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Identification and Characterization of Genes Responsible for Drought Tolerance in Rice Mediated by *Pseudomonas fluorescens*

Manjesh SAAKRE, Thirthikar Meera BABURAO, Abida Puthenpeedikal SALIM, Rose Mary FFANCIES, Valasala Poothecty ACHUTHAN, George THOMAS, Sajeevan Radha SIVARAJAN

(*Centre for Plant Biotechnology and Molecular Biology / Department of Genetics and Plant Breeding / Department of Agronomy, College of Horticulture, Kerala Agricultural University, Vellanikkara, Thrissur680656, Kerala, India*)

Abstract: Drought is one of the major abiotic stresses which adversely affect crop plants limiting growth and yield potential. Structural and functional characterization of drought stress-induced genes has contributed to a better understanding of how plants respond and adapt to the drought stress. In the present study, differential display technique was employed to study the gene expression of rice plants at the reproductive stage that were subjected to drought stress by withholding water, *Pseudomonas fluorescens* strain (*Pf1)* treated plants subjected for drought stress by withholding water and control (well-watered). Differentially expressed cDNAs of six genes (*COX1*, *PKDP*, *bZIP1*, *AP2-EREBP*, *Hsp20* and *COC1*) were identified, cloned and sequenced. Real-time qPCR analysis showed that all the six genes were upregulated in drought-stressed plants treated with *Pf1*. This revealed that the remarkable influence of *Pf1* colonization leads to drought tolerance at the reproductive stage. These results showed that high levels of gene expression in plants lacking adequate water can be remarkably influenced by *Pf1* colonization, which might be a key element for induced systemic tolerance by microbes.

Key words: rice; drought tolerance; *Pseudomonas fluorescens*; differential display reverse transcription polymerase chain reaction; quantitative real-time PCR; transcript derived fragment

Rice (*Oryza sativa* L.) is one of the major food crops for about 65% of the world's population, and is the staple food for an expansive part of the world, particularly in Asia (Ghadirnezhad and Fallah, 2014). It has been estimated that a large portion of the world's population depends wholly or partially on rice for its calorie intake.

Climatic factors play a major role in the growth and development of any crops. Among the various factors, water availability is of great significance with regard to rice cultivation (Singh et al, 2008). Rice is predominantly a kharif season crop. However, it is also grown as rabi/summer season crop with assured irrigation wherever winter is not severe. Indian rice production largely depends on monsoon rains, and only 59% area under rice cultivation has assured irrigation (Auffhammer et al, 2011).

Drought is a problem of worldwide importance, affecting the crop production and quality on a large scale, and is becoming more serious with respect to the global climate change (Halliwell, 2006). Therefore, it is associated with all parts of plant biology. As of now, research on drought stress has been one of the principle headings in the global plant biology and biological breeding.

Plant growth promoting rhizobacterias (PGPRs) are

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Corresponding author: Manjesh SAAKRE (manjesh.saakre565@gmail.com)

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soil bacteria inhabiting around/on the root surface, and can directly or indirectly involve in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere (Ahmad and Kibert, 2013). Several microbes promote plant growth, and many microbial products that stimulate plant growth have been marketed (Lugtenberg and Kamilova, 2009). Such bacteria are generally designated as PGPRs (Lugtenbergc and Kamilova, 2009), which are effective in a wide range of crops to enhance the growth and improve the crop yield (Herman et al, 2008).

Pseudomonas fluorescens is a PGPR that colonizes a wide range of ecological niches, including the rhizosphere of plants (Jose et al, 2013). By promoting seed germination, accelerating growth at early stages and inducing root initiation, *P. fluorescens* acts as a plant growth stimulator (Heinonsalo et al, 2004). Marschner and Timonen (2006) reported the production of various phytohormones by *P. fluorescens* including auxins, gibberellins and cytokinins. *P. fluorescens* is also reported to produce specific amino acids and other growth promoters that improve plant growth. Matthijs et al (2007) observed that *P. fluorescens* has a high capacity for solubilizing phosphate and also can affect in siderophore production. Deveau et al (2007) reported that *P. fluorescens* adheres and colonizes the surface of some ectomycorrhizas. This colonization of *P. fluorescens* improves the symbiotic relationship between the plant and the ectomycorrhiza, and benefits the host plant. Certain strains of *P. fluorescens* promote the 1-aminocyclopropane-1-carboxylate deaminase activity and help plants to resist the stress conditions more efficiently (Arshad et al, 2007).

An additional mechanism, by which biocontrol agents can reduce plant biotic and abiotic stresses, enhances plant growth and metabolism, in which *P. fluorescens* is a significant group of bacteria which help inducing systemic resistance (Ganeshan and Arthikala, 2005). *P. fluorescens* is also involved in controlling pathogens and forms an integral component of organic farming. The growth promotional activity of *P. fluorescens* in plants has been revealed previously under different conditions such as laboratory, glass house and field. *P. fluorescens* strain *Pf1*, developed by Kerala Agricultural University, Thrissur, India, was found to exhibit plant growth promotional activity in rice under both *in-vitro* and *in-vivo* conditions. But the mechanism underlying such promotional activity of *P. fluorescens* is not yet understood clearly. The transcriptomic and gene expression analysis can provide in depth data about the interaction between plant cells and bacteria. In this study, efforts were made to elucidate the molecular responses of rice plants to *P. fluorescens* treatment through gene expression profiling.

Currently, there are several molecular techniques for transcriptome analysis, including differential display reverse transcription polymerase chain reaction (DD-RT-PCR), cDNA-amplified fragment length polymorphism, suppression subtractive hybridization and cDNA microarrays. DD-RT-PCR is a simple, sensitive and powerful technique that can be used successfully to isolate a number of differentially expressed genes from plants. This is comparatively inexpensive method among the techniques and does not require previous sequence information. Quantitative real-time PCR (qRT-PCR) technique is highly sensitive, accurate and practically easy to use, and hence it has become a routine bioinstrumentation for gene level measurement (Provenzano and Mocellin, 2007). In the present study, six transcript derived fragments (TDFs) were identified and isolated from rice with different treatments by DD-RT-PCR. These isolates were cloned, sequenced and characterized as differentially expressed genes by validating using quantitative real-time and semi-quantitative reverse transcriptase PCR.

MATERIALS AND METHODS

Rice materials

High-yielding rice variety Matta Triveni (PTB45) was used in the present study. It is a popular rice variety in Kerala, India, but performed very poor growth in upland. Seeds were sown in plastic trays and posted 15 d, and the seedlings were transplanted to earthen pots (Supplemental Fig. 1). Pots were filled with red soil, clay and cow dung in 1:1:1. The plants were grown under a completely randomized design with three treatments and five replications. A total of 60 plants were grown (four plants in each pot) in pots and representative plants were used for molecular analysis.

Drought treatments

Rice plants were subjected for drought stress at the reproductive stage (panicle initiation stage). The first batch of control plants was maintained under well-watered condition (T1). The second batch was subjected for drought stress by withholding water for