12. Comparative expression analysis of some stress responsive genes in *indica* and *japonica* rice cultivars showing different levels of abiotic stress tolerance

A. L. RANAWAKE, F. KOBAYASHI, O. E. MANGKIL, S. TAKUMI, N. MORI and C. NAKAMURA
Laboratory of Plant Genetics, Graduate School of Agricultural Science, Kobe University, Kobe 657-8501, Japan,
Corresponding Author, E-mail: nakamura@kobe-u.ac.jp

Abiotic stresses are major constraint in crop production. For the identification and proper manipulation of the responsive genes towards development of stress tolerant rice cultivars, reliable bioassay methods for evaluating the level of tolerance against abiotic stresses and precise knowledge of induction, suppression and varietal specificity in gene expression under stresses are needed. In this study we devised new bioassay methods suitable for evaluating the level of tolerance against cold, dehydration and salinity stresses. For comparison, we used a *japonica* rice cultivar Hyogo-Kitanishiki (HGKN) and an *indica* cultivar Hokuriku142 (HOK). HGKN is a sake-brewing cultivar bred in Hyogo Institute of Agriculture, Forestry and Fishery, Japan and HOK was bred from a cross between a Korean cultivar `Milyang 21` and an IRRI line `IR-2061-214-31` in Hokuriku Agricultural Experimental Station, Japan. For the evaluation of cold tolerance, seedlings were grown under standard conditions (25 ℃, 16h/8 hr light/dark regime) for seven days in soil-filled Petri dishes and on the 7th day they were transferred to 4 ℃ with all other conditions remaining the same. Cold-stressed seedlings were transferred back to the standard conditions and grown for 1 to 5 days for recovery. After the recovery periods、under the standard conditions, green shoot length, root length, plant fresh weight and dry matter weight were measured. For the evaluation of dehydration tolerance, 7-day-old seedlings grown in soil-filled Petri dishes (7 cm in diameter and 1.5 cm in depth) under the standard conditions were subjected to complete drying for 2 days after cutting off water supplement on the 7th day of planting. After the dehydration treatment, seedlings were allowed to recover under the standard conditions by supplying enough water for 10 days. At the end of the experiment, the same four growth parameters as ones used in the bioassay for cold tolerance were recorded. Salinity tolerance was evaluated using 12-day-old seedlings that were kept for 8 days in a series of NaCl solution prepared to the EC (electric conductivity) levels equivalent to 1 dS/m (6.84 mM) to 14 dS/m (95.72 mM). NaCl was dissolved in 1 ppm Hyponex (N:P:K=1:2:1) solution. After the NaCl treatment, seedlings were allowed to recover in 1 ppm Hyponex solution. The same four growth parameters were measured on the 10th day.

In the bioassay for evaluating cold tolerance, all HOK seedlings died within 5 days of the recovery period, while HGKN did not show much damage as compared to the control plants at least until 4th day of the cold stress (Fig. 1A and 1B). In the bioassay for dehydration tolerance, the two cultivars showed significant difference at the 10th day of the recovery period after 2-days of dehydration stress; HGKN was more tolerant than HOK (Fig. 1C). In the experiment designed to determine the NaCl concentration for evaluating salinity tolerance, EC 5dS/m to EC 6dS/m were found to clearly distinguish the cultivar difference, i.e. higher tolerance in HGKN than in HOK (Fig. 1D). Under these bioassay conditions, HGKN was more tolerant than HOK against all of the three abiotic stresses, indicating that HGKN possesses common and/or different tolerance mechanisms against all these stresses.

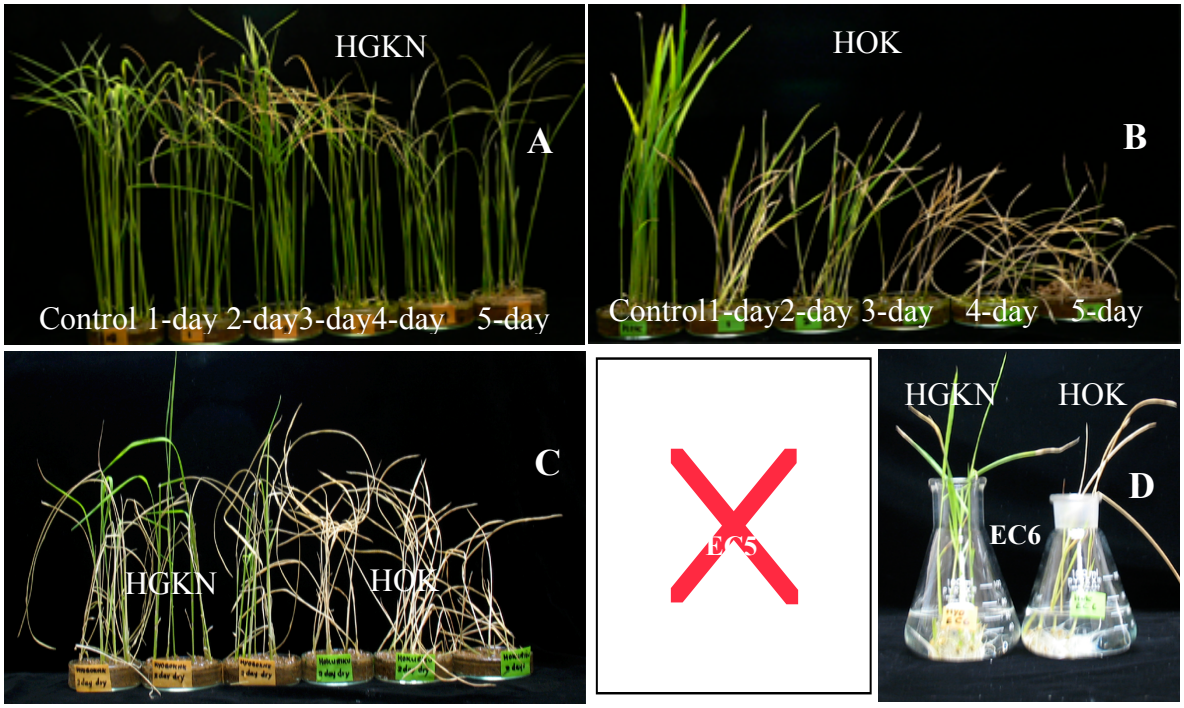

Fig.1. Comparison of the levels of stress tolerance between HGKN and HOK. A) Cold stress. 7-day-old HGKN seedlings were kept at 4 ℃ for 1-day, 2- days, 3-days, 4-days and 5-days. B) Cold stress. 7-day-old HGKN seedlings were kept at 4 ℃ for 1-day, 2- days, 3-days, 4-days and 5-days C) Dehydration stress; 7-day-old seedlings (three replications of each cultivar) were dehydrated for 2 days and recovered at the standard conditions. D) NaCl stress. 12-day-old seedlings were treated by NaCl solution at EC levels 5 dS/m (equivalent to 34mM) and 6 (40.8mM) for 8 days and recovered under the standard conditions.

We next carried out a comparative study of the gene expression levels of 12 candidate genes by RT-PCR under the stress conditions (Fig. 2). Genes we studied included those encoding transcription factors *OsDREB(Dehydration Responsive Element Binding)1A* and *OsDREB1B* (Dubouzet et al. 2003), alternative

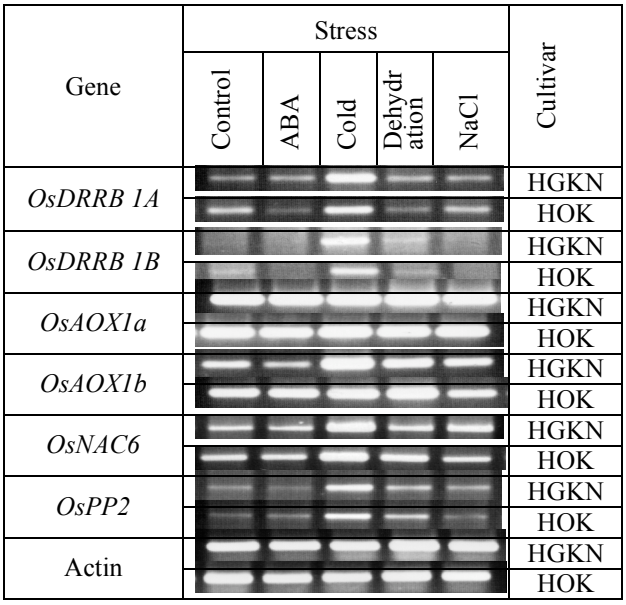

Fig. 2. RT-PCR analysis of the transcript levels of some stress responsive genes in the leaves of one-week-old seedlings under stress conditions: ABA, 200 mM exogenous ABA for 30 min; Cold, at 4 ℃ for 1 day; dehydration, drying at 25 ℃ for 6 h, NaCl, dipping up-rooted 1 week-old-seedlings in 400 mM NaCl solution for 1h at 25 ℃. PCR cycle number: *OsDREB1A* and *OsDREB1B* 25 cycles and for all the other genes 30 cycles.

oxidase paralogs *OsAOX1a* and *OsAOX1b* (Ito 1997), NAC-type DNA binding protein OsNAC6, and protein phosphatase *OsPP2* (Rabbani et al. 2003). Since ABA signaling pathway has been known to play a role in some stress responses (Shinozaki et al. 1997), the effect of exogenous ABA was also studied.

After subjecting the two cultivars to the three abiotic stresses for different periods, we found that five genes (*OsDREB1A, OsDREB1B, OsAOX1b, OsNAC6* and *OsPP2*) showed marked increases in their transcript abundance in response to at least cold stress. No apparent changes however were observed in seven other transcripts under all stress conditions studied (data not shown). The level of *OsPP2* transcript showed an increase also under dehydration stress. In all of the five responsive genes, the highest level of increase was observed against cold stress. ABA caused no apparent changes in the gene expression level in both cultivars. Despite the clear differences in the tolerance level against all three abiotic stresses between the two cultivars, no clear differences in the transcript levels were observed in all genes except for the two DREB genes, *OsDREB1A* and *OsDREB1B*, whose transcript level showed marked increases under cold stress. *CBF*(*C-repeat Binding Factor)*/*DREB1* transcription factors are known as key regulators of the cold signal transduction in various plant species (Thomashow 1999). Our observation that cold stress caused greater increases in the *DREB* transcript levels in a cold tolerant HGKN than in a cold susceptible HOK suggest that *DREB* genes play a key role in determining cold tolerance in these two rice cultivars.

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