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Engineering C_3 Plants with SbtA, a Cyanobacterial Transporter, for Enhancing CO_2 Fixation

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Abstract : The cyanobacterial CO_2 concentrating mechanism (CCM) operates to raise the levels of CO_2 in the vicinity of the main carboxylation enzyme Rubisco which is encapsulated in protein micro compartments called carboxysomes. Thus, due to the presence of CCM, cyanobacterial cells are able to work with high photosynthetic efficiency even at low Ci conditions and can accumulate 1000 folds high internal concentrations of Ci than external environment. Engineering of some useful CCM components into higher plants is one of the plausible approaches to improve their photosynthetic performance. The first step and the simplest approach for attaining this objective would be the transfer of cyanobacterial bicarbonate transporter such as SbtA to inner chloroplast envelope of C_3 plants. For this, SbtA transporter gene from Synechococcus elongatus PCC 7942 was fused to a transit peptide element to generate chimeric constructs in order to direct it to chloroplast inner envelope. Two transit peptides namely, TnaXTP (transit peptide from AT3G56160) and TMDTP (transit peptide from AT2G02590) were shortlisted from Arabidopsis thaliana genome and cloned in plant expression vector pCAMBIA1302 having mgfp5 as a reporter gene. Plant transformation was done by agro infiltration and Agrobacterium mediated co-culture. DNA, RNA, and protein were isolated from the leaves four days post infiltration, and the presence of transgene was confirmed by gene specific PCR (Polymerase Chain Reaction) analysis and by RT-PCR (Reverse Transcription Polymerase Chain Reaction). The expression was confirmed at the protein level by western blotting using anti-GFP primary antibody and horseradish peroxidase (HRP) conjugated secondary antibody. The localization of the protein was detected by confocal microscopy of isolated protoplasts. We observed chloroplastic expression for both the fusion constructs which suggest that the transit peptide sequences are capable of taking the cargo protein to the chloroplasts. These constructs are now being used to generate stable transgenic plants by Agrobacterium mediated transformation. The stability of transgene expression will be analyzed from T_0 to T_2 generation.

Keywords : agro infiltration, bicarbonate transporter, carbon concentrating mechanisms, cyanobacteria, SbtA
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