Enhanced performance of sugarcane transgenics under water limited conditions and its biosafety considerations

Dr. C. Appunu
Scientist
Genetic Transformation Lab
ICAR-Sugarcane Breeding Institute
Coimbatore 641 007 (Tamil Nadu)
Background Information

Sugarcane is the central source of sugar or sucrose in India

Sugarcane is a high water demanding tropical crop and generally grown under irrigated conditions.

Drought is one of the most challenging agricultural issues limiting sustainable sugarcane production and can potentially cause up to 50% yield loss.

In India, nearly 35% total cane areas has optimum irrigation facilities that contribute about 50% of total cane production, while 65% of the cane areas in rainfed or partially irrigated contribute about 50% of cane production (Singh et al., 2006).
The area under the crop declined from 5.04 million hectare (m ha) (2011-12) to 4.92 and 4.75 m ha during 2015-16 and 2016-17 (Cooperative Sugar, June 2017), respectively.

**Sugarcane Water requirement and productivity**

<table>
<thead>
<tr>
<th>Crop</th>
<th>Water requirement (mm)</th>
<th>Productivity per unit water consumed (Kg/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane</td>
<td>1000-1500</td>
<td>4.97</td>
</tr>
<tr>
<td>Cotton</td>
<td>550-950</td>
<td>0.22</td>
</tr>
<tr>
<td>Rice</td>
<td>300-950</td>
<td>0.42</td>
</tr>
<tr>
<td>Wheat</td>
<td>300-450</td>
<td>0.64</td>
</tr>
</tbody>
</table>
## Water requirement of sugarcane

<table>
<thead>
<tr>
<th>Growth Phase</th>
<th>Irrigation interval (days)</th>
<th>Quantum of water required (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coarse textured Soil</td>
<td>Medium textured Soil</td>
</tr>
<tr>
<td>Germination (0-60 days)</td>
<td>5-6d</td>
<td>7-8d</td>
</tr>
<tr>
<td>Tillering (60-130 days)*</td>
<td>6-7d</td>
<td>7-10d</td>
</tr>
<tr>
<td>Grand growth (130-250 days)</td>
<td>Weekly</td>
<td>10d</td>
</tr>
<tr>
<td>Ripening (250-365 days)</td>
<td>10d</td>
<td>12-15d</td>
</tr>
</tbody>
</table>

*Water stress during this period lead up to maximum of 70% yield loss in sugarcane*
Sugarcane Productivity Trend in Ahmednagar (t/ha)

Source: Climate Change Impacts in Drought and Flood Affected Areas: Case studies in India (World Bank Report No:43946-IN)

Over a period of 25 years (1970-71 to 2004-2005) sugarcane yield per hectare in Ahmednagar district, a drought prone area in Maharashtra have fallen from 115 to 60 ton
To meet the increasing demand of sugar and other byproducts, the yield and quality of sugarcane must be improved but at the same time the loss of the yield and quality due to drought and other abiotic stresses must be reduced.

World consumption will reach 173 million tons during 2025, India has the highest human consumption of about 27 million tons (24 million tons) followed by European Union (19 million tons) and is expected to increase year after year.

To meet the increasing sugar demand improvement in quality and yield of sugarcane must be doubled.
Development of drought tolerant genotypes or improve water use efficiency of genotype to the idea of water productivity ("more crop per drop").
Co 86082 was chosen because

Widely adopted in Peninsular zone

Tamil Nadu - 80%
Karnataka - 50%
Maharashtra - 50%
Andhra Pradesh - 40-45%.
# Expansins (EXPA1)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Source</th>
<th>Gene activity</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>EaEXP A1</td>
<td><em>E. arundinaceus</em></td>
<td>Induced under stress conditions</td>
<td>ICAR-SBI, Coimbatore</td>
</tr>
<tr>
<td>Port Ubi 882</td>
<td><em>Porteresia coarctata</em></td>
<td>Gene promoter</td>
<td>ICAR-SBI, Coimbatore</td>
</tr>
</tbody>
</table>

Root growth in 90 days old plants (10 days after release of drought) of a Co 86032 and *Erianthus* (Augustine et al., 2015)
Cell walls are distinctive feature of plant cell and their major functions are to determine cell structure, provide tensile strength and protection against mechanical and osmotic stresses.

- For a plant cell to grow, it must be continuously able to modify and expand its cell wall.

- Expansins are cell wall loosening proteins that regulate cell wall expansion. It is involved in cell enlargement and also in other developmental processes in which cell wall modification occurs.

- Studies reported that expansin genes involved in all biological processes related to plant growth and development including seed germination, stem elongation, stomatal opening, xylem development and root system architecture.
In addition, expansins participate in response to abiotic stresses in plants.

However, the mode of action of expansin genes are still unclear, it is assumed that expansins act non-enzymatically via disrupting non-covalent bonds between hemicelluloses and cellulose microfibrils.

On the basis of phylogenetic sequence analysis, expansins are classified into four families namely α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB).
Vector construction of EriEXPA1

### a-Expansin1 (EXPA1)

<table>
<thead>
<tr>
<th>Details</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full length</td>
<td>1330bp</td>
</tr>
<tr>
<td>CDS</td>
<td>762bp</td>
</tr>
<tr>
<td>Protein</td>
<td>254 amino acid</td>
</tr>
<tr>
<td>Intron</td>
<td>2</td>
</tr>
<tr>
<td>CDS similarity with Zea mays</td>
<td>96.2%</td>
</tr>
<tr>
<td>CDS similarity with Sorghum bicolor</td>
<td>96.6%</td>
</tr>
<tr>
<td>Protein similarity with Zea mays</td>
<td>95.3%</td>
</tr>
<tr>
<td>Protein similarity with Sorghum bicolor</td>
<td>97.2%</td>
</tr>
</tbody>
</table>
Transformation in Sugarcane

Fig A – Sugarcane leaf whorls in MS-Aceto medium

Fig B – Callusing on selection medium

Fig C – Embryogenic calli on selection medium

Fig D – Regeneration of putative transgenic sugarcane plants

Fig E – Rooting of putative transgenic plants

Fig F – Hardening of putative transgenic sugarcane plants
**Fig A** - PCR amplification of Port ubi882 promotter
Lane M - 1Kb Marker
Lane 1 - Port ubi882 promotter amplicon at 882 bp

**Fig B** - PCR amplification of EriEXPA1 in E.coli
Lane M - 1Kb Marker
Lane 1 - EriEXPA1 amplicon at 1330 bp

**Fig C** - PCR amplification of Port ubi 882-EriEXPA1 in Agrobacterium
Lane M - 1Kb Marker
Lane 1 - EriEXPA1 promotter amplicon at 1330 bp
Lane 2 - Port ubi882 promotter amplicon at 882 bp
Transgenic expansin sugarcane events (18 nos) were produced and confirmed through PCR. A total of eighteen events were taken to next generation ($V_1$).

90 days old plants were subjected to drought stress for 10 days and physiological experiments namely relative water content (RWC), photosynthetic efficiency ($F_v/F_m$), chlorophyll content, cell membrane injury (CMI) and visual scoring were carried out.

Screening for Water deficit stress

Gravimetric Method
Stand of transgenic events before soil moisture (~25 %) stress
Stand of transgenic events after soil moisture (~ 8 %) stress
Relative water content in Expansin transgenic events
Photosynthetic efficiency in Expansin transgenic events
Chlorophyll Content of Expansin transgenic events
Cell membrane Injury of Expansin Sugarcane plants
During the normal irrigated condition there is no difference of RWC in both transformed events and control plants, but after 10 days of drought RWC of transformed events is almost 15 to 20% more than the untransformed plants

- These experiments showed that over expression of EXPA1 gene helps in enhanced drought tolerance.

- Once the plant is normally irrigated it is seen that the expansin gene transformed plants were able to recover much faster than the untransformed control plants showing the gene’s involvement in the speedy recovery of the plant.

- However, root phenotype in these transgenics are yet to be studied.
Biosafety Issues are relatively low in sugarcane
Commercially sugarcane is vegetatively propagated and sexual seeds are not used for commercial cultivation.

It does not flower in many parts of the country - even if it flowers it does not set seeds.

As the sexual seed is not the propagating material no chance of contaminating neighbouring untransformed sugarcane plants
There is no report of pollen transfer from cultivated sugarcane to the wild relatives of sugarcane. Hence no chance of transgene introgression to wild Saccharum spp.

Weediness in sugarcane is not observed as it is a vegetatively propagated plant and it requires ideal conditions for establishment and growth.

Harvesting of cane before flowering is possible.

Sugar is a purified product hence very little chance for presence of the transgenic products.
For the production of sugar/Jaggary, sugarcane juice is subjected to high temperature and hence the degradation of the transgene products.

Development of *crop specific regulation* would help in reaching the technology to the farming community.
Acknowledgement

- The Department of Biotechnology (DBT) (Grant No.BT/PR6277/AGII/106/882/2012), Government of India.
- ICAR-Extramural Research project (F.No. CS/18(13)/2015-O&P)
- Sugarcane Breeding Institute, Coimbatore for the funding and infrastructure
THANK YOU