HIGH LEVEL EXPRESSION OF C4 PHOTOSYNTHESIS ENZYMES IN A C3 PLANT, RICE: THE STRATEGY FOR HIGH LEVEL EXPRESSION AND ITS PHYSIOLOGICAL IMPACTS

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Agronomically important crops are classified into C3 and C4 plants, according to the mechanism of their photosynthetic carbon assimilation. C4 plants evolved from C3 plants, acquiring a unique biochemical mechanism (the C4 photosynthesis pathway) to perform photosynthesis at a rate about two times higher than that of C3 plants. It is anticipated that the photosynthetic efficiency of C3 plants can be improved by installing the C4 pathway into C3 plants. To drive the C4 pathway in C3 plants, at least three enzymes, namely, phospho*enol*pyruvate carboxylase (PEPC), pyruvate, Pi dikinase (PPDK) and NADP malic enzyme (NADP-ME) must be introduced.

We have studied conditions for high level expression of each enzyme in a C3 plant, rice. In the case of PEPC and PPDK, introduction of the maize intact gene led to 120- and 40-fold increases in enzyme activity, respectively. The levels of the PEPC and PPDK proteins in homozygous lines amounted to 12% and 35%, respectively, of total leaf soluble protein. Such high level expression was ascribable to the presence of intron(s) and/or the 3-flanking sequence in the introduced genes. In the case of NADP-ME, by contrast, introduction of a construct containing cDNA of maize NADP-ME linked with the rice *Cab* promoter was effective in increasing the enzyme activity and the protein level. Transgenic rice plants expressing either PEPC or PPDK at high levels grew normally, while those expressing NADP-ME could not grow under photoautotrophic conditions.

It is expected that the photosynthetic properties of rice can be affected only when all the three C4 enzymes are expressed at high levels. Actually, high level expression of either PEPC or PPDK alone had no effects on photosynthesis of rice. However, we found that transgenic rice plants showed improved stress resistance. PEPC transformants were more resistant to phosphorus limitation and aluminum in soil, and PPDK transformants showed enhanced growth under high oxygen stress conditions. These effects of C4 enzymes on stress resistance are ascribable to enhancement of housekeeping functions of the C4 enzymes that are intrinsically present in rice.