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Gene expression analysis in response to low and high temperature and oxidative stresses in rice: Combination of stresses evokes different transcriptional changes as against stresses applied individually

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ABSTRACT

Transcript expression profiles of rice seedlings were analyzed in response to (a) prior exposure with oxidative stress followed by heat or cold stress and (b) simultaneous exposure to oxidative stress along with heat stress or cold stress. The numbers of genes differentially regulated during stress combination of cold and oxidative stress as well as heat and oxidative stress treatments were higher when compared with the number of genes differentially regulated in response to individual stress conditions. A large number of transcript changes were noted unique to the stress combination mode as compared with when individual stresses were applied. Specific differences in the transcript expression profiles of OsHsf and OsClp gene family members were noted during combination of stresses as against individual stresses. For instance, OsHsf26 induction was specific to stress combinations, while OsHsfA2a, OsHsfA2f, and OsHsfA3 transcript levels were additively affected during combination of stresses. Unique promoter models and transcription factor binding sites (i.e. P$KNOX3_01, P$OSBZ8_Q6) were noted in the promoters of differentially regulated genes during combination of stresses. It is proposed that stress combinations represent a novel state of abiotic stresses for rice seedlings that might involve a different type of molecular response.

1. Introduction

Abiotic stresses (i.e. water stress, salt stress, flooding stress, low and high temperature stress, oxidative stress etc.) widely affect growth and productivity of crop plants. Abiotic stresses often occur in a complex way in field conditions: several different abiotic stresses affect the plants in combination, occurring concurrently or separated temporally. Such combination of stresses may have increased detrimental effects on the plants as against individual stress types [1]. Heat stress (HS), in combination with drought, salinity, high light intensity and other related stress types results in huge losses to crop productivity [1,2]. Similarly, cold stress (CS) combined with high light intensity is highly injurious to plants. The components of oxidative stress (OS) are considered to be a common consequence of drought, salinity and CS [3]. It has been shown that abiotic stress responsive signaling pathways constitute a network that is interconnected at several levels allowing the crosstalk amongst different abiotic stresses. This crosstalk enables interaction (additive or negative regulation) of two or more signaling pathways from different stresses. Miller et al. [4] proposed that during the establishment of this crosstalk, there may be summation of various signals that may (a) cause an additive effect, (b) result in generation of a new signal as of the integration of different signals or (c) one signal might be epistatic over the other in crosstalk. The above-mentioned possibilities may co-exist and henceforth, it seems that converging gene networks regulate the abiotic stress responses of plants. The details of the stress-related crosstalk between signaling pathways and the underlying mechanisms remain to be fully understood.

The molecular and metabolic response of plants to combination of drought and heat is different from the response shown by the plants when these stresses are applied separately. Mittler [2] has shown that the response of plants to combination of stresses cannot be predicted directly from the analysis wherein different stresses are applied individually. Stress response involving temperature extremes results in production of ROS (reactive oxygen species), leading to oxidative damage [5]. The consequent ROS accumulation has been proposed as a key process that is shared not only among various abiotic stresses but between abiotic and biotic stresses as well [6,7]. Suzuki et al. [8] suggested that ROS play a key role in mediating the signal transduction events. It has been

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noted that HS is accompanied by elicitation of OS [9,10]. Cheng et al. [11] showed that a ROS-mediated regulatory module functions as an early component during CS. The accumulation of intracellular ROS following environmental stresses results in accumulation of misfolded and damaged proteins via inhibition of 26S proteasome [12,13]. Recently, Mittal et al. [14] have highlighted that ROS homeostasis plays an important role in CS and HS on rice seedlings.

In most cases, abiotic stress responses in plants are investigated by subjecting plants to a single stress type in the experiments and this situation does not reflect the conditions that are associated with field-level cultivation of crops [15–17]. In this study, we have analyzed the stress response in a mode where oxidative stress (OS) and HS and CS were combined. Genome-wide transcriptional changes in response to stress situations where CS/HS and OS co-occur were analyzed. OS was given as prior treatment (PO_CS and PO_HS) as well as during the temperature stresses (DO_CS and DO_HS). Functional classification and promoter architectures based analysis (promoter models and overrepresented transcription factor binding sites) for differentially expressed genes (DEGs) were undertaken. We also present a comparative analysis of the transcriptional changes in response to stress combinations (this study) and individual stresses based on our earlier data [14,18,19].

2. Materials and methods

2.1. Growth of rice seedlings and stress treatments

Rice [*Oryza sativa* ssp. *indica* L; cultivar Pusa Basmati (PB1)] seedlings were raised as described by Mittal et al. [14,18]. For imposing oxidative stress prior to low temperature stress (PO_CS) treatment or high temperature stress (PO_HS) treatment, uniformly grown, 10-day-old seedlings were transferred to beakers which contained 10 mM H_2O_2 at $28 \pm 2\,^{\circ}C$ for OS for 4 h followed by CS at $5 \pm 1\,^{\circ}C$ for 5 h or HS at $42 \pm 1\,^{\circ}C$ for 30 min. Both PO_CS and PO_HS treatments were given in light condition ($80\,\mu mol\,m^{-2}\,s^{-1}$). For oxidative stress during low temperature stress (DO_CS) treatment or during high temperature stress (DO_HS) treatment, seedlings were transferred to beakers, which contained 10 mM H_2O_2 maintained at $5 \pm 1\,^{\circ}C$ for 5 h (CS) or at $42 \pm 1\,^{\circ}C$ for 30 min (HS). The seedlings did not show any visual damage in response to the exposure of the above stress regimes, indicating thereby that the applied stress regimes were sub lethal in nature. Subsequent to completion of the stress intervals, tissues were harvested (whole seedlings were pooled), frozen in liquid nitrogen and kept at $-80\,^{\circ}C$. Non-stressed plants for control were handled exactly in similar manner. RNA samples from three independent biological replicates for stressed and control tissues were processed for transcript expression analysis.

2.2. Microarray, semi-quantitative RT-PCR and Q-PCR analysis

A 60mer rice 44k oligo DNA array kit (AMADID: No: 015241, Agilent Technologies) which contains 45,018 features/microarray and ~40,000 transcripts was used and processed as described earlier [14,18]. The microarray data discussed in this publication have been deposited in NCBI Gene Expression Omnibus and are accessible through GEO series accession number GSE32704 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE32704). Feature extraction software (version 10.5.1.1 Agilent Technologies, USA) was employed for the image analysis and data extraction process. The normalization was done using GeneSpring GX version 10.2 (Agilent Technologies, USA) using the recommended per chip and per gene data transformation: set measurements less than 0.01–0.01, per chip: normalize to 50th percentile per gene: normalize to specific samples (treated vs control). Data analysis was done using GeneSpring GX version 7.3.1 (Agilent Technologies, USA) and Microsoft Excel. Three biological replicates were used in the microarray analysis. We applied the criteria of at least 2.0 fold change (log2 values) in gene expression levels and *p*-value revealed by *t*-test of less than 0.05. Multiple testing correction (Benjamini and Hochberg False Discovery Rate multiple testing correction) was applied on the *t*-test *p*-values and these corrected *p*-values were used to identify the significantly changed genes. The RAP-DB IDs given in the results corresponds to the IRGSP genome build 4 (http://rapdb.dna.affrc.go.jp). The correlation coefficients of the normalized data among the biological replicates and the PCA plots are provided in Supplementary Figs. 1 and 2. The microarray data was validated using Q-PCR and semi-quantitative PCR for selected genes as described earlier [18,19]. Two biological replicates and three technical replicates were used for the Q-PCR analysis. cDNA for the real-time reactions were synthesized using the same RNA samples that were used for microarrays.

2.3. Upstream analysis and functional classification

Composite promoter models (CPMs) were identified using CMA software. Transcription factor (TF) binding sites in the promoter sequences of differentially expressing genes were studied with

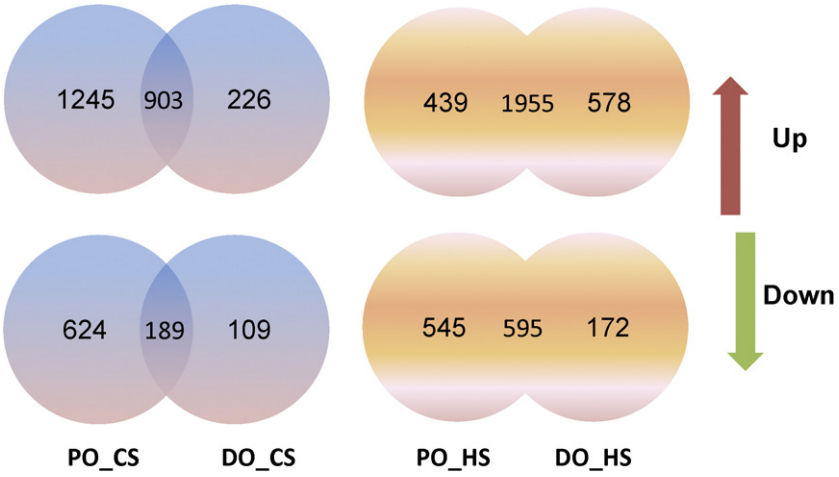

Fig. 1. Global changes in gene expression profiles during combination of stresses. Numbers represent the differentially regulated genes. PO_CS; OS4h prior to CS5h, DO_CS; OS4h during CS5h, PO_HS; OS4h prior to HS30min and DO_HS; OS4h during HS30min. Detailed gene lists with fold change values, hierarchical clusters pertaining to these numbers are provided in Supplementary Data 1.