Gene-technological enhancement of stress tolerance of plants by biosynthesis of glycinebetaine

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Many halotolerant plants, bacteria and algae accumulate the compatible solute glycinebetaine (betaine) in response to environmental stresses, such as high concentrations of salt, low temperature and drought. Betaine acts by stabilizing the cellular structure and maintaining the function of proteins and membranes. To generate plants capable of the biosynthesis of betaine, the *codA* gene encoding choline oxidase, the enzyme that synthesizes betaine, was isolated from the soil bacterium *Arthrobacter globiformis*. *Arabidopsis thaliana*was transformed with a modified *codA* gene containing an additional leader sequence for transport into chloroplasts. The resultant transgenic plants were more tolerant to salt, cold and heat stress than wild-type plants during both germination of seeds and the growth of young and mature plants. Seeds from the transgenic plants also tolerated cold and heat stress during imbibition. Moreover, transformation with the *codA* gene enhanced tolerance to salt, cold and high-light stress in the photosyntheticmachinery and freezing tolerance of matured plants.

Recently, we examined the tolerance of transformed plants to salt stress at the reproductive stage, which is when plants are most sensitive to environmental stress. Salt-shock treatment of wild-type plants for 3 days resulted in abortion of flower buds and decreased the number of seeds per silique. These deleterious effects were clearly visible 6 days after the termination of the salt-shock treatment. Microscopic examination of floral structures revealed that salt stress inhibited the development of anthers, pistils and petals. In particular, the productionof pollen grains and ovules was dramatically inhibited. These effects of salt stress were significantly reduced by transformation with the *codA* gene, and our observations suggest that the enhanced tolerance of the transgenic plants is a result of the accumulation of betaine in the reproductive organs. Transformation with the *codA* gene has an additional unique characteristic. Such transformed plants grow faster and produce a more number of seeds than wild-type plants even under non-stress conditions, enhancing the productivity of the plant.

We also transformed the rice (*Oryza sativa* L.) japonica variety with the codA gene. Levels of betaine were as high as 1 and 5 mol per gram fresh weight of leaves in plants where choline oxidase was targeted to the chloroplast and the cytosol, respectively. Treatment of plants with 0.15 M NaCl inhibited growth of both wild-type and transgenic plants. However, after removal of the salt stress the transgenic plants began to grow againat the normal rate after a significantly shorter time than wild-type plants. Inactivation of photosynthesis indicated that plants exposed to salt stress and low temperature were more tolerant to photoinhibition if they contained a chloroplast-targeted choline oxidase than a

cytosol-targetedone. This indicates that the subcellular compartmentalization of the biosynthesis of betaine is a critical factor in the efficient enhancement of tolerance to stress in the engineered plants. We have also transformed the indica variety with the *codA* gene. This transgene also had enhanced tolerance of salt stress in young plants.

Very recently we transformed tomato (*Lycopersicon esculentum* Mill.) plants, which normally do not accumulate betaine and are susceptible to chilling stress, with the *codA* gene. Exposure to temperatures below 10 °C causes various injuries and greatly decreases fruit set in most cultivars. Transgenic plants expressing *cod*A accumulatebetaine in their leaves and reproductive organs at levels of upto 0.3 and 1.2 $mol \cdot g^{-1}$ fresh weight, respectively. Over various developmental phases, from seed imbibition to fruit production, these betaine-accumulating plants are more tolerant tochilling stress than their wild-type counterparts. During reproduction, they yield, on average, 10-30% more fruits following chilling stress.

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RESEARCH INTERESTS

(1) Molecular biology of stress physiology
(2) Biophysics and molecular biology of membrane lipids
(3) Regulatory aspects of photosynthesis
(4) Gene engineering of stress tolerance of plants
(5) Signal transduction pathway of environmental stress