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Effect of aluminum on protein oxidation, non-protein thiols and protease activity in seedlings of rice cultivars differing in aluminum tolerance

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SUMMARY

The effect of toxic concentrations of aluminum (Al) was investigated on contents of protein-thiols, non-protein and total thiols, protein carbonylation and protease activity in the seedlings of Al-sensitive (Al-S) Indica rice cv. HUR-105 and Al-tolerant (Al-T) cv. Vandana grown in sand cultures. Al treatment of 178 μM and 421 μM for 3–12 days caused a significant decline in the level of protein thiols, rise in non-protein thiols (NPTs) as well as protein carbonyl content and an insignificant alteration in the level of total thiols in cv. HUR-105 seedlings. However, in the seedlings of Al-T cv. Vandana, no significant alteration could be observed on any of these parameters with Al treatment. Al treatment inhibited protease activity in roots, whereas the opposite trend was seen in shoots. New isozymes of protease appeared in shoots of cv. Vandana with increased level of Al treatment. Our results show a link between protein thiols and NPTs and suggest the role of NPTs in the repair and protection of protein thiols. Inhibitory effect of Al on protease activity in roots could be a major reason for Al rhizotoxic effects. Al tolerance in rice appears to be associated with lesser content of protein thiols in roots, smaller amount of carbonylated proteins in roots as well as shoots and higher protease activity in shoots.

Introduction

Aluminum (Al) is the most abundant metal in the earth's crust and represents a major constraint to crop production in acid soils (Matsumoto and Motoda, 2012). All over the world, up to 70% of cultivable land is acidic (von Uexkull and Mutert, 1995) and the problem of soil acidification further aggravates due to high usage of ammonium fertilizers, mining activities and industrial development. In addition, a study conducted by the FAO concludes that the world's population will increase by more than 2 billion by 2050 (Food and Agriculture Organisation of the United Nations, 2009). Therefore, we will be in need to extend our farming land to meet the increased demand of food for the increasing population. Rice is a major food crop for the majority of world population. Hence, studies related to understanding the molecular mechanisms associated with Al toxicity and tolerance in rice will be of great relevance for growing rice crop in acid soils.

Enhanced production of reactive oxygen species (ROS) leading to oxidative stress in the cells is a common consequence when plants are exposed to many abiotic stresses including Al toxicity (Sharma and Dubey, 2007; Pandey et al., 2013). Though Al is not a transition metal, it has pro-oxidant property. Inside the cells, Al forms the semi-reduced radical ion of aluminum superoxide, which is a more potent oxidant than the superoxide anion itself and also promotes the formation of other ROS, such as hydrogen peroxide (H_2O_2) and hydroxyl radicals ($HO^{\bullet-}$), that contribute to an oxidizing environment in plant cells (Matsumoto and Motoda, 2012). Previous studies conducted in our laboratory and many others indicate that Al causes oxidative stress in the tissues by increasing production of ROS and that oxidative stress is a major component in expression of Al toxicity in plants (Sharma and Dubey, 2007; Achary et al., 2012; Matsumoto and Motoda, 2012; Pandey et al., 2013).

Proteins are one of the major cellular constituents, targeted by ROS, owing to their great abundance and high rate constants for a range of reactive radicals (Davies, 2005). As proteins are present ubiquitously in the cell and play diverse roles ranging from structural, catalytic and regulatory functions, the oxidation of proteins may affect almost all the important functions of the cell. Furthermore, protein oxidation processes are predominantly irreversible except only a few, involving sulfur containing amino acids (Møller et al., 2007). Therefore, excessive protein oxidation is a deleterious event in the cells and is often used as a crucial indicator of oxidative stress. Among different types of protein

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Abbreviations: Al-S, aluminum-sensitive; Al-T, aluminum-tolerant; NPT, non-protein thiol; ROS, reactive oxygen species.

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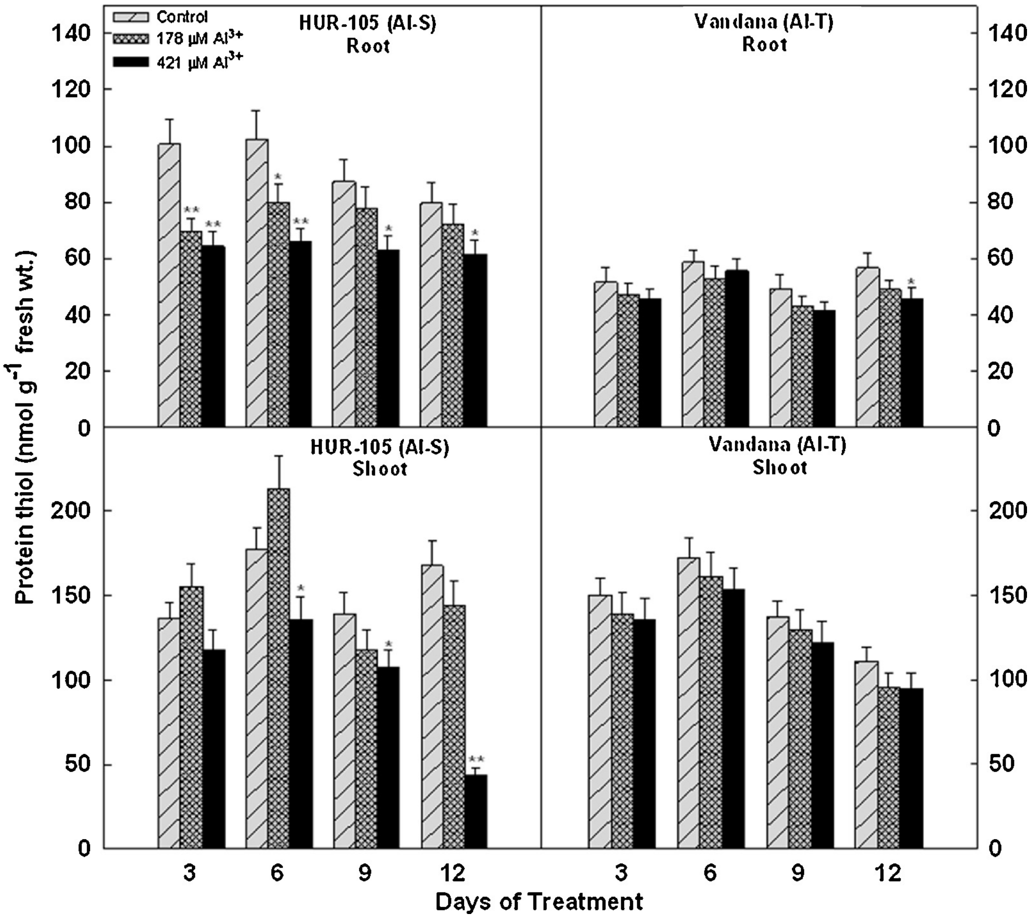

Fig. 1. Effect of increasing concentrations of Al^{3+} (control, 178 μM Al^{3+} and 421 μM Al^{3+}) in sand cultures on protein thiol content of roots and shoots of the seedlings of Al-sensitive rice *cv.* HUR-105 and Al-tolerant *cv.* Vandana at 3–12 days of Al treatment. Values are mean ± SD based on three independent determinations and bars indicate standard deviations. * and ** represent significant differences compared to controls at $p \leq 0.05$ and $p \leq 0.01$, respectively, according to Tukey's multiple range test.

modifications, oxidative modifications arising from environmentally induced oxidative stress such as oxidation of thiol residues and formation of carbonyl derivatives are of great interest from an ecotoxicological assessment viewpoint (Braconi et al., 2011).

In a peptide chain, sulfur containing amino acids cysteine and methionine and more specifically thiol groups are the most susceptible sites for ROS attack due to their high redox activities (Davies, 2005; Sharma et al., 2012). These amino acids can be oxidized to several products such as disulfides, sulfenate, sulfinate, sulfonate or sulfenamide; mixed disulfides and glutathionylated residues (glutathionylation). Loss of protein —SH groups can be induced by ROS and is regarded as one of the most instant responses occurring due to oxidative stress. As a consequence of —SH loss, protein misfolding, catalytic inactivation, decreased antioxidative capacity and loss of certain specific function such as binding to a metal ion may occur (Davies, 2005). Oxidation of cysteine to cysteic acid and formation of sulfinate or sulfonate forms are irreversible; however, the oxidation of cysteine thiol group to disulfide is a reversible modification where the reduced form can be regenerated by the thioredoxin or glutaredoxin systems. Glutaredoxin reduces disulfide bonds using glutathione (GSH) as an electron donor which is a key non-protein thiol (NPT) in the cell (Møller et al., 2007; Pena et al., 2012). Other than GSH (γ-Glu-Cys-Gly; γ-glutamylcysteinyl glycine), cells also contain several other NPTs such as cysteine, γ-glutamyl cysteine, hydroxyl-methyl GSH (hm-GSH, γ-Glu-Cys-Serine), phytochelatins [PCs, γ-(Glu-Cys)n-Gly] and hydroxyl-methyl phytochelatins (hm-PCs), which play crucial roles in metal detoxification mainly by chelating metal ions (Maheshwari and Dubey, 2009).

Another important oxidative modification of proteins is the formation of carbonyl (reactive aldehydes and ketones) derivatives. ROS can directly produce free carbonyl groups by reacting with amino acid side chains of protein molecules, particularly lysine, arginine, proline, histidine, tryptophan and threonine residues. Protein carbonyl derivatives can also be formed indirectly on cysteine, lysine and histidine residues by formation of adducts with reactive carbonyl compounds on carbohydrates (glycoxidation products), lipids and advanced glycation/lipoxidation end products (Pena et al., 2012). Protein carbonylation is an irreversible process and serves as a good indicator of oxidative stress as the formation of carbonyl groups requires more stringent oxidation conditions than for the reversible oxidation of thiols or lipid peroxidation (Davies, 2005; Møller et al., 2007; Juszczuk et al., 2008).

Due to irreversibility of most of the oxidative modifications of proteins, the ultimate fate of these oxidized proteins is their degradation/proteolysis. Proteolysis of oxidized proteins is also necessary to protect cells from further damage as these are potentially toxic to the cells and due to the presence of reactive groups, they can cause further damage to other molecules such as DNA and other proteins (Davies, 2005). To perform proteolysis, several types of proteases are present in different cellular compartments, i.e., cytosol, mitochondria and chloroplasts. Depending on the site of hydrolytic cleavage, proteolytic enzymes have been classified into two major classes, namely exopeptidases and endopeptidases, where endopeptidases are simply known as proteases. Proteases are responsible for cellular housekeeping of proteins, removing the abnormal or misfolded proteins, supplying free amino acids