Genetic and biochemical studies on mechanisms for salt accumulation and tolerance in the facultative CAM halophyte, *Mesembryanthemum crystallinum*

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Summary

Mesembryanthemum crystallinum, a facultative CAM halophyte, can survive and complete its life cycle under high salinity condition. Following salt stress, *M. crystallinum* shift its photosynthetic mode from C3 to CAM, and develop epidermal bladder cells (EBC) to accumulate salt into these cells. To elucidate the biochemical and molecular mechanisms that regulate these processes, we have established the large-scale mutant collections generated by irradiation of fast-neutron in *M. crystallinum*. We screened more than 30,000 plants over three generation, and we have isolated the mutants that are defective in their ability to develop CAM and EBC. Genetic and biochemical characteristics of those mutants are as follows:

1. CAM defective mutant

The M3 plants of the mutant showed the even less accumulation of starch and malate than in wild type plants. We have simultaneously analyzed the expression pattern of 1600 selected genes at dawn in the wild type and the mutant using cDNA microarray. Approximately 5% of transcripts showed significant up- and down-regulation in the mutant. Seventeen unique transcripts showed at least a two-fold increase in abundance, whereas 65 genes were down regulated to a similar extent. Up-regulated genes played functional roles in sugars/polysaccharide metabolism, growth regulators, defense-related, and stress responses. In contrast, down-regulated genes were mainly involved in photosynthesis processes, vacuolar H^+-ATPase and proteolysis, which are known to be expressd at high levels in the wild type leaves.

2. EBC mutant

The EBC mutant has small EBCs with 10 to 20% volumes of the wild type EBCs, which are found only on its adaxial surfaces. The growth of the mutant was reduced by 60% of that in the wild type plants under 400 mM NaCl for three weeks. The wild type plants accumulated salt in higher extent than in the mutant leaves, but grew more than the mutant. Consequently, NaCl contents on whole plant basis were higher in wild type. These results suggest that the EBCs accumulated NaCl, and hence sequestered salt away from photosynthetic tissues still contributing to maintain photosynthetic ability under the salinity condition.

This work should provide new insights into the genetic basis of the response mechanisms that contribute to survival following exposure to high salinity stress.