

Acknowledgements

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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 C₃ pathway for photosynthetic CO₂ 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 CO₂ 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 (C₄ or CAM pathway) or from certain lower organisms such as cyanobacteria and algae in to the existing C₃ photosynthetic system.

The research work described in this thesis basically deals with the cyanobacterial CCM which comprises of several transporters for the uptake of CO₂ and HCO₃⁻ 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 C₃ 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. *pRII01-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 *pRII01* 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 C₃ plant machinery.

List of Figures

No.	Caption	Page No.
1-1	The representation of cyanobacterial carbon concentrating mechanism (CCM) components	1-7
1-2	Diagrammatic representation of topology map of SbtA transporter; sodium dependent bicarbonate transporter from <i>Synechocystis</i> PCC 6803	1-9
1-3	Diagrammatic representation of topology map of BicA transporter; sodium dependent bicarbonate transporter from <i>Synechococcus</i> PCC 7002	1-11
1-4	Regulation of cyanobacterial CCM at transcriptional level	1-14
1-5	Regulation of cyanobacterial CCM at post transcriptional level	1-16
1-6	Regulation of cyanobacterial CCM at post translational level	1-16
2-1	Various growth stages of <i>A. thaliana</i> plantlets grown on MS basal medium	2-5
2-2	Various growth stages of <i>A. thaliana</i> plantlets grown in soilrite mixture	2-5
2-3	The agarose gel electrophoresis image for the genomic DNA isolated from <i>A. thaliana</i> leaves	2-7
2-4	A schematic representation of the complete cloning strategy of <i>bicA</i> gene to generate fusion constructs for genetic transformation of model C ₃ plants	2-15
2-5	Schematic representation of the amplification of transit peptides	2-15
2-6	Schematic representation of the splicing of <i>bicA</i> gene from <i>pGEM</i> [®] - <i>T</i> clone	2-16
2-7A	Recombinant vector map showing cloning of fusion genes (TICTP/MEXTP + <i>bicA</i> gene) into <i>pCold-IV</i> vector	2-17
2-7B	Recombinant vector map showing cloning of fusion genes (TICTP/MEXTP + <i>bicA</i> gene) into <i>pET-15b</i> vector	2-18
2-7C	Recombinant vector map showing cloning of <i>gus</i> gene into <i>pRI 101-AN</i> vector	2-19
2-7D	Recombinant vector map showing cloning of fusion genes	2-20

	(TICTP/MEXTP + <i>bicA</i> gene) upstream of the <i>gus</i> gene into <i>pRI 101-AN</i> vector	
2-7E	Recombinant vector map showing cloning of fusion genes (TICTP/MEXTP + <i>bicA</i> gene) upstream of the <i>mgfp5</i> gene into <i>pCAMBIA-1302</i> vector	2-21
2-8	Various growth stages of <i>N. tabacum</i> plantlets grown on MS basal medium	2-30
2-9	Standard curve for the relative fluorescence of 4-methyl umbelliferone	2-33
2-10	Various growth stages of <i>N. tabacum</i> plantlets grown in soil mixture	2-34
2-11	Various growth stages of <i>N. benthamiana</i> plantlets grown in soil mixture	2-37
3-1	Outline of the analysis carried out to find ancestral BicA homologues	3-5
3-2	Molecular phylogenetic analysis by Maximum Likelihood method	3-11
3-3	A line diagram representing the sequence coverage of all the proteins and presence of their conserved domains with respect to BicA protein sequence	3-15
3-4	A schematic representation of correlation between cyanobacterial transporter BicA and transporters of various other lower organisms	3-20
3-5	Categorization of <i>A. thaliana</i> proteins and criteria for selection of relevant protein candidates	3-22
3-6A	Relative adaptiveness plots for each amino acid of <i>bicA</i> gene (shown in red color) with genome of <i>Synechocystis</i> PCC 6803 (shown in black color)	3-32
3-6B	Relative adaptiveness plots for each amino acid of <i>bicA</i> gene (shown in red color) with genome of <i>A. thaliana</i> (shown in black color)	3-33
3-6C	Relative adaptiveness plots for each amino acid of <i>bicA</i> gene (shown in red color) with genome of <i>N. tabacum</i> (shown in black color)	3-34
3-7	Schematic representation of the sequence arrangement of the chimeric transformation construct, the source organisms and various expression hosts	3-35
3-8	The agarose gel (1.5%) electrophoresis image for the PCR amplified products of transit peptides	3-36

3-9	BicA- <i>pGEM</i> [®] - <i>T</i> clone vector map	3-36
3-10	Restriction analysis to confirm the presence of <i>bicA</i> gene in BicA- <i>pGEM</i> [®] - <i>T</i> clone	3-37
3-11	Strategy used for cloning of transit peptides (TICTP and MEXTP) and transporter gene (<i>bicA</i>) into few intermediate cloning vectors and plant expression vector (<i>pRII01</i>)	3-38
3-12	Schematic representation of the chimeric constructs of <i>bicA</i> gene from <i>Synechococcus</i> 7002, chosen transit peptide sequences (TICTP and MEXTP) and reporter gene (<i>gus</i>) in recombinant <i>pRII01</i> constructs (A and B)	3-39
3-13	Restriction analysis to confirm the cloning of TICTP- <i>bicA</i> into <i>pCold-IV</i> vector	3-40
3-14	Restriction analysis to confirm the cloning of MEXTP- <i>bicA</i> into <i>pCold-IV</i> vector	3-41
3-15	Restriction analysis to confirm the cloning of TICTP- <i>bicA</i> and MEXTP- <i>bicA</i> into <i>pET-15b</i> vector	3-42
3-16	Restriction analysis to confirm the cloning of <i>gus</i> gene into <i>pRII01</i> vector	3-43
3-17	Restriction analysis to confirm the cloning of fusion genes [transit peptides (TICTP/MEXTP) + transporter gene (<i>bicA</i>)] into <i>pRII01</i> vector	3-44
3-18	The chemical reaction of the GUS fluorometric assay	3-46
3-19	Expression of the methylumbelliferone (in terms of specific GUS activity) for wild type control, TICTP- <i>bicA-gus</i> and MEXTP- <i>bicA-</i> <i>gus</i> constructs	3-46
3-20	Colony PCR amplified <i>bicA</i> gene products using recombinant <i>pRII01</i> plasmid DNA as template	3-47
3-21	Transformation of <i>N. tabacum</i> by <i>Agrobacterium</i> mediated co-culture method using leaves as explants	3-48
3-22	Transformation of <i>N. tabacum</i> by <i>Agrobacterium</i> mediated co-culture method using calli as explants	3-49
3-23	The chemical reaction of the GUS histochemical assay	3-50
3-24	Microscopic observation of the GUS histochemical staining of callus	3-50

	tissue regenerated from agroinfected leaves and calli explants and its comparison with the non agroinfected control callus	
3-25	Transformation of <i>A. thaliana</i> and <i>N. tabacum</i> by <i>Agrobacterium</i> mediated co-culture method	3-52
3-26	GUS histochemical assay results were analysed by observing the transformed and non-transformed callus tissue of <i>N. tabacum</i> under the compound microscope	3-55
3-27	Various growth stages of shoots regeneration from <i>N. tabacum</i> leaves co-cultured with <i>Agrobacterium</i> suspension harbouring recombinant <i>pRI101</i> plasmid	3-56
3-28	Genomic DNA isolated from <i>N. tabacum</i> leaves (wild type: WT and co-cultured with <i>Agrobacterium</i> suspension harboring recombinant <i>pRI101</i> plasmid)	3-57
3-29	PCR products (transit peptides) amplified from <i>N. tabacum</i> genomic DNA, isolated from wild type (WT) and agroinfected leaves	3-57
3-30	PCR products (<i>bicA</i> partial gene) amplified from <i>N. tabacum</i> genomic DNA, isolated from wild type (WT) and agroinfected leaves	3-58
3-31	A schematic representation of the various regions of fusion genes (TP + <i>bicA</i> gene) and their PCR amplification results	3-59
3-32	Transformation of <i>A. thaliana</i> by <i>Agrobacterium</i> mediated floral dip method	3-60
3-33	Screening and selection of <i>A. thaliana</i> transgenic seeds on MS medium containing kanamycin and regeneration of antibiotic resistant seedling on soilrite mixture	3-61
3-34	Strategy used for cloning of fusion genes (TICTP+ <i>bicA</i> and MEXTP+ <i>bicA</i>) into plant expression vector (<i>pCAMBIA</i>)	3-63
3-35	Schematic representation of the chimeric constructs of <i>bicA</i> gene from <i>Synechococcus</i> 7002, chosen transit peptide sequences (TICTP and MEXTP) and reporter gene (<i>mgfp5</i>) in recombinant <i>pCAMBIA</i> constructs	3-63
3-36	The agarose gel electrophoresis image of the PCR amplified fusion gene products	3-64
3-37	Restriction analysis to confirm the cloning of fusion constructs	3-65

	(transit peptide: TICTP/MEXTP + transporter gene: <i>bicA</i>) into <i>pCAMBIA</i> vector	
3-38	Colony PCR amplified products (fusion gene) using recombinant <i>pCAMBIA</i> plasmid and empty <i>pCAMBIA</i> plasmid DNA as template	3-66
3-39	Transformation of <i>N. benthamiana</i> by agroinfiltration method	3-67
3-40	Genomic DNA of <i>N. benthamiana</i> leaves isolated from wild type and agroinfiltrated leaves	3-68
3-41	PCR products amplified from genomic DNA of agroinfiltrated leaves of <i>N. benthamiana</i> . PCR was performed using sequence specific primers for (A) TICTP transit peptide, (B) <i>bicA</i> and (C) <i>mgfp5</i>	3-69
3-42	PCR products amplified from genomic DNA of agroinfiltrated leaves of <i>N. benthamiana</i> . PCR was performed using sequence specific primers for (A) MEXTP transit peptide, (B) <i>bicA</i> and (C) <i>mgfp5</i>	3-70
3-43	Total RNA isolated from wild type and agroinfiltrated leaves of <i>N. benthamiana</i>	3-71
3-44	PCR products amplified from cDNA of agroinfiltrated leaves of <i>N. benthamiana</i> . PCR was performed using sequence specific primers for (A) TICTP transit peptide, (B and D) <i>bicA</i> internal region, (C) MEXTP transit peptide and (E) <i>mgfp5</i> internal region	3-73
3-45	Agarose gel electrophoresis image showing absence of any PCR amplified products	3-74
3-46	SDS-PAGE image of total protein extracted from wild type and agroinfiltrated leaves of <i>N. benthamiana</i>	3-75
3-47	Western blot analysis of GFP tagged cyanobacterial BicA transporter protein displaying transient expression in agroinfiltrated leaves of <i>N. benthamiana</i>	3-78
3-48	Confocal microscopy images of protoplast samples prepared from wild type and agroinfiltrated leaves of <i>N. benthamiana</i>	3-81

Abbreviations

μL	Microliter
μm	Micrometer
2, 4-D	2, 4-Dichlorophenoxyacetic acid
ABC	ATP Binding Cassette
APS	Ammonium persulfate
AT	<i>Arabidopsis thaliana</i>
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	Polyvinylpyrrolidone
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 <i>Arabidopsis</i>
TAE	Tris Acetate EDTA
TAIR	The Arabidopsis Information Resource
Taq	<i>Thermus aquaticus</i>
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.	<i>Agrobacterium</i>
2.	Agroinfection
3.	Agroinfiltration
4.	<i>Arabidopsis thaliana</i>
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.	<i>Nicotiana benthamiana</i>
20.	<i>Nicotiana tabacum</i>
21.	N-terminal transit peptides
22.	Particle bombardment
23.	Photosynthesis
24.	Plant expression vector
25.	RuBisCO
26.	<i>Synechococcus</i> sp. PCC 7002
27.	TICTP

Declaration

I hereby declare that this Ph.D. thesis entitled “**Targeting Cyanobacterial Bicarbonate Transporter BicA into Chloroplast of Model C₃ 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.

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CONTENTS

List of Tables	i
List of Figures	iii
Abbreviations	viii
Key Words	xiii
Chapter 1 Introduction and Review of Literature	
1.1 Introduction	1-1
1.2 C ₃ photosynthesis	1-3
1.3 Carbon Concentrating Mechanisms (CCM)	1-4
1.3.1 CCM in higher plants	1-4
(a) C ₄ pathway	1-4
(b) CAM pathway	1-5
1.3.2 CCM in algae	1-6
1.3.3 CCM in cyanobacteria	1-6
1.3.3.1 Ci transporters	1-8
(a) BCT1 transporter	1-8
(b) SbtA transporter	1-9
(c) BicA transporter	1-10
(d) NDH-I ₃ complex	1-11
(e) NDH-I ₄ complex	1-11
1.3.3.2 Carboxysome	1-12
1.3.3.3 Regulatory mechanisms of cyanobacterial CCM	1-13
(a) At transcriptional level	1-13
(b) At post transcriptional level	1-15
(c) At post translational level	1-16
1.3.4 Various approaches for improving C ₃ photosynthesis	1-17
1.3.4.1 Manipulation/Modification in the RuBisCO	1-17
1.3.4.2 Increasing RuBP regeneration	1-18
1.3.4.3 Reducing/bypassing photorespiratory pathway	1-19
1.3.4.4 Incorporating C ₄ pathway into C ₃ plants	1-21
1.3.4.5 Incorporating CAM mechanisms into C ₃ plants	1-22

1.3.4.6 Incorporating algal CCM into C ₃ plants	1-23
1.3.4.7 Incorporating cyanobacterial CCM components into C ₃ plants	1-24
1.4 Gaps in existing research	1-26
1.5 Objectives of proposed research	1-26
Chapter 2 Materials and Methods	
2.1 Similarity searches and retrieval of other information using Cyanobase Database	2-1
2.2 PSI blast and retrieval of various protein sequences	2-1
2.3 Conserved domain (CD) analysis	2-2
2.4 Phylogenetic tree construction	2-2
2.5 Information retrieval for inner chloroplast located proteins of <i>Arabidopsis thaliana</i>	2-2
2.6 Prediction of transit peptides	2-3
2.7 Prediction of TM helices and orientation of N- and C-termini	2-3
2.8 Codon usage and rare codon analysis	2-3
2.9 Standardizing growth conditions for <i>A. thaliana</i>	2-4
2.10 Genomic DNA isolation from plant leaves using DNeasy Plant Mini Kit	2-5
2.11 Plasmid DNA isolation by alkaline lysis method	2-7
2.12 Preparation of genetic constructs	2-8
2.12.1 Primer designing and PCR amplification	2-8
2.12.2 Agarose gel electrophoresis	2-11
2.12.3 Digestion of PCR amplicons and plasmid DNA using appropriate restriction enzymes	2-11
2.12.4 Gel elution of PCR amplicons and digested DNA fragments using QIAquick [®] Gel Extraction Kit	2-12
2.12.5 Ligation of genes of interest into cloning and plant expression vectors	2-13
2.12.6 Cloning strategy and selection of multiple vector systems	2-14
2.12.6.1 Amplification of transit peptide sequences from <i>A. thaliana</i> genome	2-15
2.12.6.2 Splicing of <i>bicA</i> gene segment from pGEM [®] -T clone	2-16
2.12.7 Preparation of competent cells of various bacterial hosts	2-22

2.12.7.1 Preparation of chemically competent cells of <i>E. coli</i> DH5 α	2-23
2.12.7.2 Preparation of chemically competent cells of <i>Agrobacterium tumefaciens</i> strain GV3101	2-23
2.12.7.3 Preparation of electrocompetent cells of <i>Agrobacterium tumefaciens</i> strain GV3101	2-24
2.12.8 Transformation of chemically competent <i>E. coli</i> DH5 α cells with recombinant plasmids	2-25
2.12.8.1 Screening of transformed colonies	2-25
2.12.8.2 Glycerol stocks preparation	2-26
2.12.9 Transformation of chemically competent <i>Agrobacterium</i> cells with recombinant plasmids	2-26
2.12.10 Transformation of electrocompetent <i>Agrobacterium</i> cells with recombinant plasmids	2-27
2.13 Transformation of <i>N. tabacum</i> by particle bombardment method	2-28
2.13.1 Preparation and purification of recombinant plasmid constructs using Plasmid Mini Kit	2-28
2.13.2 Preparation of plant material for transformation by particle bombardment	2-29
2.13.3 Preparation of gold particle (microcarriers) suspension and coating onto DNA	2-30
2.13.4 Particle bombardment	2-31
2.13.5 GUS fluorometric assay	2-32
2.14 Transformation of <i>A. thaliana</i> and <i>N. tabacum</i> by <i>Agrobacterium</i> mediated co-culture method	2-34
2.14.1 GUS histochemical assay	2-35
2.15 Transformation of <i>A. thaliana</i> by floral dip method	2-36
2.16 Transformation of <i>N. benthamiana</i> by <i>Agrobacterium</i> mediated agroinfiltration method	2-37
2.16.1 Genomic DNA isolation using DNeasy Plant Mini Kit	2-38
2.16.2 Total RNA isolation using RNeasy Plant Mini Kit	2-38
2.16.2.1 cDNA synthesis using QuantiTect [®] Reverse Transcription kit	2-40
2.16.3 Diagnostic PCR using genomic DNA and cDNA as template	2-40

2.16.4 Total protein extraction using G-Biosciences kit	2-40
2.16.4.1 Sodium Dodecyl Sulfate (SDS) Polyacrylamide Gel Electrophoresis	2-41
2.16.5 Western blot analysis	2-42
2.16.6 Protoplast isolation from agroinfiltrated leaves	2-44
2.16.6.1 Confocal microscopy analysis	2-44
 Chapter 3 Results and Discussions	
3.1 Identification of BicA transporter in various cyanobacterial species	3-1
3.2 Mining ancestral homologues of cyanobacterial bicarbonate transporter protein BicA in lower organisms	3-4
3.2.1 Identification of distant homologues of <i>bicA</i> using PSI blast searches	3-6
3.2.2 Conserved domain analysis using NCBI CD search and HHpred	3-12
3.2.3 Construction of phylogenetic tree to analyze the divergence of BicA transporter from proteins of other lower organisms	3-16
3.2.4 Elucidating the link between the function of the proteins under study and habitat/mode of nutrition of their respective organisms	3-16
3.3 Selection of Transit Peptide (TP) sequences from <i>A. thaliana</i> proteome	3-21
3.3.1 Transit peptide analysis	3-25
3.3.2 Membrane topology analysis	3-27
3.4 Codon usage analysis of BicA transporter gene of <i>Synechococcus</i> 7002	3-29
3.5 PCR amplification of transit peptide sequences	3-35
3.6 Preparation of BicA transporter gene of <i>Synechococcus</i> 7002	3-36
3.7 Generation of genetic constructs tagged with <i>gus</i> reporter gene	3-37
3.7.1 Cloning of TP and <i>bicA</i> gene in <i>pCold-IV</i> vector	3-39
3.7.2 Cloning of fusion gene (TP+ <i>bicA</i>) in <i>pET-15b</i> vector	3-42
3.7.3 Cloning of <i>gus</i> reporter gene in <i>pRII01</i> vector	3-43
3.7.4 Cloning of fusion gene (TP+ <i>bicA</i>) in <i>gus</i> gene containing <i>pRII01</i> vector	3-43
3.8 Transformation of recombinant <i>pRII01</i> constructs into <i>Nicotiana tabacum</i> and <i>Arabidopsis thaliana</i> for studying transient and stable gene expression	3-44
3.8.1 Transformation of <i>Nicotiana tabacum</i> by particle bombardment method	3-45

3.8.2 Transformation of <i>Nicotiana tabacum</i> by <i>Agrobacterium</i> mediated co-culture method	3-46
3.8.3 Transformation of <i>Arabidopsis thaliana</i> by <i>Agrobacterium</i> mediated co-culture method and comparative analysis of relative efficiencies of transient transformation of <i>A. thaliana</i> and <i>N. tabacum</i>	3-51
3.8.4 Transformation of <i>Nicotiana tabacum</i> by <i>Agrobacterium</i> mediated co-culture method for generation of stable transgenic plants	3-55
3.8.5 Transformation of <i>Arabidopsis thaliana</i> by <i>Agrobacterium</i> mediated floral dip method for generation of stable transgenic plants	3-59
3.9 Generation of transformation constructs tagged with <i>gfp</i> reporter gene	3-62
3.9.1 PCR amplification of fusion genes (TP+ <i>bicA</i>)	3-64
3.9.2 Cloning of fusion genes (TP+ <i>bicA</i>) in <i>pCAMBIA</i> vector	3-64
3.10 Transformation of recombinant <i>pCAMBIA</i> constructs into <i>Nicotiana benthamiana</i> by <i>Agrobacterium</i> mediated agroinfiltration method	3-65
3.10.1 Detection of transgene expression at DNA level in the agroinfiltrated leaves of <i>Nicotiana benthamiana</i>	3-67
3.10.2 Detection of transgene expression at mRNA level in the agroinfiltrated leaves of <i>Nicotiana benthamiana</i>	3-71
3.10.3 Detection of transgene expression at protein level in the agroinfiltrated leaves of <i>Nicotiana benthamiana</i>	3-75
3.10.4 Determination of intracellular location of the targeted transporter protein by confocal microscopy	3-79
Conclusions	C-1
Summary	S-1
Bibliography	B-1
Appendices	
➤ List of publications	A-1
➤ List of conferences and workshops attended	A-2
➤ Biography of supervisor	A-4
➤ Biography of candidate	A-5
Reprint of publications	

List of Tables

No.	Caption	Page No.
2-1	List of primers used throughout the course of this study for the amplification of various transit peptide sequences, genetic constructs for transformation and analyses of transformants	2-9
2-2	PCR conditions for various amplifications carried out throughout the study	2-10
2-3	Details of all the restriction enzymes used in the study	2-12
2-4	Details of all the inserts, their sources, cloning vectors, plant expression vectors and restriction enzymes used for clonings	2-22
2-5	The composition of polyacrylamide gel	2-41
3-1A	List of cyanobacterial species which contain BicA transporter (information retrieved from Cyanobase database)	3-1
3-1B	List of cyanobacterial species which contain BicA transporter (information retrieved from NCBI database)	3-3
3-2	A list of proteins under study, their source organism, results of PSI blast searches which represent identities and E-values of each BLAST hit obtained and results of NCBI conserved domain analysis which represents specific hits	3-6
3-3	Results of specific hits obtained from HHpred CDD analysis, performed using BicA transporter protein of <i>Synechococcus</i> sp. PCC 7002 as query	3-13
3-4	Details about the habitat and mode of nutrition for all the organisms which belong to the proteins under study	3-17
3-5	List of transporter proteins present on the inner envelope membrane of chloroplast of <i>A. thaliana</i>	3-22
3-6	Results of N-terminal transit peptide prediction for proteins under study using ChloroP 1.1 software	3-25
3-7	Results of N-terminal transit peptide prediction for proteins under study using TargetP 1.1 software	3-26
3-8	Detection of transmembrane helices for proteins under study using TMHMM 2.0 software	3-27

3-9	Prediction of transmembrane helices and the location of N- and C-termini for proteins under study using TOPCONS software	3-28
3-10	Details of candidate proteins, used as a source of transit peptide sequence and the description of their respective transit peptides	3-29
3-11	Comparative codon usage analysis of <i>bicA</i> transporter gene for expression in different host species	3-30
3-12	Comparative analysis of various experimental parameters used for transformation of <i>A. thaliana</i> and <i>N. tabacum</i> by co-culture method	3-51
3-13	Details of various reaction components and conditions used for performing diagnostic PCRs using genomic DNA template	3-68
3-14	Details of various reaction components and conditions used for performing diagnostic PCRs using cDNA template	3-72