Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Comparative analysis of gene expression in response to cold stress in diverse rice genotypes

Gabriela Peres Moraes de Freitas [a, b], Supratim Basu [a], Venkategowda Ramegowda [a], Eugenia Bolacel Braga [b], Andy Pereira [a, *]

[a] Crop, Soil, and Environmental Sciences, University of Arkansas, Fayetteville, AR 72701, USA
[b] Department of Botany, Federal University of Pelotas, Pelotas, Brazil

ARTICLE INFO

Article history:
Received 31 January 2016
Accepted 1 February 2016
Available online xxx

Keywords:
ABA
Cold
Photosynthesis
ROS
Transcription factor

ABSTRACT

Cold stress is a major factor affecting rice (*Oryza sativa*) growth and productivity, limiting its distribution worldwide. Rice production is affected primarily due to its vulnerability to cold stress at seedling stage, as well as reproductive stage leading to spikelet sterility. We report here the analysis of 21 diverse rice genotypes from the USDA mini-core collection for cold tolerance and categorized their tolerance levels on the basis of reduction in growth measured by root and shoot length. The screening identified 12 cold tolerant genotypes from which six tolerant genotypes were characterized at the vegetative stage for cold tolerance and gas-exchange parameters. Two tolerant and two sensitive genotypes were used further for gene expression analysis. Lipid Transfer Protein (LTP) genes showed a clear difference in expression between cold tolerant and sensitive genotypes suggesting that they are good candidates for engineering cold tolerance in rice. Nipponbare was identified as a cold tolerant genotype with stress tolerance mechanism potentially operating via both ABA dependent and independent pathways.

1. Introduction

Cold stress, classified as freezing (<0 °C) or chilling (0–15 °C) plays a key role in determining the growing season and geographical distribution of plants [1]. The sensitivity and symptoms of plant responses to cold stress varies with the growth stage. Plants subjected to cold stress at the germination stage show delayed and lower percentage of germination, while at the vegetative stage symptoms are expressed through yellowing of leaves, lower stature, and decreased tillering of the rice plants [2]. Exposure of plants to cold stress severely affects the photosynthetic machinery, more specifically the ultrastructure of chloroplasts, altering the light harvesting chlorophyll antenna complexes [3] and/or modifying thylakoid structures [4]. The restriction of photosynthetic processes by cold temperatures thereby leads to a lack of plant energy resources. Cold stress induced ROS accumulation [5] is capable of causing severe damage to various cellular components such as altering membrane lipid composition due to excess accumulation of malondialdehyde (MDA), structural proteins and enzymes. The survival of plants under low temperatures involves two distinct mechanisms: chilling tolerance and cold acclimation. Chilling tolerance can be defined as the intrinsic ability of the plant to survive under low temperature without injury or damage [6] while cold acclimation is the enhanced potential of the plant to tolerate the physical and physiochemical abnormalities of cold stress [7].

Plants upon perceiving cold stress stimulus become proactive to restore normal metabolite levels, and most importantly, metabolic fluxes [8]. The modification of metabolic responses via increased accumulation of antioxidants and osmolytes is coupled to enhanced tolerance, achieved through an intricate stress signaling network. Previous studies on cold tolerance have identified several regulons comprising of transcription factor (TF) CBF/DREB (C-repeat binding factors, also known as *DEHYDRATION-RESPONSIVE ELEMENT-BINDING* protein or *DREBs*) and its cold-inducible target genes, *KIN* (cold-induced), or *LTI* (low-temperature-induced) [9,10]. In addition to the TFs, several other genes like FRO1 (FROSTBITE 1) encoding ferric reduction oxidase 1, and OsFAD2 encoding fatty acid desaturase 2 have been identified in Arabidopsis and rice, respectively, that contribute to cold tolerance through increased expression of *cold responsive* (*COR*) genes or by maintaining

---

* Corresponding author. 115 Plant Sciences Building Crop, Soil and Environmental Sciences, University of Arkansas, Fayetteville, Arkansas 72701, USA.
E-mail address: apereira@uark.edu (A. Pereira).

http://dx.doi.org/10.1016/j.bbrc.2016.02.004
0006-291X/© 2016 Elsevier Inc. All rights reserved.

Please cite this article in press as: G.P. Moraes de Freitas, et al., Comparative analysis of gene expression in response to cold stress in diverse rice genotypes, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.02.004

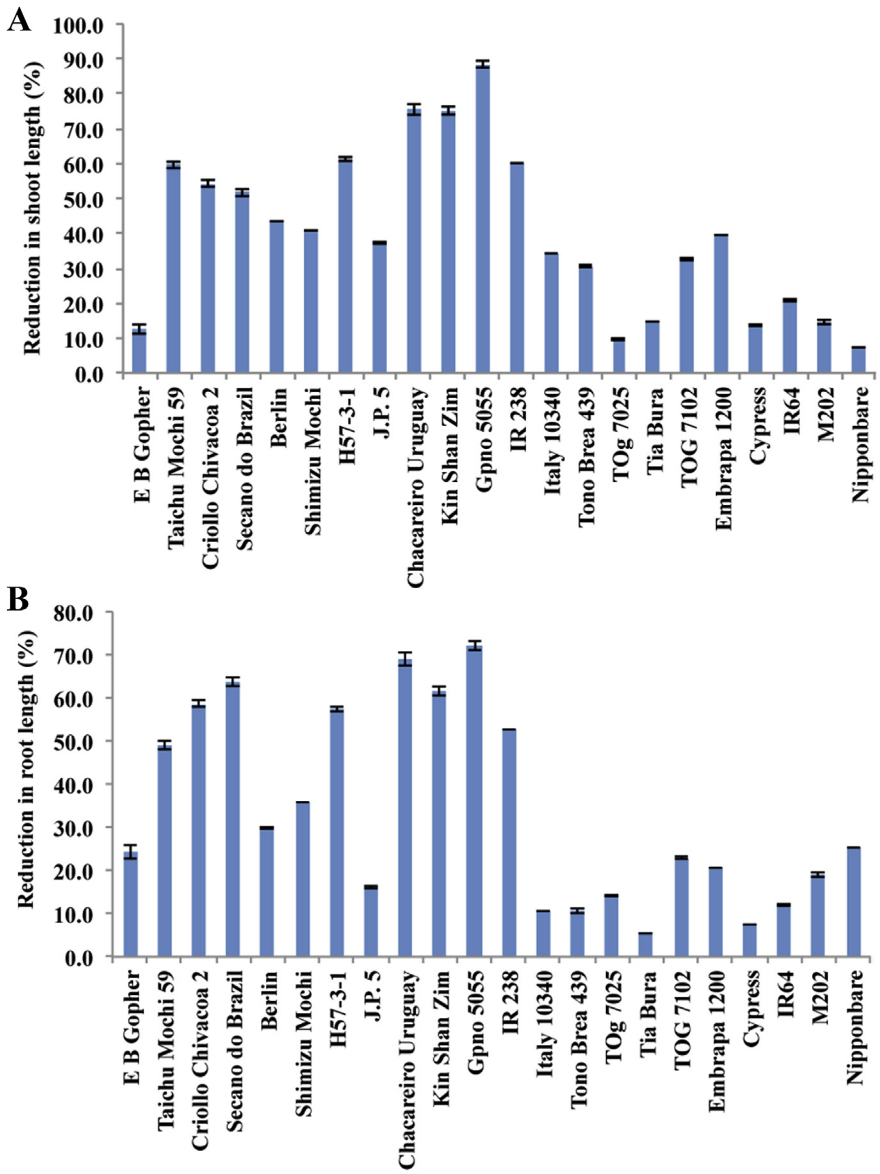

Fig. 1. Phenotypic changes of rice seedlings in response to cold stress. Dehusked and sterilized seeds were germinated in ½ MS medium for 7 days. Cold stress was applied by keeping the plants at 10 °C for 96 h. Plants kept at 28 °C served as control. Cold stress tolerance of different genotypes was estimated by measuring the shoot and root length. Response of different rice genotypes to cold stress was indicated by reduction in (A) Shoot length, and (B) Root length relative to the control.

membrane fluidity [11,12].

Rice is more vulnerable to cold stress at the reproductive stage not only showing spikelet sterility but also incomplete panicle exertion and spikelet abortion [13]. During the course of evolution, the continuous selection and breeding efforts have led to the development of rice cultivars that are abiotic stress tolerant. Some rice genotypes have evolved naturally which are tolerant to cold, salinity and other stresses, but are low yielders, however serve as excellent genetic resources for stress tolerance. Previous research has identified 40 quantitative trait loci (QTLs) in different combinations contributing to cold tolerance [14]. Hence, it becomes essential to identify naturally occurring cold tolerant rice genotypes with diverse mechanisms from the rice germplasm for developing cold tolerant rice. In this study we screened a diverse panel of rice genotypes for cold tolerance at the seedling stage. Selected genotypes were tested for cold tolerance response parameters at the vegetative stage followed by the gene expression analysis to dissect the potential cold tolerance mechanisms operating in these genotypes.

2. Materials and methods

2.1. Screening for cold stress tolerance at vegetative stage

For cold stress treatment, the plants were kept at 10 °C for 48 h. Leaf rolling, a symptom of cold stress, was monitored. Measurement of photosynthesis and photochemical efficiency of PSII during steady state illumination were taken on the second fully expanded leaf using a portable photosynthesis meter (LI-6400XT; LI-COR) at a CO_2 concentration of 370 μmol mol^{-1}, light intensity of 1000 μmol $m^{-2}s^{-1}$, and 55%–60% RH. Instantaneous water use efficiency (iWUE) was calculated using data obtained from the photosynthesis meter as = (photosynthesis/transpiration rate). RWC was measured with modifications as described [15] in the

Please cite this article in press as: G.P. Moraes de Freitas, et al., Comparative analysis of gene expression in response to cold stress in diverse rice genotypes, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.02.004