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Date: Signature

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.

List of Figures

Abbreviations

µL	Microliter
µm	Micrometer
2, 4-D	2, 4-Dichlorophenoxyacetic acid
ABC	ATP Binding Cassette
APS	Ammonium persulfate
AT	*Arabidopsis thaliana*
ATP	Adenosine triphosphate
BAP	6-Benzylaminopurine
BIC	Bayesian Information Criterion
BLAST	Basic Local Alignment Search Tool
bp/kb	Base pair/kilo base pair
BPB	Bromophenol blue
BSA	Bovine Serum Albumin
CA/CAH	Carbonic Anhydrase
CAM	Crassulacean Acid Metabolism
CBB	Calvin Benson Bassham
CBB	Coomassie Brilliant Blue
CCM	Carbon Concentrating Mechanism
CD	Conserved Domain
cDNA	Complementary DNA
Ci	Inorganic Carbon
CO_2	Carbon Dioxide
CS	Cleavage Site
cTP	Chloroplast Transit Peptide
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol

EDTA	Ethylenediaminetetraacetic acid
EEO	Electroendoosmosis
EtBr	Ethidium Bromide
FBPase	Fructose-1, 6-bisphosphatase
gDNA	Genomic DNA
GFP	Green Fluorescent Protein
GUS	β-glucuronidase
HCl	Hydrogen Chloride
HCO_3^-	Bicarbonate ion
He	Helium
HMM	Hidden Markov Model
Hr/hrs	Hour/hours
IEM	Inner Envelope of Chloroplast
KCl	Potassium Chloride
kDa	Kilodalton
KG	Ketoglutarate
kV	Kilovolts
LB	Luria Bertani
LTTR	LysR Type Transcriptional Regulators
MCS	Multiple Cloning Site
MEGA	Molecular Evolutionary Genetics Analysis
MEME	Multiple EM for motif elicitation
MES-K	2-(N-morpholino) ethanesulfonic acid
MEXTP	Transit peptide from “maltose excess 1 protein”
MFS	Major Facilitator Superfamily
mg	Milligram
mGFP	Modified Green Fluorescent Protein
Min	Minute/minutes

ML	Maximum Likelihood
mL	Milliliter
mM	Millimolar
mRNA	Messenger RNA
MS	Murashige and Skoog
MSA	Multiple Sequence Alignment
MU	4-Methyl Umbelliferone
MUG	4-methylumbelliferyl-β-D-glucuronide
NAA	1-Naphthaleneacetic acid
NAD	Nicotinamide Adenine Dinucleotide
NADP	Nicotinamide Adenine Dinucleotide Phosphate
NCBI	National Center for Biotechnology Information
NEB	New England Biolabs
NFW	Nuclease Free Water
ng	Nanogram
nm	Nanometer
nmol	Nanomole
NN	Neural Network
OD	Optical Density
OGs	Putative Orthologous Groups
PAGE	Polyacrylamide Gel Electrophoresis
PCK	Phosphoenolpyruvate carboxykinase
PCR	Polymerase Chain Reaction
PDS	Particle Delivery System
PEP	Phosphoenolpyruvate
PG	Phosphoglycolate
PGA	Phosphoglyceric acid
PGR	Plant Growth Regulators

pM	Picomolar
PPDB	Plant Proteome Database
PSI	Position Specific Iterative
PVDF	Polyvinylidene difluoride
PVPP	Polyvinylpolypyrrolidone
RFU	Relative Fluorescent Unit
RNA	Ribonucleic acid
RPM	Rotations per minute
RT	Room temperature
RuBisCO	Ribulose-1, 5-Bisphosphate Carboxylase/Oxygenase
RuBP	Ribulose-1, 5-bisphosphate
SBPase	Sedoheptulose-1, 7-bisphosphatase
SDS	Sodium Dodecyl Sulfate
Sec	Seconds
SP	Signal Peptide
SSS	Sodium Solute Symporter
SSU	Small subunits
STAS	Sulphate transporter anti-sigma factor-like domain
SUBA	The subcellular localization database for *Arabidopsis*
TAE	Tris Acetate EDTA
TAIR	The Arabidopsis Information Resource
Taq	*Thermus aquaticus*
TBS	Tris Buffer Saline
TCDB	Transporter Classification Database
T-DNA	Transfer DNA
TE	Tris EDTA
TEMED	N, N, N', N'-Tetramethylethylenediamine
TICTP	Transit peptide from “translocon at inner chloroplast 55 protein”

T_m	Melting Temperature
TM	Transmembrane
TMB	3, 3′, 5, 5′-Tetramethylbenzidine
TP	Transit Peptide
UV	Ultraviolet
v/v	Volume/volume
wt/v	Weight/volume
X-Gluc	5-Bromo-4-Chloro-3-Indolyl-β-D glucuronide
β-ME	Beta mercaptoethanol

Key Words

Sr. No.	Key Words
1.	*Agrobacterium*
2.	Agroinfection
3.	Agroinfiltration
4.	*Arabidopsis thaliana*
5.	BicA transporter
6.	Bicarbonate ion
7.	C_3 plants
8.	Carbon concentrating mechanisms
9.	Carbon dioxide
10.	Chloroplast
11.	Co-culture
12.	Cyanobacteria
13.	Floral dip
14.	GUS reporter
15.	Heterologous expression
16.	Inner envelope of chloroplast
17.	MEXTP
18.	mGFP5 reporter
19.	*Nicotiana benthamiana*
20.	*Nicotiana tabacum*
21.	N-terminal transit peptides
22.	Particle bombardment
23.	Photosynthesis
24.	Plant expression vector
25.	RuBisCO
26.	*Synechococcus* sp. PCC 7002
27.	TICTP

Declaration

I hereby declare that this Ph.D. thesis entitled **"Targeting Cyanobacterial Bicarbonate Transporter BicA into Chloroplast of Model C_3 Plants and Analyzing Transient Expression and Localization"** is my original work which was carried out for the degree of Doctor of Philosophy under the supervision of Dr. Sandhya Mehrotra at Birla Institute of Technology and Science, Pilani, Pilani campus, India. I have duly acknowledged all the sources of information used in the work presented in the thesis.

Signature:

Name: Panchsheela Nogia

Date:

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Chapter 2 Materials and Methods

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