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学 位 論 文 要 旨

氏名: Lina Yin

題目: **Improvement of aluminum tolerance through scavenging reactive oxygen species and lipid peroxide-derived aldehydes**

活性酸素と過酸化脂質由来アルデヒド消去によるアルミニウム耐性の改善

Aluminum (Al) toxicity is a major factor limiting plant growth and productivity in acid soils. Al ions inhibit plant growth partly by causing oxidative damage that is promoted by reactive oxygen species (ROS) and can be prevented by improving antioxidant capacity. Ascorbic acid (AsA) and glutathione (GSH) are the major antioxidants in plants, which are regenerated by the action of monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR). However, the functions of DHAR, MDAR and GR in Al tolerance have not been characterized. In the present study, I investigated the role of MDAR, DHAR and GR in AsA and GSH regeneration during Al stress using transgenic tobacco (*Nicotiana tabacum*) plants overexpressing AtMDAR (MDAR-OX) or AtDHAR (DHAR-OX), and transgenic Arabidopsis (*Arabidopsis thaliana*) overexpressing AtGR (GR-OE), and the wild-type tobacco SR-1 and Arabidopsis Columbia (Col) plants were used as control plants.

DHAR-OX plants showed rapid root growth than wild-type (SR-1) plants after exposure to $AlCl_3$ for 14 d on agar plate, but MDAR-OX plants did not. There was no difference in Al distribution and accumulation in the root tips among SR-1, DHAR-OX and MDAR-OX plants after treatment with 500 μM $AlCl_3$ for 24 h in hydroponic medium. However, DHAR-OX plants showed lower hydrogen peroxide content, less lipid peroxidation and lower level of oxidative DNA damage than wild-type SR-1 plants, whereas MDAR-OX plants showed the same extent of damage as SR-1 plants. Compared with SR-1 plants, DHAR-OX plants consistently maintained a higher AsA level both with and without Al exposure, while MDAR-OX plants maintained a higher AsA level only without Al exposure. Also, DHAR-OX plants maintained higher ascorbate peroxidase (APX) activity under Al stress. The higher AsA level and APX activity in DHAR-OX plants contributed to their higher antioxidant capacity and higher tolerance to Al stress. These findings show that the overexpression of DHAR, but not of MDAR, confers Al tolerance, and that maintenance of a high AsA level is important to Al tolerance.

Arabidopsis plants overexpression GR also showed Al tolerance as compared with wild-type Col plants. Under Al stress, GR transgenic plants exhibited better root elongation, lower hydrogen peroxide content and less lipid peroxidation compared to wild-type plants. Although no difference in Al accumulation and the activities of superoxide disumutase (SOD), catalase (CAT) and dehydroascorbate reductase (DHAR) were observed in roots of transgenic and wild-type plants after 24-h Al treatment, GR transgenic plants showed higher activities of GR and ascorbate peroxidase (APX), and higher

levels of GSH and AsA than wild-type plants. Thus, overexpression of GR did not affect Al accumulation and the activities of other antioxidant enzymes. This results demonstrate that overexpression of GR improves the antioxidant capacity of Arabidopsis through increasing GSH and AsA level in the cell, leads to suppression of H_2O_2 generation and lipid peroxidation, and results in enhanced tolerance to Al stress.

Lipid peroxidation, in the downstream of ROS, is a common symptom of Al toxicity, and it increases with increasing Al concentration. From animal cell studies, it is now recognized that the toxicity of lipid peroxide (LOOH) is largely ascribed to LOOH-derived aldehydes. In plants, a close correlation between the level of LOOH-derived aldehydes (determined as thiobarbituric acid-reactive substances (TBARS)) and cellular damage has been shown in environmental stresses caused by heat, chilling, UV-B radiation, salinity, heavy metals and Al. Thus, it is possible that LOOH-derived aldehydes are involved in Al toxicity. In this study, I verified the hypothesis that LOOH-derived aldehydes, especially highly electrophilic *α,β*–unsaturated aldehydes (2-alkenals), participate in Al toxicity. Transgenic tobaccos overexpressiong *Arabidopsis thaliana* 2-alkenal reductase (AER-OE plants), wild-type SR-1, and an empty vector-transformed control line (SR-Vec) were exposed to Al on their roots. Compared with the two control plants, AER-OE plants suffered less retardation of root elongation under Al treatment and showed rapid regrowth upon Al removal. Under Al treatment, the roots of AER-OE plants accumulated Al and hydrogen peroxide (H_2O_2) to the same levels as did the sensitive controls, while they accumulated lower level of aldehydes and suffered less cell death than SR1 and SR-Vec roots. In SR1 roots, Al treatment markedly increased the contents of the highly reactive 2-alkenals acrolein, 4-hydroxy-(*E*)-2-hexenal, and 4-hydroxy-(*E*)-2-nonenal and other aldehydes such as malondialdehyde and formaldehyde. In AER-OE roots, accumulation of these aldehydes was significantly less. Growth of the roots exposed to 4-hydroxy-(*E*)-2-hexenal and (E)-2-hexenal were retarded more in SR1 than in AER-OE plants. Thus, the lipid peroxide-derived aldehydes, formed in the downstream of ROS, injured root cells directly. Their suppression by AER provides a new defense mechanism against Al toxicity.

This study indicates that Al toxicity induced irreversible oxidative damage in tobacco and Arabidopsis. Plants with overexpressed antioxidant enzyme genes DHAR and GR showed enhanced Al tolerance in tobacco and Arabidopsis. However, MDAR showed no protective effect on improving Al tolerance in tobacco. Both DHAR-OX and GR-OE plants showed increased AsA level and APX activity in their roots as compared with wild-type plants, indicating AsA and APX play a paramount role in Al tolerance. Furthermore, tobacco plants with overexpressed AER gene showed improving tolerance to Al. AER-OE plants accumulated less LOOH-derived aldehydes, especially 2-alkenals, than that in wild-type plants, indicating the LOOH-derived aldehydes are the cause of Al-induced injury, and enhanced aldehydes scavenging capacity could alleviate Al toxicity. Taken together, oxidative injuries caused both by reactive oxygen species and LOOH-derived aldehydes, are the important causes of Al toxicity. Our study provide a new mechanism for understanding Al toxicity in plants, meanwhile, new strategies for breeding Al tolerant plants are suggested. This will benefit improving plant productivity on acid soils in the world.