STUDIES ON MUSAWRKY71, A MULTIPLE STRESS INDUCIBLE TRANSCRIPTION FACTOR GENE FROM BANANA WHICH IS INVOLVED IN DIVERSE STRESS RESPONSES

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Abstract

WRKY transcription factor proteins play conspicuously important roles in diverse plant stress responses. We have identified and characterized a novel WRKY gene, MusaWRKY71 from banana cultivar Musa spp. cv Karibale Monthan (ABB group). MusaWRKY71 shares significant similarity with other monocot WRKY proteins and is translocated to nucleus. MusaWRKY71 transcripts are induced in banana in response to exposure to cold, drought, salt, ABA, H2O2, ethylene, salicylic acid and methyl jasmonate. Transgenic banana plants overexpressing MusaWRKY71 display better endurance to salt and oxidative stress. Overexpression of MusaWRKY71 transcripts in these plants resulted in differential expression of several putative target genes of WRKY proteins like other WRKY genes, pathogenesis-related (PR) protein genes, non-expressor of pathogenesis-related genes 1 (NPR1) and chitinase genes. This study confirms that MusaWRKY71 is an important player in the transcriptional reprogramming following the perception of diverse stress stimuli in banana plants.

Introduction

Plant growth and wellbeing are negatively influenced by various abiotic and biotic stress stimuli. To survive these challenges, plants possess functional capacity to perceive and react to these signals using sophisticated physiological and biochemical strategies Shinozaki and Shinozaki (2000). Typically once the incidence of stress is sensed, plants set upon a chain of cellular events wherein multiple parallel transduction pathways together modulate the activity of specific transcription factors leading to differential expression of genes responsible for synthesis of effector proteins and/ or metabolites which ultimately contribute to stress tolerance (Chen and Zhu et al., 2004). Important among these transcription factors are plant specific WRKY transcription factors which have been widely studied in several plant species in context of both abiotic and biotic stress responses (Rushton et al., 2010). We have identified and characterized a multiple stress responsive WRKY transcription factor gene from banana, MusaWRKY71. Apart from its expression and localisation pattern in native banana plants (Shekhawat et al., 2010), we also undertook investigations on its role in stress tolerance by generating banana plants overexpressing this transcription factor.

MusaWRKY71 is multiple stress inducible and nuclear localized

MusaWRKY71 gene encodes a protein consisting of 280 amino acids and contains a characteristic WRKY domain in its C-terminal half. Semi-quantitative expression profiling indicated that low levels of
MusaWRKY71 transcripts are present in the unstressed leaves and roots of banana plants. In contrast, when banana plants are exposed to cold, dehydration, or oxidative stress or are treated with NaCl, ABA, ethylene, SA or MeJA, MusaWRKY71 transcripts are substantially induced in both leaves and roots although the exact quantum of this induction varies between different stress stimuli. Transcript level induction in response to different stress stimuli indicate that MusaWRKY71 is probably a part of the complex signaling networks controlling abiotic and biotic stress responses of banana plant. Being a transcription factor MusaWRKY71 protein should localize to the nucleus of the banana cells and this was proved by transforming a expression cassette consisting of MusaWRKY71::GFP fusion cDNA into banana suspension cells. In these transiently transformed cells, GFP fluorescence was prominently noticed in the nuclei whereas in cells transformed with GFP alone, the fluorescence was present throughout the entire cell.

MusaWRKY71 overexpressing plants are salt and oxidative stress tolerant

Banana plants overexpressing MusaWRKY71 were generated by Agrobacterium mediated genetic transformation of banana embryogenic cells (Fig. 1) using a binary vector wherein CaMV 35S promoter was used to express MusaWRKY71 cDNA essentially as described previously (Ganapathi et al., 2001). Once the putatively transformed plants were confirmed to be transgenic using Southern blotting, these plants were subjected to oxidative and salt stress treatments. The detached leaf assays for enhanced salt and oxidative stress tolerance indicated that the MusaWRKY71 overexpressing plants endured the stress conditions better than the equivalent control plants. This was further proved by better photosynthesis efficiency (Fv/Fm) and lower membrane damage of the assayed transgenic leaves.

Fig. 1: Agrobacterium-mediated genetic transformation of banana embryogenic cells to overexpress MusaWRKY71 in transgenic banana plants. (A) Putatively transformed banana embryos on banana embryo induction medium (B) Germinating embryos on embryo germination medium (C) Multiple shoot cultures derived from putatively transformed tissues (D) Rooted plantlets (E) Hardened transformed plants in greenhouse.
MusaWRKY71 influences the expression of several related genes

In order to understand the role of MusaWRKY71 in biotic stress reception and regulation of banana plant, we identified several defense related genes whose expression was modulated by MusaWRKY71 overexpression in the transgenic banana plants. Differential regulation of these putative targets of MusaWRKY71 protein were studied using real-time RT-PCR expression analysis of genes from WRKY, non-expressor of pathogenesis-related genes 1 (NPR1), pathogenesis-related (PR) protein genes and chitinase families. Out of a total of 122 genes tested belonging to these families (identified from banana genome database), 10 genes (6 WRKY genes, 3 PR genes and 1 chitinase gene) were found to display significant differential expression in MusaWRKY71 overexpressing banana plants.

Discussion and conclusions

In the last decade there have been several reports detailing overexpression and downregulation of select specific WRKY genes in transgenic plants (Song et al., 2010; Ren et al., 2010). These studies have proved that WRKY proteins are involved in a multitude of processes in plants especially biotic and abiotic stress responses. MusaWRKY71 is proposed to be one of the important members of the WRKY gene family in banana which is involved in both abiotic and biotic stress responses and possesses the capacity to regulate other WRKY genes as also other genes involved in biotic stress signaling pathways. Also, since overexpression of MusaWRKY71 imparts the transgenic plants improved oxidative and salt stress tolerance, it is predicted to be a significant linkage in the interface between the various stress response pathways in banana plant.

References