Abstract

Attainment of improved crop yield by conventional crop breeding strategies is proving to be insufficient in coping with world population growth and hence there is a need to enhance crop yield via some novel strategies. Majority of the cereal crops such as rice, wheat, barley etc., possess C_3 pathway for photosynthetic CO_2 fixation and which is found to be relatively inefficient due to dual substrate specificity and low catalytic turnover rate of the primary enzyme of Calvin cycle i.e., RuBisCO.

For the enhancement of photosynthetic yield in the crops, there have been several strategies tried till date which include manipulation in the RuBisCO and/or other enzymes involved in photosynthesis, reducing/bypassing the photorespiratory losses, improving CO_2 diffusion inside the leaves, increasing substrate (RuBP) availability or most importantly the incorporation of more efficient photosynthetic components/ machinery commonly known as carbon concentrating mechanisms (CCM), from other plants (C_4 or CAM pathway) or from certain lower organisms such as cyanobacteria and algae in to the existing C_3 photosynthetic system.

The research work described in this thesis basically deals with the cyanobacterial CCM which comprises of several transporters for the uptake of CO_2 and HCO_3^- and a proteinaceous compartment named carboxysome which has RuBisCO and carbonic anhydrases in it. Specifically, the study is focused on targeting the simplest component of cyanobacterial CCM i.e., sodium dependent bicarbonate transporter BicA into various C_3 model plant species *viz. Nicotiana tabacum* and *Nicotiana benthamiana*. The long-term objective of the crop improvement research would be the incorporation of whole CCM assembly into the agronomically important cereal crops. It is believed that cereals are difficult to transform and hence to understand and standardize the plant transformation procedures, the initial studies have to be carried out on model plant species.

As per the objectives of the research work, firstly, the BicA homologues were traced in cyanobacteria as well as other lower organisms using various bioinformatics tools to speculate the evolutionary relationship of BicA with various other transporters of diverse substrate specificities and functionalities. Phylogenetic analysis and presence of common

conserved domains confirmed the ancestral relatedness of SulP family member BicA with archaeal NCS-2 family proteins which are involved in nucleotide metabolism.

The major aspect of the study was to establish and express cyanobacterial BicA transporter in model plants. Hence, suitable genetic constructs were synthesized using appropriate chloroplast targeting sequences (transit peptides) which were taken from N-terminus of chloroplast inner envelope proteins of *Arabidopsis thaliana* i.e., Inner translocon complex or TIC55 transporter (AT2G24820) and maltose transporter (AT5G17520) and named as TICTP and MEXTP respectively. Transit peptides were first fused upstream of the transporter gene and thereafter fusion genes (TICTP/MEXTP+*bicA* gene) were finally cloned into plant expression vectors *viz. pRI101-AN* and *pCAMBIA-1302* containing *gus* and *mgfp5* reporter gene respectively. Several plant transformation methods were tried and standardized to successfully transform the fusion genes into various model plants. Initial studies were carried out using recombinant *pRI101* constructs by transforming calli and leaves of *N. tabacum* plants, in which the transient protein expression was confirmed by GUS reporter gene assay. Further, *N. benthamiana* plant leaves were transiently transformed using recombinant *pCAMBIA* constructs and the incorporation of the targeted fusion genes was confirmed at DNA and mRNA levels by performing diagnostic PCRs using gene specific primers. Protein (BicA-mGFP5 fusion) expression was confirmed by performing western blotting using anti-GFP antibodies. The localization of fusion proteins was visualized in mesophyll protoplast cells by confocal laser scanning microscopy which indicated their targeting in chloroplasts.

This study would definitely serve as an important step towards the accomplishment of plant productivity enhancement by manipulating the C_3 plant machinery.