Improving C3 Plant productivity
By Using Cyanobacterial Bicarbonate Transporters

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Food security

Population growth- Crop yields need to nearly double over the next 35 years.

Improving photosynthesis: genetic engineering strategies

Increased photosynthetic yield

&

Water-use efficiency (WUE)
$\text{CO}_2$ concentrating mechanism (CCM)
Cyanobacteria based approaches

✅ Carboxysomes
✅ Ci transporters
RubisCO and Great Oxidation event

Evolution of oxygenic photosynthesis (GOE) in cyanobacteria ~2.5 billion years ago
Carboxysomes

Ci transporters

Three $\text{HCO}_3^-$ transporters

Two $\text{CO}_2$ uptake systems
The evolution of photosynthetic cyanobacteria, algae, higher plants, and chemoautotrophic proteobacteria over the past 3.5 billion years.
Contd.....

Price G. D. *et al.* 2011

Concept of incorporating cyanobacterial bicarbonate transporters to chloroplast inner envelope to check the effect on plant photosynthesis
Problems
1. Correct targeting to chloroplast envelope
2. Uncertainty about the post translational activation of transporter protein.

Advantages
1. Possibly 5% to 15% improvement in photosynthetic CO$_2$ fixation.
2. Energetically efficient process.
3. Increased water and nitrogen use efficiency.
Phases in engineering a cyanobacterial CCM into C$_3$ chloroplasts

**Phase 1a.** Transferring active HCO$_3^-$ pumps to the chloroplast envelope.

**Phase 1b.** Building a functional cyanobacterial carboxysome in the chloroplast stroma.

**Phase 2.** Combining the traits from phase 1a and 1b.

**Phase 3.** Eliminating carbonic anhydrase from the stroma.

**Phase 4.** Building a functional NDH-1 CO$_2$ uptake complex in the thylakoid membranes.
Types of *Ci* Transporters

**Inorganic Carbon transporters**

- **Bicarbonate transporters** (Substrate - HCO$_3^-$)
  - BCT1
  - BicA
  - SbtA

- **CO$_2$ uptake complexes** (Substrate- CO$_2$)
  - NDH-I$_3$ (ndhF$_3$D$_3$chpY)
  - NDH-I$_4$ (ndhF$_4$D$_4$chpX)
- BicA belongs to SulP/SLC26 family
- BicA is a low/medium affinity bicarbonate transporter
- Cyanobacterial BicA family shows the relation to the Arabidopsis SulP sulphate transporter family, the human SLC26A family and a few of bacterial BicA-like proteins

Price G.D. and Howitt S. M. 2011
12 transmembrane helices, both termini cytoplasmic
Size (gene) - 1.701 kb, 566 amino acids, Mol.wt. - 59.6 kD
SbtA transporter

- SbtA belongs to Sodium solute symporter (SSS) family
- SbtA is a high affinity bicarbonate transporter
- 95-member family of SbtA transporter has related homologs within a large number of β-cyanobacteria and a number of α-cyanobacteria and proteobacteria

Price G.D., 2013
Contd…..

- 10 transmembrane helices, both termini periplasmic
- 5+5 structure
- Size (gene) - 1.12 kb, 374 amino acids, Mol. wt. - 39.7 kD
BicA and SbtA transporters, as most suitable candidates

- In cytosol of a leaf cell approximately 250 μM HCO₃⁻ is present and this is maintained by cytosolic carbonic anhydrase activity.

- Uptake affinities for sodium dependent bicarbonate transporter
  
  (i) SbtA : 5-15 μM
  (ii) BicA : 90-170 μM

- SbtA and BicA require about 1 mM Na⁺, while leaf cytosol contains 1-3 mM Na⁺ so this will form a sufficient gradient across chloroplast envelope leads to energization of transporter.

- *Arabidopsis thaliana* proteome analysis have shown that several Na⁺/H⁺ antiporters are present on chloroplast envelope.
Objectives

- Analysis of bicA/sbtA and screening of transit peptide elements
- Synthesis of fusion construct of bicA/sbtA transporter gene with a transit peptide element and its transformation into C₃ host plant/s followed by reporter gene assay
- Evaluation of successful transformants for increased growth rate/biomass yield
Categorization of *Arabidopsis thaliana* proteins to find out transit peptide elements from suitable tentative candidates.

- **Total 1190 proteins**
  - **Envelope (487)**
  - **Stromal (483)**
  - **Thylakoid (220)**

- 46 inner envelope membrane proteins, transport facilitation, transit peptide detected, most of them with TM helices
- Selection of suitable proteins
List of various bioinformatics tools, online databases and software used for analysis

1. Localization in inner envelope
2. Transport facilitation

3. Transit peptide prediction

4. Transmembrane domain prediction

- **AT_chloro database**
  (http://grenoble.prabi.fr/at_chloro): Maintains information for all proteins that have been identified from *Arabidopsis thaliana*.

- **ChloroP 1.1 server**
  (http://www.cbs.dtu.dk/services/ChloroP/): Predicts chloroplast transit peptides.

- **TargetP 1.1 server**
  (http://www.cbs.dtu.dk/services/TargetP/): Predicts N-terminal presequence for chloroplastic, mitochondrial and secretory proteins.

- **OCTOPUS** (http://octopus.cbr.su.se/)
- **TMHMM**
  (http://www.cbs.dtu.dk/services/TMHMM/)
- **TOPCONS** (http://topcons.cbr.su.se/)
# List of protein candidates used as a source of TP (Transit Peptide) elements for \textit{bicA} gene

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Name of Transit Peptide</th>
<th>Gene accession Number</th>
<th>Function of the Source protein of Transit Peptide</th>
<th>Length of Transit Peptide</th>
<th>No. of TM helices</th>
<th>Location of N- &amp; C-terminus</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TP1</td>
<td>AT1G70610</td>
<td>ATPase transporter</td>
<td>177 bp</td>
<td>6</td>
<td>Cytosolic</td>
</tr>
<tr>
<td>2</td>
<td>TP5</td>
<td>AT2G24820</td>
<td>Tic55, protein precursor import</td>
<td>144 bp</td>
<td>2</td>
<td>Cytosolic</td>
</tr>
<tr>
<td>3</td>
<td>TP9</td>
<td>AT5G17520</td>
<td>Maltose transporter RCP1</td>
<td>141 bp</td>
<td>10</td>
<td>Cytosolic</td>
</tr>
<tr>
<td>4</td>
<td>TP10</td>
<td>AT5G22830</td>
<td>Mg transporter</td>
<td>186 bp</td>
<td>2</td>
<td>Cytosolic</td>
</tr>
<tr>
<td>Sr. No.</td>
<td>Name of Transit peptide</td>
<td>Gene accession Number</td>
<td>Function of the Source protein of Transit Peptide</td>
<td>Length of Transit Peptide</td>
<td>No. of TM helices</td>
<td>Location of N- &amp; C-terminus</td>
</tr>
<tr>
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<td>-----------------------------</td>
</tr>
<tr>
<td>1</td>
<td>TP3</td>
<td>AT3G56160</td>
<td>Transporter Na/X</td>
<td>141 bp</td>
<td>7</td>
<td>Periplasmic</td>
</tr>
<tr>
<td>2</td>
<td>TP4</td>
<td>AT2G02590</td>
<td>Transporter multidrug</td>
<td>234 bp</td>
<td>6</td>
<td>Periplasmic</td>
</tr>
</tbody>
</table>

**List of protein candidates used as a source of TP (Transit Peptide) elements for *sbtA* gene**
Preparation of *bicA* gene

*bicA* gene construct (~4.7kb)

- *bicA* (*Synechococcus* sp. PCC 7002)
- Cloned in pGEM®-T vector
- pGEM®-T vector (3 kb)+*bicA* gene (1.701 kb)
- Procured as a kind gift from Dr. Price’s lab

pGEM®-T vector map

![pGEM®-T vector map](image)
Preparation of *sbtA* gene

*sbtA* gene construct (~4.12 kb)

- *sbtA* (*Synechococcus elongatus* PCC 7942)
- Cloned in *pGEM®*-T vector
- *pGEM®*-T vector (3 kb)+ *sbtA* gene (1.12kb)
- Procured as a kind gift from Dr. Price’s lab
Cloning strategy for bicA gene

bicA gene was taken out from pGEM®-T vector using SacI & XbaI restriction enzymes

TP elements were amplified by primers which include restriction enzyme sites of NdeI and SacI at 5' and 3' end respectively.

TP element and bicA gene were cloned into pCold IV vector B/W NdeI and XbaI sites

Sequencing of fused segment (TP+bicA gene) to confirm cloning

Fused segment was cloned into plant expression vector pRI 101-AN

Analysis of transformants
Cloning strategy for *sbtA* gene

- *sbtA* gene was taken out from pGEM®-T vector using PCR with primers having *KpnI* & *BamHI* restriction enzymes
- TP elements were amplified by primers which include restriction enzyme sites of *NdeI* and *KpnI* at 5’ and 3’ end respectively
- TP element and *sbtA* gene were cloned into pCold I vector B/W *NdeI* and *BamHI* sites
- Sequencing of fused segment (TP+*sbtA* gene) to confirm cloning
- Fused segment was cloned into plant expression vector pRI 101-AN
- Analysis of transformants
Primer sequences used for amplification of TP elements for *bicA* gene

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Primer name</th>
<th>Primer sequence (5’ to 3’)</th>
<th>Primer length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BicA -TP1-Ndel-F</td>
<td>CGA ACA CAT ATG GCT CAG CAA GTA CTC</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td>BicA -TP1-Sacl-R</td>
<td>CAC CGT GAG CTC ATT AAT CGA AGG C</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>BicA -TP5-Ndel-F</td>
<td>GCG TCC CAT ATG GCT GTT CCA TTT CTA</td>
<td>27</td>
</tr>
<tr>
<td>4</td>
<td>BicA -TP5-Sacl-R</td>
<td>ATA GAG CTC GCG GAG GAG ACG TAG</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>BicA -TP9-Ndel-F</td>
<td>CGC CAT ATG GAA GGT AAA GCC ATC</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>BicA -TP9-Sacl-R</td>
<td>ATA GAG CTC AGA GAC AGC ACC ACC</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>BicA -TP10-Ndel-F</td>
<td>GGC CCA CAT ATG GCG TTA ACT CC</td>
<td>23</td>
</tr>
<tr>
<td>8</td>
<td>BicA -TP10-Sacl-R</td>
<td>GGC GAG CTC TTT CGA TCT CGA TAA AAC</td>
<td>27</td>
</tr>
</tbody>
</table>
Primer sequences used for amplification of TP elements for *sbtA* gene

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Primer name</th>
<th>Primer sequences (5’ to 3’)</th>
<th>Primer length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SbtA - KpnI-F</td>
<td>AGAGGTACCCTGATTTTCTTG</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>SbtA –BamHI-R</td>
<td>TAAGGATCCGGCGCCCAATGG</td>
<td>20</td>
</tr>
<tr>
<td>3</td>
<td>SbtA –TP3-Ndel-F</td>
<td>ACTCATATGGCGATAGCTAGTA</td>
<td>22</td>
</tr>
<tr>
<td>4</td>
<td>SbtA –TP3-KpnI-R</td>
<td>TTGTTACCTCTAATGGATCTG</td>
<td>22</td>
</tr>
<tr>
<td>5</td>
<td>SbtA –TP4-Ndel-F</td>
<td>ATACATATGGCGATTTCTACA</td>
<td>21</td>
</tr>
<tr>
<td>6</td>
<td>SbtA –TP4-KpnI-R</td>
<td>TTCGTTACCTTTGGTTTT</td>
<td>19</td>
</tr>
</tbody>
</table>
a. PCR product of TP1 (product size ~ 177 bp)
b. PCR product of TP5 (product size ~ 144 bp)
c. PCR product of TP9 (product size ~ 141 bp)
d. PCR product of TP10 (product size ~ 186 bp)
PCR amplification of TP3, TP4 and sbtA

a. PCR product of TP3 (product size ~ 141 bp)
b. PCR product of TP4 (product size ~ 234 bp)
c. PCR of sbtA (product size ~ 1.12 kb)
Synthesis of fusion construct of TP elements and \textbf{bicA/sbtA gene}

Fusion constructs containing different transit peptide elements with \textit{bicA/sbtA} genes were generated
Cloning of TP element and \textit{bicA} gene into pCold IV vector between \textit{NdeI} and \textit{XbaI} sites.

TP5/9 along with \textit{bicA} was cloned between \textit{NdeI} and \textit{XbaI} sites.
Cloning of TP element and sbtA gene into pCold I vector between NdeI and BamHI sites

TP3/4 along with sbtA was cloned between NdeI and BamHI sites.
Clone confirmation (TP5+bicA+pCold IV) by restriction analysis

Lane 1: Uncut pCold IV plasmid
Lane 2: Double digestion of pCold IV plasmid with NdeI and XbaI restriction enzymes
Lane 3 and 6: 100bp tp 10kb DNA ladder
Lane 4: Double digestion of transformed/recombinant pCold IV plasmid with NdeI and XbaI restriction enzymes
Lane 5: bicA gene double digested with SacI+XbaI restriction enzymes
Lane 7: Double digestion of transformed/recombinant pCold IV plasmid with SacI+XbaI restriction enzymes
Lane 8: Digestion of transformed/recombinant pCold IV plasmid with SacI enzyme
A. Chromatogram of forward sequencing

B. Chromatogram of reverse sequencing
Clone confirmation (TP9+bicA+pCold IV) by restriction analysis

Lane 1: Uncut pCold IV plasmid
Lane 2: Double digestion of pCold IV plasmid with NdeI and XbaI restriction enzymes
Lane 3 and 6: 100bp tp 10kb DNA ladder
Lane 4: Double digestion of transformed/recombinant pCold IV plasmid with NdeI and XbaI restriction enzymes
Lane 5: bicA gene double digested with SacI+XbaI restriction enzymes
Lane 7: Double digestion of transformed/recombinant pCold IV plasmid with SacI+XbaI restriction enzymes
Lane 8: Digestion of transformed/recombinant pCold IV plasmid with SacI enzyme
A. Chromatogram of forward sequencing  
B. Chromatogram of reverse sequencing
Clone (TP3 + *sbtA* + pCold I) confirmation by restriction analysis

**Lane 1** - Marker (SM0331)
**Lane 2** – pCold I + TP3+ *sbtA* plasmid
**Lane 3** – pCold I + TP3+ *sbtA* plasmid digested with *BamHI*
**Lane 4** – pCold I + TP3+ *sbtA* double digested with *NdeI* and *BamHI*
**Lane 5** – pCold I + TP3+ *sbtA* double digested with *NdeI* and *KpnI*
**Lane 6** – pCold I + TP3+ *sbtA* double digested with *KpnI* and *BamHI*
**Lane 7** – PCR elute of *sbtA*
**Lane 8** – Marker (SM0331)
A. Chromatogram of forward sequencing  
B. Chromatogram of reverse sequencing
Clone (TP4 + sbtA + pCold I) confirmation by restriction analysis

**Lane 1** - Marker (SM0331)
**Lane 2** – pCold I+ TP4+ sbtA plasmid uncut
**Lane 3** – pCold I + TP4+ sbtA plasmid digested with *BamHI*
**Lane 4** – pCold I + TP4 + sbtA double digested with *NdeI* and *BamHI*
**Lane 5** – pCold I + TP4+ sbtA double digested with *NdeI* and *KpnI*
**Lane 6** – pCold I + TP4+ sbtA double digested with *KpnI* and *BamHI*
**Lane 7** – PCR elute of sbtA
**Lane 8** - Marker (SM0331)
A. Chromatogram of forward sequencing

B. Chromatogram of reverse sequencing
Clone confirmation (gus + pRI 101-AN) by restriction analysis

Lane 1: SM0331 DNA ladder (100bp to 10kb)

Lane 2: Double digestion of transformed/recombinant pRI 101-AN plasmid with BamHI and SacI enzymes

Lane 3: Digestion of transformed/recombinant pRI 101-AN with BamHI enzyme

Lane 4: Transformed/recombinant pRI 101-AN plasmid uncut

Lane 5: Double digestion pRI 101-AN plasmid with BamHI and SacI enzymes
Fused genes (TP+bicA/sbtA gene) were cloned into *gus* reporter gene containing pRI 101-AN plant expression vector by sticky end cloning.
Cloning of fusion constructs in plant expression vector (pRI 101-AN DNA)
**Fusion Construct preparation**

**Transporter gene seq.**
*(bicA/sbtA gene)*

**Reportor gene seq.**
*(gus gene)*

**Transit peptide seq.**

**Construct 1:** TP5+ bicA gene+gus+ pRI 101-AN vector  
**Construct 2:** TP9+ bicA gene+gus+ pRI 101-AN vector  
**Construct 3:** TP3+ sbtA gene+gus+ pRI 101-AN vector  
**Construct 4:** TP4+ sbtA gene+gus+ pRI 101-AN vector

**pRI 101-AN Plant Expression Vector**
Clone (pRI 101 + bicA+TP5 /TP9) confirmation by restriction analysis

**Lane 1:** Digestion of pRI 101 vector with *BamHI* enzyme

**Lane 2:** Digestion of “gus gene+ pRI 101” with *BamHI* enzyme

**Lane 3 & 5:** SM0331 DNA ladder (100bp to 10kb)

**Lane 4:** Double digestion of “TP5+bicA+ gus gene+ pRI 101” with *NdeI+BamHI* enzymes

**Lane 6:** Double digestion of “TP9+bicA+ gus gene+ pRI 101” with *NdeI+BamHI* enzymes
Clone (pRI 101 + sbtA+TP3) confirmation by restriction analysis

Lane 1 - Marker (SM0331)
Lane 2 - pRI101+ TP3+ sbtA plasmid uncut
Lane 3 - pRI101+ TP3+ sbtA plasmid digested with BamHI
Lane 4 - pRI101+ TP3+ sbtA double digested with NdeI and BamHI
Lane 5 - pRI101+ TP3+ sbtA double digested with KpnI and BamHI
Lane 6 - pRI101+ TP3+ sbtA double digested with NdeI and KpnI
Lane 7 - Marker(SM0331)
Clone (pRI 101 + sbtA+TP4) confirmation by restriction analysis

M - Marker(SM0331)
Lane 1 – pRI101+ TP4+ sbtA plasmid uncut
Lane 2 – pRI101+ TP4+ sbtA plasmid digested with BamHI
Lane 3 – pRI101+ TP4+ sbtA double digested with NdeI and BamHI
Lane 4 – pRI101+ TP4+ sbtA double digested with KpnI and BamHI
Lane 5 – pRI101+ TP4+ sbtA double digested with NdeI and KpnI
M - Marker(SM0331)
Recombinant pRI 101-AN constructs were transformed into *Agrobacterium tumefaciens* GV3101/LBA4404 strain by electroporation.

**Electroporation conditions:**
- Program: Agr (For *Agrobacterium tumefaciens*)
- Cuvette size: 0.1mm
- Voltage: 2.20kv
- Pulses: 5 pulses (each with 5ms)

*Agrobacterium* mediated plant transformation was performed using below mentioned methods:

1. Co-culture method
2. Floral-dip method
Plant transformation using bicA/sbtA construct

**Particle bombardment**

- **Explant**: *Nicotiana tabacum* leaf
- **Constructs**: bicA+TP5, bicA+TP9, sbtA+TP3, sbtA+TP4

**Agro-infection**

- **Explant**: *Nicotiana tabacum* leaf and callus
- **Constructs**: bicA+TP5, bicA+TP9, sbtA+TP3, sbtA+TP4

**Floral dip**

- **Explant**: *Arabidopsis thaliana* floral buds
- **Constructs**: bicA+TP5, bicA+TP9, sbtA+TP3, sbtA+TP4
Protocol of fluorometric GUS assay

Tobacco leaves were bombarded with recombinant plasmids of bicA and sbtA

Bombarded leaves were incubated for 48 hrs (16/8hr photoperiod) at 25°C

Leaves were crushed in liq.N₂ and suspended in extraction buffer

Centrifuged at 13,000 rpm for 20 min at 4°C, 90 μl supernatant was added to 10 μl pre-warmed assay buffer, incubated at 55°C for 20 mins

20 μl methanol was added and incubated at 37°C for 2 hrs followed by addition of 900 μl carbonate stop buffer

Measured the fluorescence in Spectrofluorometer Fluoroskan Ascent (Ex. 365nm, Em. 455nm), Specific GUS activity was calculated and plotted on graph
Gus specific activity:
no. of nano moles of MU/mg of protein/minute = 0.07 \( \frac{X}{Y} \)

X=rfu of each construct
Y=total protein concentration for each construct
GUS fluorometric assay for bicA/sbtA constructs

Specific gus activity nM-4MU/mg/min

[Bar graph showing specific gus activity for different constructs: TP5+bicA, Tp9+bicA, TP3+sbtA, TP4+sbtA, and Control. The x-axis represents the constructs, and the y-axis represents the specific gus activity in nM-4MU/mg/min.]
Agrobacterium mediated gene transformation of *Nicotiana tabacum* using leaf explants

A. Potted plant of *Nicotiana tabacum*
B. Leaf explant
C. Co-culture of explants with Agrobacterium containing gene of interest
D. Inoculation of the leaf discs on to co-cultivation media MS + BAP (2.0 mg/L) + NAA (0.2 mg/L)
E. Transfer of the explants to the regeneration medium (MS + BAP (2.0 mg/L) + NAA (0.2 mg/L), Kanamycin (50 mg/L) and Cefotaxime (250 mg/L)
Regeneration of shoots in Agro-infected tobacco leaves with Agro GV3101 (pRI 101-AN+ TP3/TP4+ sbtA) construct

Media composition: MS + BAP (2.0 mg/L) + NAA (0.2 mg/L), Kanamycin 50 mg/L and Cefotaxime 250 mg/L
**Agrobacterium mediated plant transformation using callus tissue as explant (pRI 101-AN+ TP9+ bicA)**

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**A. Nicotiana tabacum plant**

**B. 4-5 week old callus obtained from leaf explants of Nicotiana tabacum**

**C. Callus tissue dipped in Agrobacterium culture for agroinfection**

**D. Culturing of agroinfected callus on regeneration media (MS+2,4-D,4 mg/lit, kanamycin 50 mg/lit, cefotaxime 75 mg/lit)**
Agrobacterium mediated plant transformation using leaf tissue as explants (pRI 101-AN+ TP5+ bicA)

A. Nicotiana tabacum plant.
B. Leaf explants of Nicotiana tabacum dipped in Agrobacterium culture for agroinfection.
C. Culturing of agroinfected leaves on regeneration media (MS+2,4-D, 4 mg/L, kanamycin 50 mg/L, cefotaxime 75 mg/L)
Analysis of transformants by GUS histochemical assay

1. A small amount of fresh callus tissue (From test and experimental control both) into a centrifuge tube, immersed into 500 µl of staining solution

2. Incubated sample at 37°C for 12-18 hrs. (If there is excessive staining then washed the solution with 50% ethanol until tissue cleared)

3. A small section of callus tissue (both test and control); fixed on a slide with a drop of 100% glycerol and observed under microscope and compared the results with experimental control
5-bromo-4-chloro-3-indolyl glucuronide (X-Gluc) + GUS (beta-glucuronidase) enzyme

- Hydrolysis
- Indoxyl derivative
- Oxidative dimerization
- Insoluble indigo coloured product
- Observation under microscope
Results of GUS staining assay

[A] Negative control

[B] TP5+BicA construct

Negative control – Callus which has not been infected with *Agrobacterium*

Construct – Callus which has been infected with *Agrobacterium* containing recombinant (TP5/9+bicA+gus) pRI 101 construct

[C] Negative control

[D] TP9+BicA construct
a) Control Callus (Untransformed)
b) Transformed callus sbtA + TP4 construct
c) Transformed callus sbtA + TP3 construct
1. *Arabidopsis thaliana* plant
2. Dipping of *Arabidopsis* flowers in to *Agrobacterium* culture (20-30 seconds)
3. Plant covered in plastic bag, after floral dip (To maintain humidity for 12 hrs)
4. Plant after 3 weeks of floral dip (Showing silique development)

Selection of transgenic plants
Screening of the seeds for positive transformants on $\frac{1}{2}$ MS media containing kanamycin

Transformed Untransformed
SbtA

Transformed Untransformed
BicA
T1 plants transformed with bicA/ sbtA fusion constructs

T₂ generation would be raised using T₁ seeds for selection of stable transformants
Future Plans

- Analysis of transformants (generated via *Agrobacterium* mediated method) by reporter gene assay

- Screening of $T_1$ seeds (generated via floral dip method) and further generation of $T_2$ plants

- Southern blot hybridization and qPCR assays would be performed to check the transgene expression
References


Thank You