

GENE EXPRESSION ANALYSIS IN WEEDY AND CULTIVATED *INDICA* RICE IN RESPONSE TO COLD TREATMENT

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Palavras-chave: *Oryza sativa*, abiotic stress, seedling stage.

INTRODUÇÃO

Temperature plays a key role in the metabolism of organisms and thereby is pivotal in determining the growing season and geographical distribution of plants. Cold stress can be classified as chilling (<20°C) and freezing (<0°C) stress (CHINNUSAMY et al., 2006). Cold stress adversely affects growth and development of warm-season plants, such as rice, and thus limits crop productivity. *Oryza sativa* (rice) diverged into two major subspecies – *Indica* and *Japonica*. The majority of rice cultivars grown in Brazil are *Indica* subspecies, more susceptible to cold stress, which exhibits reduced germination under cold stress (SOUZA, 1990). Injury from cold is reflected in reduced values of germination speed index and emergence speed index (TERRES, 1991). Developing cold-tolerant cultivars will improve the yield of early-planted rice.

We aimed to 1) categorize rice accessions as *Japonica* or *Indica*; 2) classify *Indica* subspecies as cold-sensitive or –tolerant; and 3) analyze the expression of cold-inducible genes under cold treatment.

MATERIAL E MÉTODOS

Accessions were classified as *Indica/Japonica* by phenol test. To evaluate tolerance to cold stress, seeds were germinated and grown for two weeks either at 25°C or at 18/13°C. Twenty-one weedy red rice and rice accessions (from Brazil and USA) were tested in 3 replicates. Evaluation was done after 14 days by measuring shoot length. Accessions were compared based on % reduction of shoot length under cold stress relative to the control (normal temperature).

Expression of cold-inducible genes was analyzed by qRT-PCR with total RNA isolated from the cold-sensitive and –tolerant *Indica* accessions after exposing 10-day-old seedlings to cold stress (10°C) for 6, 24, and 96 h. Plants were washed, coleoptiles were harvested; samples of equal fresh weight were frozen in liquid nitrogen and immediately homogenized for total RNA extraction.

A total of 16 cold-inducible and other selected genes including *NAC*, *MYB*, *Germin*, and *Rab16* were analyzed. To determine fold-change differences in gene expression, the Ct value for each gene was normalized relative to the Ct value for ubiquitin and was calculated using the equation $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001).

RESULTADOS E DISCUSSÃO

Of 21 accessions tested, 9 were *Japonica* and 12 were *Indica*. Two *Indica* and one *Japonica* were tolerant to cold stress; the rest were sensitive. We then selected three *Indica* accessions (sensitive

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CHI08-C; tolerant GRE08-D01 and 1602) to analyze the expression profiles of cold-inducible genes (Figure 1).

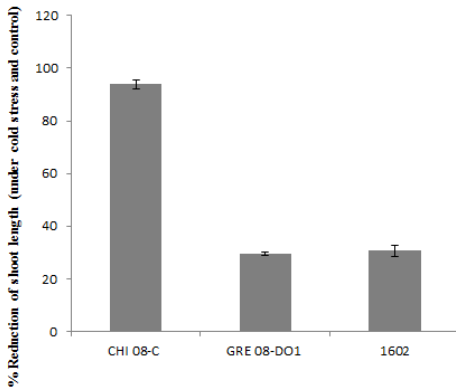


Figure 1. Response of selected tolerant and sensitive rice genotypes to cold treatment. University of Arkansas, 2013.

The transcription factor genes *NAM*, *DRE2A*, *MYB* and *F-box* were all up-regulated under cold stress in the cold-tolerant accession from the GRE08-D01, whereas only *NAM* and *F-box* were highly induced in the sensitive accession CHI08-C. The data also indicate that the cold-tolerant accession 1602 has a different tolerance mechanism from the GRE08-D01 accession (Figure 2).

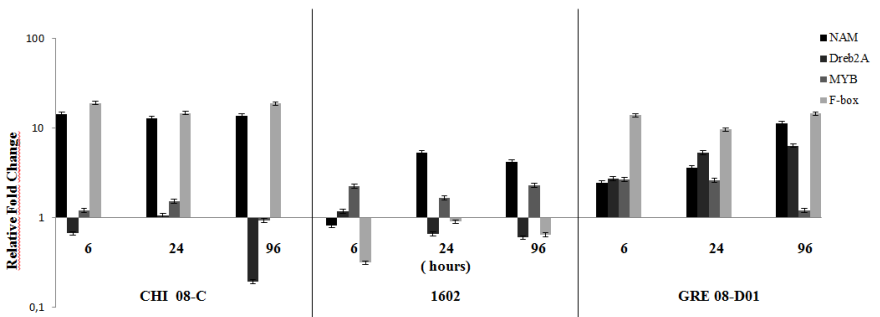


Figure 2. Effect of cold stress (10°C) on transcription factors *NAM*, *DREB2A*, *MYB* and *F-box* gene expression. Coleoptiles were dissected from 10-d-old seedlings of sensitive (CHI08-C) and tolerant (1602 and GRE08-D01) *Indica* subspecies. Seedlings were exposed to cold stress for 6, 24, and 96 hours before RNA extraction. University of Arkansas, 2013.

All cytosolic protein genes were up-regulated in both GRE08-D01 (tolerant, USA) and CHI08-C (sensitive, USA) within 24 h of cold exposure; however, the amount of transcript accumulation was higher in GRE08-D01 (Figure 3). These genes remained highly expressed in the tolerant USA

accession at 96 h of cold exposure, but only *Rab16* remained up-regulated in the sensitive accession. The expression profiles of cytosolic protein genes in 1602 differed from that of the tolerant GRE08-D01 accession. In general, transcript accumulation was higher in GRE08-D01 than in 1602 especially for *Glutamate dehydrogenase* and *Germin*. The only gene that was highly expressed in 1602 than in GRE08-D01 at any time point was *H⁺ pyrophosphatase* at 6 h of cold treatment.

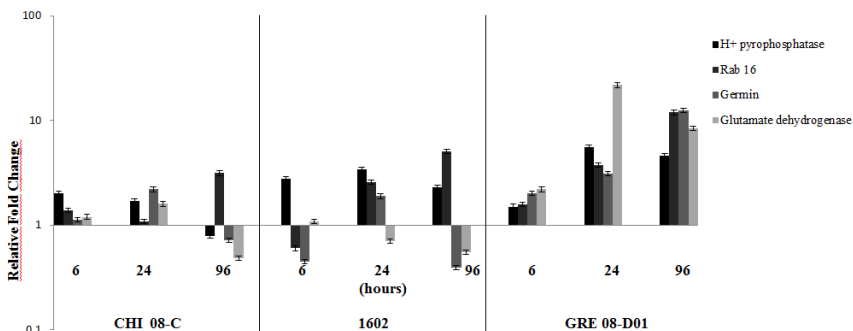


Figure 3. Effects of cold stress (10C) on H⁺ pyrophosphatase, Rab 16, GERMIN and glutamate dehydrogenase gene expression. Coleoptiles were dissected from 10-d-old seedlings of sensitive (CHI08-C) and tolerant (1602 and GRE08-D01) *Indica* subspecies. Seedlings were exposed to cold stress for 6, 24, and 96 h before RNA extraction. University of Arkansas, 2013.

Studying the expression patterns of cold-inducible genes help elucidate how rice plants cope with cold stress. Follow-up research needs to identify primary gene networks involved in cold tolerance and adaptation, unique gene expression patterns in cold-tolerant rice, key genes or genetic elements controlling such response, and harnessing such genes to infuse cold tolerance to elite cultivars. These patterns of differential transcript accumulation certainly indicate a signalling cascade that involves activation of ABA-dependent pathways, among others. This is not unlike those reported under osmotic stress (Mahajan and Tuteja, 2005), which indicates some universal stress adaptation mechanisms. Tolerance could be the manifestation of differential regulation of these pathways.

CONCLUSÃO

The rice accessions were categorized as *Japonica* or *Indica*. The evaluation of genotypes identified some cold-tolerant *Indica* rice from Brazil and the USA. Differential transcript accumulation was observed for the genes investigated. *DREB2A* and *NAM* showed a large difference in expression between cold-tolerant and cold-susceptible accessions; the same was observed with *H⁺ pyrophosphatase*, *Rab 16*, *GERMIN* and *glutamate dehydrogenase* transcript accumulation. Future experiments can focus on these genes.

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