol (Ma) or proline (Pro), 30 mM mannitol or 20 mM proline were applied as a foliar spray at leaves of *A. canescens*. To ensure penetration of chemicals into leaf tissues tween-20 (0.01% v/v) was used as a surfactant for the foliar spray.

2.3. Determination of reactive oxygen species

Superoxide radical ($O_2^{\bullet}-$) production was measured as described by Able et al. (1998) by monitoring the reduction of Na,3′-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-bis(4-methoxy-6-nitro) benzenesulphonic acid hydrate (XTT) in the presence of $O_2^{\bullet}-$, with some modifications.

The content of hydrogen peroxide (H_2O_2) was measured by monitoring the A_{415} of the titanium-peroxide complex following the method described by Brennan and Frenkel (1977). Absorbance values were calibrated to a standard graph generated with known concentrations of H_2O_2.

The generation of $^{\bullet}OH$ was measured according to Halliwell et al. (1987). The absorbance was measured at 532 nm and $^{\bullet}OH$ content was expressed as nmol g^{-1} dw. Corrections were made for endogenous MDA.

2.4. Determination of lipid peroxidation and electrolyte leakage

Oxidative damage to lipids was estimated by measuring the content of malondialdehyde (MDA) in leaf segment homogenates as in Hodges et al. (1999).

Oxidative damage to proteins was estimated as the content of protein carbonyl (PCO) groups by reaction with 2,4-dinitrophenylhydrazine (Levine et al., 1990).

2.5. Statistical analysis

All values reported in this study are means of three replicates. *ANOVA* was performed by using a statistical package SPSS v. 16.0 (SPSS, Chicago, IL, USA). Duncan's multiple range test was done to determine the significant difference ($P \leq 0.05$) between means.

3. Results

3.1. Characterization of alkaline bauxite residue and additives

pH and electrical conductivity (EC) measured in the alkaline bauxite residue (ABR) paste extract were found to be 12.4 and 25.12 dS m^{-1}. Aluminum concentration in the ABR paste extract was 232.8 mg dm^{-3}. Exchangeable sodium percentage (ESP) in the ABR paste extract was 46.6% (Fig. 1). ABR was added with local cinnamon soil (CS) in the ratio of 1:2, w/w, which reduced the pH of mixture extract from 12.3 to 9.8. Then this single mixture was added with gypsum (Gy) in the ratio of 9:1, w/w. The addition of gypsum reduced the pH of the composite mixture paste extract to 8.3 (Fig. 1A). The EC of gypsum-amended composite mixture extract was 14.52 dS m^{-1} which was slightly increased compared with single mixture estimated (Fig. 1B). Aluminum concentration in the paste extract of the CS/ABR/Gy mixture was reduced from 78.2 mg dm^{-3} to 0.7 mg dm^{-3} compared with CS/ABR mixture. ESP of the extract of the CS/ABR/Gy mixture was reduced from 35.6% to 11.8% compared with CS/ABR mixture. (Fig. 1C–D).

3.2. Effects of ABR and additives on reactive oxygen species

After transplantation, the leaves of *A. canescens* were sampled at different time points for ROS analysis. The level of superoxide radical ($O_2^{\bullet}-$) was found to be significantly high in CS/ABR (2:1, w/w) treated *A. canescens* and reached maximum on the 3rd day after treatment, thereafter decreased more than 25% in two days. CS/ABR/Gy (6:3:1, w/w) treated *A. canescens* showed similar and marginally weaker peak response in $O_2^{\bullet}-$ production until the end

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