Over-expression of sugarcane MYB18 transcription factors deciphering tolerance to drought and salinity

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Genesis

- The complexity of plants especially sugarcane in response to salinity and drought stress, suggests that a large number of genes are involved in stress response pathways.
- Identifying critical transcription factor genes that contribute to the abiotic stress tolerance in sugarcane.
- MYB transcription factors (ScMYABAS1) differentially expressed in response to biotic and abiotic stresses having the role in triggering the stress tolerance pathways were previously reported in our laboratory using subtraction hybridization (SSH) and AP-PCR approaches (Prabu et al. 2010; Kawar et al. 2009; Pagariya et al. 2011;2012).
- Identification, isolation and molecular characterization of MYB transcription factor gene from wild relative species may provide an efficient stress tolerant gene.
- Theses will be a useful tool for developing stress tolerant sugarcane cultivars to minimize the heavy losses caused in present day sugarcane and other crops.

Aims and objectives

- Isolation, bioinformatic analysis and cloning of MYB transcription factor gene from wild relative species of sugarcane.
- Vector construction, transformation and functional characterization of Sacchrum complex MYB transcription factor gene in model plant system (Nicotiana sp.) in relation to environmental stresses.
- Physio-biochemical and molecular expression studies of MYB transcription factor gene in model plant system.

Materials and methods

Plant material

Sugarcane Co740, *S. spontaneum* and *E. arundinaceus* for the experimental work was collected from Vasantdada Sugar Institute, Pune, India fields.

Methodology

Isolation of full length MYB18 genes from *Saccharum* complex

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PCR amplification of MYB18 genes from *S. spontaneum* and *E. arundinaceus*

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Cloning and sequencing of MYB18 genes

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Sequence analysis and determination of ORF for MYB18 genes from *Saccharum* complex

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Preparation of constructs over expressing *SoMYB18*, *SsMYB18* and *EaMYB18* genes and its transformation in *Agrobacterium tumefaciens*

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Agrobacterium mediated Plant transformation & screening of putative transformants through antibiotic selection

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Confirmation of transformants through PCR, RT-PCR and Southern blot analysis

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Development of stable transgenic tobacco plants over expressing *SoMYB18*, *SsMYB18* and *EaMYB18* transcription factor genes

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In vitro drought and salinity stress treatment of control and all three transgenic tobacco plants

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Comparative physio-biochemical analysis of stressed MYB18 over expressing transgenic tobacco plants

Table: Primers used in this study

Sr. no.	Primer	Sequence	Amplicon size
	MYB18FP	5'-GCTTCGTGCTACTGGAGAAG-3'	
1.	MYB18RP	5'-GATTTCTGTATCAACTTAATGTATCTATGTAAGC-3'	1792 bp
2.	35S promoter sequencing	5'-CTATCCTTCGCAAGACCCTTC-3'	
3.	SoMYB18 FP	5'-ATGGGAAGGCATTCTTGC-3'	
	SoMYB18 SR	5'-CTCGTGCTCTGTGGTTCAAA-3'	1557bp
4.	SsMYB18 FP	5'-ATGGCTGCTGGAGTTCTGTTC-3'	
	SsMYB18 RP	5'-CTAGATATTCTCAAAAGACAGTTGCAT-3'	1470bp
5.	EaMYB18 FP	5'-ATGGGAAGGCATTCTTGC-3'	
	EaMYB18 RP	5'-CTAGATATTCTCAAAAGACAGTTGCAT-3'	1466bp

Results

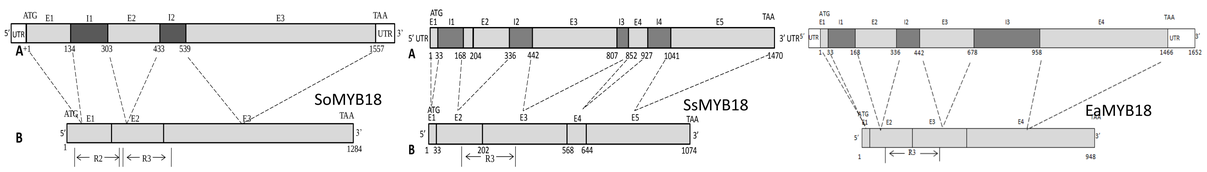

Fig 1. Depiction of mRNA transcript architecture of all three *MYB18* genes through FGENESH
A. Genomic DNA containing introns (I) and exons (E). mRNA sequence with marked coding region are shown. Exons are shown as [ ] while introns are shown as [ ] boxes and [ ] box represents UTR region. **B.** FGENESH predicted mRNA transcript includes exons E. The putative open reading frame starts from the start codon (ATG 1[st]bp), stop codon (TAA). The R2-R3 and R3 MYB domain repeats are designated by the arrows.

Conclusion

- Over expression of all three MYB18 transcription factors led to lesser accumulation of MDA than that of un-transfrormed tobacco plants
- MYB18 over expression also improved the proline biosynthesis together with less accumulation of H_2O_2 under stress.

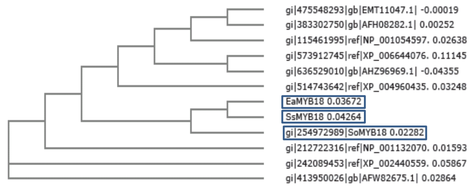

Fig 2. Neighbour–Joining tree of predicted MYB proteins.
The phylogeny was based on an alignment derived using the Clustal W program and the Neighbour-joining method (1000 bootstraps). The phylogeny with respect to the conserved SANT could be segregated into 2 groups viz; A and B. The GenBank accession numbers with respective MYBs *Oryza brachyantha* [XP_006644076.1], *Oryza sativa* NP_001054597.1], *Zea mays* [NP_001132070.1, ACF79741.1] and *Sorghum bicolour*[XP_002440559.1].

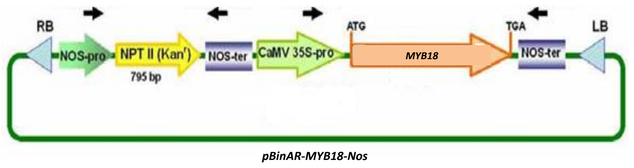

Fig 3. pBinAR Vector and pBinAR::SoMYB18 expressing construct

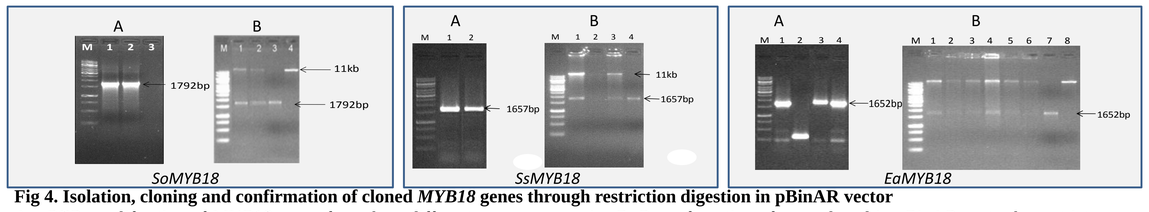

Fig 4. Isolation, cloning and confirmation of cloned *MYB18* genes through restriction digestion in pBinAR vector
A. PCR amplification of MYB18 genes from three different sugarcane species B. Reconfirmation of gene cloned in pBinAR vector by restricts digestion using *Kpn*I and *BamH*I enzymes.

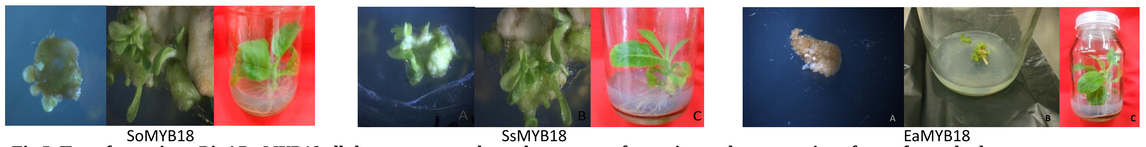

Fig 5. Transformation *pBinAR::MYB18* all three construct through agro-transformation and regeneration of transformed tobacco.

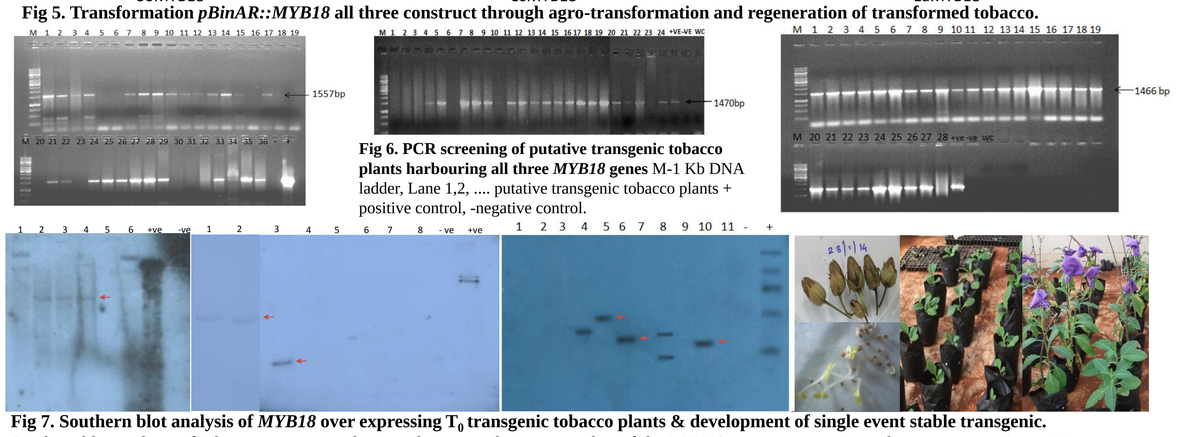

Fig 6. PCR screening of putative transgenic tobacco plants harbouring all three *MYB18* genes M-1 Kb DNA ladder, Lane 1,2, ... putative transgenic tobacco plants + positive control, -negative control.

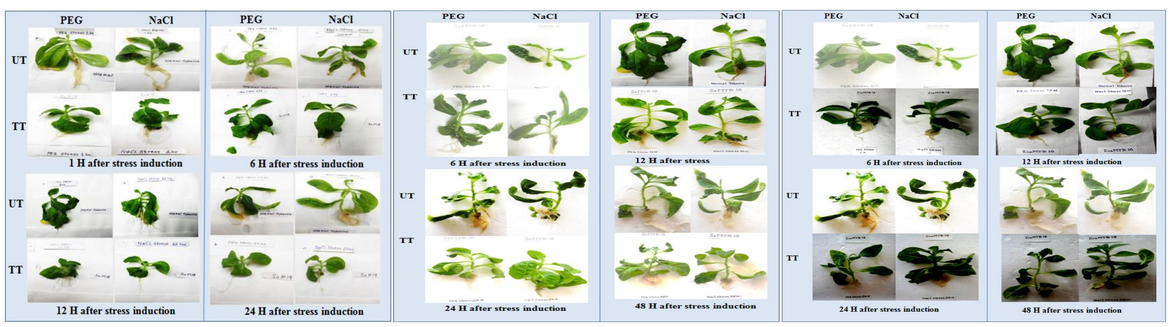

Fig 7. Southern blot analysis of *MYB18* over expressing T_0 transgenic tobacco plants & development of single event stable transgenic.
Southern blot analysis of tobacco transgenic plants to determine the copy number of the *MYB18* gene integration in tobacco genome. Lane 1-11 transgenic tobacco G DNA , + respective pBinAR::MYB18 plasmid and - Un-transformed tobacco.

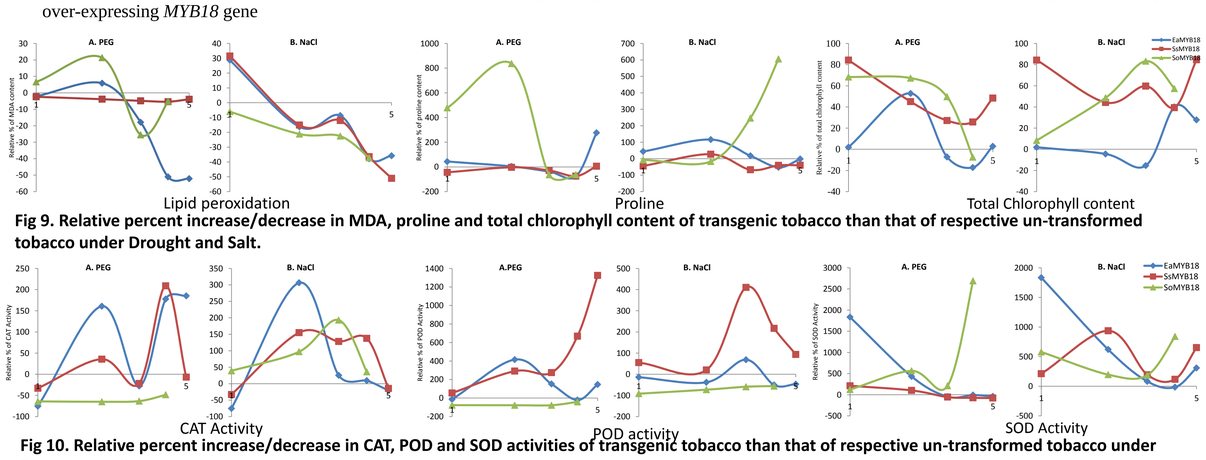

Fig 8. PEG and salt stress analysis of all three *MYB18* over expressing tobacco lines UT-Untransformed tobacco plant, TT- Transgenic tobacco plant over-expressing *MYB18* gene

Fig 9. Relative percent increase/decrease in MDA, proline and total chlorophyll content of transgenic tobacco than that of respective un-transformed tobacco under Drought and Salt.

Fig 10. Relative percent increase/decrease in CAT, POD and SOD activities of transgenic tobacco than that of respective un-transformed tobacco under Drought and Salt stresses..

- Thus, our results indicated that over expression of all three MYB18 transcription factors playing a key role to combat drought and salinity stresses through regulation of antioxidants, membrane biosynthesis and osmolyte synthesis.
- Theses transcription factors may be a potential candidates in improving the stress tolerance in sugarcane and other crops. Further validation of these genes at molecular level is under progress.

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