# Engineering Abiotic Stress Tolerance in Transgenic Banana plants by overexpressing Effector/ Transcription Factor Genes

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**Abstract**

Banana *(Musa spp.)* is the most important fruit worldwide. Among the factors which are responsible for lower productivity in banana plantations, abiotic stress factors like water limitation, soil salinity and temperature extremes are the most significant. We have attempted to use transgenic approach for development of abiotic stress tolerant banana plants. In this pursuit, we have overexpressed multiple abiotic stress related native gene sequences in transgenic banana and characterized the responses of the resulting transgenic plants in response to application of simulated abiotic stress conditions. Physiological and biochemical assays performed on these transgenic plants have proved the efficacy of using this approach to develop abiotic stress tolerant banana plants.

## Introduction

Modern-day plants have evolved over hundred of years from simple organisms in response to continuous abiotic and biotic environmental alterations. Among the abiotic cues that have affected plant evolution, water availability, soil salt content and incidence of extreme temperatures are the most important. In order to survive these challenges to their survival, plants have developed the capacity to react to these external signals by using specialized physiological and biochemical strategies. Upon stress perception, plants activate a cascade of cellular events involving several signal transduction pathways that lead to modification levels of specific transcription factors resulting in the up- or down-regulation of specific genes responsible for synthesis of effector proteins and/ or metabolites which contribute to stress tolerance.

Banana is the most important fruit crop in the world. It also fulfills a huge food security role for millions of people mainly in the African continent. India is the largest producer of banana in the world with a production of 28.45 million tonnes from an area of 0.796 million hectares with a productivity of 35.7 MT/ha. Bananas are especially susceptible towards any sort of water scarcity and in fact they rarely attain their full genetic yield potential due to limitations imposed by water availability. A shallow root system together with a permanently green canopy comprising of large leaves and numerous stomata translates into enormous water requirements for the banana plant. Additionally, bananas are also sensitive to soil salinity and low temperatures.

We have been studying multiple banana abiotic stress related transcription factors as well as effector genes in order to engineer abiotic stress tolerance in transgenic banana plants. Some of the genes characterized are briefly described in the following sections.

### Banana Dehydrin *(MusaDHN-1)*

Dehydrins are highly hydrophilic proteins which are known to be involved in playing adaptive roles in abiotic stress conditions which have dehydration as a common component. We identified a novel banana SK3-type dehydrin, MusaDHN-1 from banana EST database maintained at NCBI and later characterized this dehydrin by overexpressing it in transgenic banana plants (Shekhawat et al. 2011). In native untransformed banana plants MusaDHN-1 was found to be induced in leaves and roots by drought, salinity, cold, oxidative and heavy metal stress and by treatment with abscisic acid, ethylene and methyl jasmonate. This inducible expression in abiotic stress conditions indicated its involvement in stress tolerance in banana plants. MusaDHN-1 promoter was isolated from banana genomic DNA by employing TAIL-PCR technique. Promoter analysis performed by constructing a MusaDHN-1 promoter: β-glucuronidase fusion construct and its transformation in tobacco leaf discs confirmed the abiotic stress inducibility of MusaDHN-1. Transgenic banana plants which were conformed to overexpress MusaDHN-1 constitutively were found to be phenotypically normal in their growth and development. When these plants were subjected to drought and salt-stress treatments in in vitro and ex vitro assays, they performed better than the equivalent controls. Biochemical analysis of these transgenic plants showed enhanced accumulation of proline and reduced malondialdehyde levels in stress conditions.

**Banana Stress Associated Protein *(MusaSAP1)***

A20/AN1 zinc finger domain containing Stress Associated Proteins (SAP) are known to be involved in different stress response pathways in higher plants. We have identified a novel banana SAP gene, MusaSAP1, in banana EST database and this gene was subsequently characterized by carrying out its overexpression in transgenic banana plants (Sreedharan et al. 2012). Expression profiling of MusaSAP1 performed in native untransformed plants indicated that it was up-regulated by drought, salt, cold, heat and oxidative stress and by treatment with abscisic acid. To confirm the cellular localization of this protein a Musa-SAP1::GFP fusion protein was designed and transformed into onion peel cells using *Agrobacterium* mediated genetic transformation. The fluorescence analysis indicated that MusaSAP1 is incompletely translocated to nucleus. Gene copy number analysis of MusaSAP1 performed by real time PCR and Southern blotting showed that MusaSAP1 gene occurs as a single copy per 11 chromosome set in banana nucleus. Transgenic banana plants which constitutively overexpressed MusaSAP1 were phenotypically indistinguishable from the untransformed controls and also displayed better stress tolerance features as compared to equivalent controls in both in vitro and ex vivo assays. Further, strong up-regulation of a polyphenol oxidase (PPO) coding transcript seen in MusaSAP1 overexpressing plants along with induction of MusaSAP1 by wounding and methyl jasmonate treatment pointed towards probable involvement of MusaSAP1 in banana biotic stress responses where PPOs are expected to perform major functions in multiple defense pathways.

**Banana bZIP transcription factor *(MusabZIP53)***

bZIP transcription factors have been shown to be involved in diverse cellular processes in plants. We identified a bZIP gene, MusabZIP53, from banana EST database and later characterized it by overexpressing in transgenic banana plants of cultivar Rasthali (Shekhawat et al. 2014). This gene was found to be upregulated in native untransformed banana plants in response to cold and drought stress and also by ABA treatment in both leaf and root tissues. Transgenic banana plants which constitutively overexpressed MusabZIP53 showed prominent growth retardation from very early stages of banana transformation/regeneration procedure and later the mature greenhouse hardened transgenic plants were found to display a distinct dwarf phenotype. These transgenic plants showed differential regulation of genes belonging to several families known to be involved in abiotic stress perception and mitigation. These included genes responsible for dehydration response element binding proteins, late embryogenesis abundant proteins, anti-oxidant enzymes, aquaporins, polyphenol oxidases, Aux/IAA proteins and some of the proteins involved in amino acid metabolism. We detected a strong up-regulation of four polyphenol oxidase coding genes in MusabZIP53 overexpressing plants together with high induction of these transcripts in native banana leaves by cold stress and ABA treatments which pointed towards potential involvement of MusabZIP53 in the master control of polyphenol oxidase activity in banana plants.

Banana Plasma Membrane Intrinsic Protein *(MusaPIP2;6)*

High soil salinity is considered to be a major abiotic stress for plants and it is an important limiting factor in nurturing of crop plants worldwide. We identified an aquaporin gene, MusaPIP2;6 and characterized it by overexpressing in transgenic banana plants (Sreedharan et al. 2015). MusaPIP2;6 was initially identified using a comparative study of stressed and

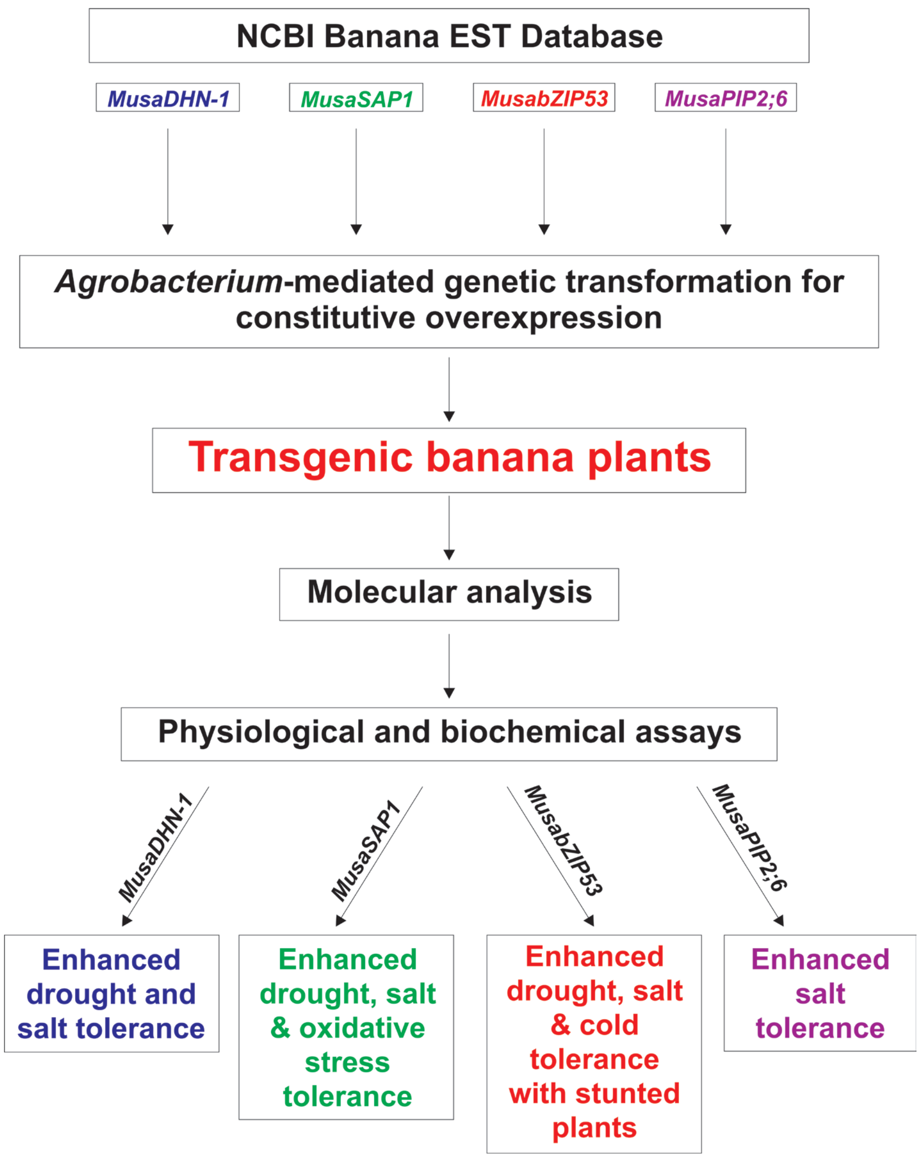


**Fig. 1: Schematic representation of development of abiotic stress tolerant transgenic banana plants using four native banana genes.**

non-stressed banana tissues derived EST databases. Its overexpression in transgenic banana plants was undertaken to study its probable functions in banana. It was found that overexpression of MusaPIP2;6 in transgenic banana plants by employing a constitutive or an inducible promoter (pMusaDHN-1) lead to higher salt tolerance in comparison with equivalent untransformed control plants. To confirm the exact cellular localization of MusaPIP2;6 protein we used transiently transformed onion peel cells and found that MusaPIP2;6 protein tagged with GFP was translocated to the cellular plasma membrane. MusaPIP2;6-overexpressing transgenic banana plants displayed comparatively better photosynthetic efficiency (Fv/Fm ratios) and lower membrane damage (MDA equivalents) under salt stressed conditions.

## Conclusion

The characterization studies performed on four genes in our laboratory over the last couple of years prove the efficacy of using native genes for development of abiotic stress tolerant transgenic banana plants (Figure 1). Apart from abiotic stress tolerance banana plants, we have also developed disease resistance establishing the versatility of our approach. We plan to demonstrate the efficacy of transgenic banana lines developed in our laboratory through limited field trials in near future.

## References

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