Elucidating mechanisms for salt accumulation and salt tolerance in the facultative CAM halophyte, *Mesembryanthemum crystallinum* using a mutant lacking the salt storage organ, epidermal bladder cells

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Summary

A facultative CAM halophyte, the common ice plant (*Mesembryanthemum crystallinum*), have adapted to extremely high salinity condition. Following salt stress, ice plant develops epidermal bladder cells (EBC) on all aerial parts and accumulates salt into the cells. The EBC has been thought to be a salt excreting system. However, the contribution of the bladder cells to salt regulation in growth of ice plant is less understood. We have induced and isolated the mutant lacking the EBC, and examined the roles of the EBC for salt tolerance and salt integration of the ice plant through anatomical observations, measurements of biomass and salt content under the hypersaline environment. In addition, we have tried to isolate the genes responsible for development of the EBC using representational difference analysis (RDA).

Observation of the structure of the lamina surface using stereoscopic microscope and scanning electron microscope showed that the mutant has only little EBC on the surfaces of leaves and stems. Whereas the wild type has the elliptical (sausage-like) and circular type EBC on the surface of leaves and stems, respectively. Another anatomical features were almost identical between the wild type and the mutant, suggesting that mutation was occurred only in EBC formation. Observation of lamina cross sections by the transmission electron microscope showed that the size of one EBC corresponded to about 4 to 8 epidermal cells. Following salt stress with 400 mM NaCl, the growth was inhibited in larger extent in the mutant than in wild type; the fresh weight of mutant at 7, 14, and 21 days after the salt stress were 66%, 58%, and 55% of that in wild type, respectively. The NaCl content per plant in the wild type at 21 days after salt treatment (400mM NaCl) was about 2g, corresponding to about 1.5 times of that in the mutant. The NaCl content in EBC occupied the almost half of the whole leaf NaCl content, and the content of wild type leaves was about 1.5 times of that in the mutant leaves. Using the RDA, we have obtained 11 PCR products as mutation region, which exist in the mutant. The homology search by BLAST in the database of GenBank showed that five clones were novel genes and other five clones have homologies with chloroplast genome or 23S ribosomal RNA, and one clone has homology with ubiquitin protein ligase or MAP kinase.

These results clearly showed that the EBC largely contributed to salt tolerance and salt storage of the ice plant. The EBC may sequestrate salt from inside the leaf, and it seemed to enable ice plant to survive under the hypersaline environment. The increased growth under the hypersaline condition increased the quantity of the salt absorption of plant, in turn, of salt removal from saline soil by the plant. The analysis of genes for EBC development may help to generate transgenic ice plant harboring extremely high salt removability from saline soil, and to advance environmental cleanup using the plant, i.e. phytoremediation.