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SUMMARY OF DOCTORAL THESIS

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Title: Improving plant tolerance to environmental stresses by overexpression and identification of stress tolerance genes

ストレス耐性遺伝子の過剰発現と同定による植物の環境ストレス耐性の改良

Ascorbate (vitamin C) is a potent antioxidant protecting plants against oxidative damage imposed by environmental stresses such as ozone, salt or drought. Dehydroascorbate reductase (DHAR; EC 1.8.5.1) and Monodehydroascorbate reductase (MDAR; EC 1.6.5.4) are crucial for ascorbate regeneration and essential for maintaining a reduced pool of ascorbate (AsA). To examine whether an overexpressed levels of DHAR and MDAR could minimize the deleterious effects of environmental stresses, we developed transgenic tobacco plants overexpressing either cytosolic DHAR or MDAR gene from *Arabidopsis thaliana.* Incorporation of transgenes in the genome of transgenic tobacco plants was confirmed by PCR and Southern blot analysis and their expression was confirmed by Northern and Western blot analyses. DHAR transgenic plants exhibited 2.3 to 3.1 folds higher DHAR activity and 1.9 to 2.1 folds higher level of reduced AsA compared to non-transformed control plants. These transgenic plants showed maintained redox status of AsA and exhibited an enhanced tolerance to ozone, drought, salt, and polyethylene glycol stresses in term of significantly higher net photosynthesis. MDAR transgenic plants exhibited up to 2.1 folds higher MDAR activity and 2.2 folds higher level of reduced AsA compared to non-transformed control plants. These transgenic plants showed enhanced stress tolerance in term of significantly higher net photosynthesis rates under ozone, salt and PEG stresses and greater PSII effective quantum yield under ozone and salt stresses. Furthermore, these transgenic plants exhibited significantly lower hydrogen peroxide level when tested under salt stress. These results demonstrate that elevation of AsA level by targeting either DHAR or MDAR overexpression in cytosol properly provides a significantly enhanced tolerance against ozone, drought and salt stresses.

To further investigate plants mechanisms to tolerate environmental stresses, we used Suppression Subtraction Hybridization (SSH) approach to identify stress tolerance genes induced by silicon element under salt or drought stress from cucumber (*Cucumis sativus* L.). Silicon is well recognized as a beneficial element for plant tolerance to biotic and abiotic stresses. mRNA isolated from leaves tissues of cucumber plants grown with or without added silicon under drought or salt stress was used to construct SSH libraries. Differential screening using forward and reverse subtracted probes was performed to further confirm the differential expression of the obtained fragments. A cucumber aquaporin and calcium binding protein were among twenty clones shown to be differentially expressed due to addition of silicon under drought or salt stress. These results suggest that the beneficial effect of silicon on enhancing plant stress tolerance might be due to the involvement of silicon in inducing these genes under stressing environments.