Development and Identification of Transgenic Rice Lines with Abiotic Stress Tolerance by using a Full-length Overexpressor Gene Hunting System

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ABSTRACT The latest report on the draft genome of *Brassica rapa* sequence has been published. To elucidate the functions of these genes and to efficiently search for agriculturally useful genes, a Full-length cDNA Over-eXpressor (FOX) gene hunting system was used. The FOX library from Chinese cabbage was introduced into rice via *Agrobacterium*-mediated transformation. Approximately 1,150 FOX-rice lines were generated. Genomic PCR analysis indicated that the average length of FL-cDNAs introduced into individual lines was 900~1,200 bp. Basic Local Alignment System Tool (BLAST) analysis of the FL-cDNA genes revealed that 35.5% have unknown function. Most of the randomly selected transgenic rice lines showed overexpression (92%) of these genes relative to the wild-type Gopum. Moreover, 94% of the 850 transgenic rice lines were moderately tolerant (slightly yellow) to cold and 9 lines were tolerant (seedlings were light green). Morphological evaluation of the transgenic rice lines showed minimal phenotypic alteration (12%). Approximately 25.1% and 22% of the plants were significantly ahead in the days to heading and had elevated chlorophyll content, respectively. Other agronomic traits such as filled grains, number of tiller, panicle length, and culm and plant height were relatively less variable among the transgenic lines. These results provide a resource for defining genes that are associated with tolerance in transgenic rice lines.

Keywords FOX-hunting system, Full-length cDNA, Abiotic stress, Transgenic plant, Rice

INTRODUCTION

Chinese cabbage (*Brassica rapa* subsp. *pekinensis*) is one of the most important *Brassica* species vegetables found in Asian countries. The draft genome sequence of *B. rapa* has been recently published (The *Brassica rapa* Genome Sequencing Project Consortium 2011). The Chinese cabbage genome contains 41,174 protein-coding regions, whereas 27,379 genes are currently believed to exist in the *Arabidopsis* genome (TAIR9 information: <http://www.arabidopsis.org/>). Appropriate biological experiments are needed to uncover the functions of these genes.

To accelerate the identification and characterization of

gene function, various resources, including *Arabidopsis* and rice information, are publicly available (Nakamura et al. 2007). The *Arabidopsis* Information Resource (TAIR) maintains a database of genetic and molecular biology data for the model higher plant *Arabidopsis thaliana* (www.arabidopsis.org). Data available from TAIR includes the complete genome sequence along with gene structure, gene product information, metabolism, gene expression, DNA and seed stocks, genome maps, genetic and physical markers, publications, and information about the *Arabidopsis* research community. Along with this, infrastructure for rice bioinformatics (reviewed in Sasaki et al. 2005), including integrated databases such as RAP-DB (Ohyanagi et al.

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2006; Rice Annotation Project 2007) and the TIGR Rice Genome Annotation Database (Ouyang et al. 2007), BGI-RIS (Zhao et al. 2004), and MOsDB (Karlowski et al. 2003), is also publicly available. The available information provides an important resource for the analysis of *Brassica rapa* genes.

For large-scale identification of gene function, several systems and tools have been developed to generate loss-of-function mutants (Hirochika et al. 2004; An et al. 2005). These loss-of-function resources and databases are very important for tagging and hunting non-redundant genes in the rice genome. However, 29% of rice genes are estimated to exist within clustered and redundant gene families (IRGSP 2005). Therefore, it is difficult to find loss-of-function phenotypes for these duplicated genes. Moreover, when knockouts of genes confer embryonic lethality or severe developmental defects upon plants, it is impossible to the phenotypes associated with mutation or deletion of these genes (Nakamura et al. 2007). To solve this problem, FL-cDNA clones (Seki et al. 2002; Ichikawa et al. 2006) were recently developed as an alternative gain-of-function approach for the systematic elucidation of gene functions in *Arabidopsis*; the system is the so-called Full-length cDNA Over-eXpressor gene hunting system (FOX hunting system). FOX uses ectopic overexpression of a single or limited numbers of FL-cDNA(s) in individual transgenic plants. It generates large numbers of dominant mutations, enabling comprehensive characterization of novel and important traits and identification of the causal genes.

Studies on genetic transformation of plants have revealed an unexpected finding; the loss of mRNA encoded by either the introduced transgene or the transgene and endogenous homologous gene(s) (Vaucheret and Fagard 2001). These gene-silencing phenomena are believed to be due to methylation induced premeiotically (MIP), repeat-induced point mutation (RIP), RNA interference (RNAi), and repeat-induced gene silencing (RIGS) (Fagard and Vaucheret 2000; Marathe et al. 2000). In order to avoid this problem during our study of gene function, FL-cDNAs genes from Chinese cabbage were transformed into the rice cultivar Gopum.

In this study, clones with full-length cDNA from Chinese cabbage were sequenced, and transgenic rice

plants were subsequently generated via *Agrobacterium*-mediated transformation. The function of each clone was classified according to the annotation function based on BLAST analysis (National Center for Biotechnology Information; www.ncbi.nlm.gov). Moreover, the FOX rice lines generated were evaluated in the fields to determine their morpho-agronomic characteristics and phenotypic traits, as well as their responses to cold and salinity.

MATERIALS AND METHODS

Plant materials and field trials

The rice variety Gopum was used as the wild-type strain in the generation of transgenic rice. Regenerated plants were transplanted in soil consisting of 50% compost and 50% earth soil and acclimatized for 2 weeks in a greenhouse. Young leaves were collected for genomic DNA analysis using *pBigs_sfiI* and *hpt* primers (GenBank Accession No. AF234296). Subsequently, plants that were confirmed to carry the gene insert were harvested, and the T₁ seeds were used in the succeeding generation in the field following the protocol of Sun et al. (2011). The varieties were sown in a seedling-growing tray and placed in the greenhouse for 3 weeks in May, 2011. Grown seedlings were transplanted in the experimental farm spaced at 30×15 cm with one seedling per hill and arranged in randomized complete block design replicated three times. The cultivation and management were performed according to the standards of the experimental farm in Chungbuk National University.

Vector construction with full-length cDNAs

Brassica rapa clones with full-length cDNA were ligated into the *pBigs_sfiI* vector. The recombinant vector carrying these clones was constructed under the control of the *pUBI-1* promoter and NOS terminator, as shown in Fig. 1. Each clone was grown in Luria-Bertani medium containing 50 mg/L kanamycin sulfate and incubated at 28°C on a shaker for 24–36 h. Plasmid DNA, extracted according to the Qiagen plasmid kit (Cat. No. 12125) protocol, was used in the sequence analysis.

Fig. 1. Schematic diagram of the binary Ti plasmid pFLCIII containing the full length cDNA from Chinese cabbage. pBigs vector was consisted of two different SfiI sites [SfiI(A) and SfiI(B)].

Generation of transgenic rice

Transgenic rice was generated following the protocol of Toki et al. (2006), with some modifications (Lee et al. 2011). Briefly, dehulled mature seeds of the *japonica* rice cultivar Gopum were sterilized with 70% ethanol for 5 min. These seeds were further sterilized by strong shaking in 30-35 mL of 2.5% sodium hypochlorite containing one drop of Tween 20 for 15 min. The sterilized seeds were

washed 20 times in distilled water, dried in the clean bench for 30 min, and then inoculated on embryogenic callus induction (2N6) medium (Table 1). This set up was incubated in the dark at 32°C for 7-10 days.

Agrobacterium strain EHA105 harboring binary Ti plasmid pFLCIII (Fig. 1) containing the full-length cDNA gene was cultured on AB medium containing 50 mg/L kanamycin sulfate solidified with 1.5% agar incubated for

Table 1. Identified transgenic rice with cold, salinity and drought tolerance.

Clone	Tolerance to NaCl stresses (130 mM)	Tolerance to Cold	Drought (20% PEG)	Gene function (based on BLAST)
BR5	-	++	-	Unknown function
BR7	+	++	+	DEAD BOX RNA helicase RH15
BR15	-	+	+	UDP-glucose 4-epimerase
BR37	+	++	+	CBL-interacting protein kinase 1
BR39	-	++	-	Carbon-monoxide oxygenase (ACYB-1)
BR40	+	+	-	Phosphatase 2C, putative / PP2C
BR74	+	+	-	Haloacid dehydrogenase-like hydrolase
BR91	+	+	-	Unknown protein (AT3G12210)
BR115	+	++	+	At5g25810 mRNA
BR119	+	++	+	Unknown function
BR503	-	++	-	Unknown function
BR522	-	++	-	Hypothetical protein
BR541	-	++	-	Light harvesting complex PSII
BR547	-	++	-	UDP galactose transporter 3

+, tolerant; ++, highly tolerant; -, susceptible. Wild type, Gopum is moderately tolerant to cold but susceptible to either salinity and drought. Evaluation was based on SES for Rice (IRRI-SES, 2002)

3 days at 28°C in the dark. *Agrobacterium* culture was scraped from the plates and suspended in AAM medium to yield an OD₆₀₀ of approximately 0.3. Pre-cultured seeds were immersed in the *Agrobacterium* suspension by gently inverting the tube for 15 min, blotted dry with a sterilized filter paper to remove excess bacteria. The seeds were transferred onto a sterilized filter paper that had been moistened with 0.5 mL of AAM medium placed on 2N6-AS medium solidified with 0.4 % Gelrite. After 3 days of co-cultivation at 25°C in the dark, seeds were washed 5 times in sterilized water, and then washed once in sterilized water containing 500 mg/L carbenicillin to remove *Agrobacterium* cells. The seeds were rapidly blotted dry on a sterilized filter paper and cultured on N6D (Table 2) medium containing 50 mg/L hygromycin and 400 mg/L carbenicillin under continuous light at 32°C for 2 weeks. Proliferating calli arising from the scutellum were transferred to REIII (Table 2) medium. Plantlets arising from the calli were transferred to HF medium to induce root formation.

DNA extraction and PCR analysis

Genomic DNA was extracted as described by Cho et al. (2007) with some modifications. The relative purity and concentration of extracted DNA was estimated using NanoDrop-1000 (NanoDrop Technologies, Inc. USA). PCR analyses were performed using HPT-Fw (5'-GGA-TTTCGGCTCCAATGTCCTGACGGA-3') and HPT-Rv (5'-CTTCTACACAGCCATCGGTCCAGA-3') primers to check the introgression of hygromycin phosphotransferase gene (*hpt*) and *pBigs_sfil*-Fw (5'-TATTCGGAGAGGG-TACGTATTTTAC-3') and *pBigs_sfil*-Rv (5'-GCA-ACAGGATTCAATCTTAAGAAACT-3') to check the introduction of the full-length cDNA gene.

Southern blot analysis

For DNA blot analysis, 10 µg of genomic DNA was extracted as mentioned above. A digoxigenin (DIG) probe was synthesized according to the manufacturer's instructions (PCR DIG Probe Synthesis Kit; Roche Molecular Biochemicals, USA). The template for the probe synthesis was

Table 2. Composition of media used for tissue culture and transformation of rice.

Medium	Composition
2N6 (Embryogenic callus induction)	4 g/L Chu (N ₆) medium (Micro and macro elements including vitamins) (Cat. No. C0204, Duchefa Biochemie B.V.), 0.3 g/L Casein hydrolysate, 0.5 g/L Glutamine, 0.5 g/L Proline, 30 g/L Sucrose, 2 mg/L 2.4-D, 2.5 g/L Gelrite (pH 5.8)
AB (Agrobacterium culture)	5 g/L Glucose, 15 g/L Bacto agar, 3 g/L K ₂ HPO ₄ , 1 g/L NaH ₂ PO ₄ , 1 g/L NH ₄ Cl, 0.3 g/L MgSO ₄ · 7H ₂ O, 0.15 g/L KCl, 13.25 mg/L CaCl ₂ · 2H ₂ O, 2.5 mg/L FeSO ₄ · 7H ₂ O (pH 7.2)
AAM-AS (Agrobacterium suspension and infection)	0.12 g/L MgSO ₄ , 0.15 g/L CaCl ₂ · 2H ₂ O, 0.15 g/L NaH ₂ PO ₄ · 2H ₂ O, 3 g/L KCl, 0.5 g/L Casamino acid, 0.177 g/L L-Arginine, 0.04 g/L EDTA Ferric sodium, 7.6 mg/L MnSO ₄ · H ₂ O, 2 mg/L ZnSO ₄ · 7H ₂ O, 0.025 mg/L CuSO ₄ · 5H ₂ O, 0.025 mg/L CoCl ₂ · 6H ₂ O, 0.75 mg/L KI, 3 mg/L H ₃ BO ₃ , 0.25 mg/L Na ₂ MoO ₄ · 2H ₂ O, 0.75 mg/L Glycine, 0.09 g/L L-Glutamine, 0.03 g/L L-Aspartic acid, 0.01 g/L Myo-Inositol, 0.1 mg/L Nicotinic acid, 0.1 mg/L Pyridoxine HCl, 1 mg/L Thiamine HCl, 68.5 g/L Sucrose, 36 g/L D-Glucose, 20 mg/L Acetosyringone (pH 5.2)
2N6-AS (Co-culture)	4 g/L Chu (N ₆) medium (Micro and macro elements including vitamins) (Cat. No. C0204, Duchefa Biochemie B.V.), 0.3 g/L Casein hydrolysate, 0.5 g/L Glutamine, 0.5 g/L Proline, 30 g/L Sucrose, 10 g/L Glucose, 2 mg/L 2.4-D, 20 mg/L Acetosyringone, 0.15 g/L Dithiothreitol, 0.25 g/L Sodium thiosulfate, 5 mg/L Silver nitrate (pH 5.2)
N6D (Transgenic callus selection)	4 g/L Chu (N ₆) medium (Micro and macro elements including vitamins) (Cat. No. C0204, Duchefa Biochemie B.V.), 0.3 g/L Casein hydrolysate, 0.5 g/L Glutamine, 0.5 g/L Proline, 30 g/L Sucrose, 2 mg/L 2.4-D, 2.5 g/L Gelrite, 250 mg/L Cefotaxime, 50 mg/L Hygromycin (pH 5.8)
REIII (Shoot formation)	4.4 g/L MS medium including vitamins (Cat. No. M0222, Duchefa Biochemie B.V.), 0.5 mg/L NAA, 2 mg/L 6-BA, 20 g/L Sucrose, 30 g/L Sorbitol, 2 g/L Casamino acid, 250 mg/L Cefotaxime, 80 mg/L Hygromycin, 4 g/L Gelrite (pH 5.8)
HF (Root formation)	4.4 g/L MS medium including vitamins (Cat. No. M0222, Duchefa Biochemie B.V.), 30 g/L Sucrose, 7.5 g/L Plant agar, 250 mg/L Cefotaxime, 80 mg/L Hygromycin (pH 5.8)

from the vector containing the full-length cDNA clone. The forward/ reverse primers used for the synthesis of the probe were 5'-GATTAAGCATCCAAATGTTGTTCA-3' and 5'-CCTACGAGCCTCATCTTCT-3'. The PCR amplification was initiated with a denaturation at 95°C for 2 min, followed by 30 cycles of 95°C for 30 s, 56°C for 30 s, and 72°C for 60 s, and the final elongation step at 72°C for 7 min. Ten micrograms of genomic DNA was digested overnight at 37°C with the restriction enzymes *Bam*HI and *Eco*RI. The digested DNA was subjected to electrophoresis in a 0.8% agarose gel and transferred to a Hybond-N⁺ membrane (Amersham Biosciences, USA), according to the manufacturer's instructions. The membrane was pre-hybridized at 42°C in a DIG East Hyb buffer for 1 h. A DIG-labeled probe was then added (2 µL of probe/mL of buffer) and hybridization was carried out overnight at 42°C. The membrane was washed twice with a low-stringency washing solution (2× SSC, 0.1% SDS) at 25°C for 5 min, and then washed twice with a high-stringency solution (0.1× SSC, 0.5% SDS) at 68°C for 15 min. The DIG-labeled DNA was detected according to the manufacturer's instructions (DIG Nucleic Acid Detection Kit; Roche Molecular Biochemicals, USA).

Cold, salinity, and drought tolerance screen

Cold evaluation was conducted at the Chuncheon Experimental Station of the Rural Development Administration, Korea. About 50 seeds were directly seeded into germination trays (60 × 30 × 3.5 cm) with a soil-free medium of steam-sterilized sand with peat compost and vermiculite and allowed to grow for 7 weeks before cold treatment at a water temperature of approximately 13°C, and an air temperature of 30-35°C.

For the salinity and drought tolerance test, styrofoam seedling floats were fabricated as described by Manigbas et al. (2011). Holes were drilled at the surface of the styrofoam with a nylon net supporting the bottom. Two pre-germinated seeds were placed on each hole. Seedling floats were placed on each tray comprising a replication of two, with 20 seedlings per entry. The growing plants were supplied with Murashige and Skoog (MS) solution at 4.4 gram per liter to maintain good growth and healthy

seedlings throughout the experiment. After 2 weeks, solutions of 130 mM NaCl and 20% PEG were used to induce salinity and drought, respectively on rice seedlings; this solution was changed after 8 days. The lines were scored after 2 weeks, or when the susceptible check showed high susceptibility by using the standard evaluation system for rice (IRRI-SES 2007).

RT-PCR analysis

Total RNA was extracted from the transgenic lines and wild-type Gopum plant by using the RNeasy Plant Mini Kit (QIAGEN, Maryland, USA), according to the manufacturer's instructions. This process was repeated in all experiments requiring RNA extraction. The relative purity and concentration of extracted RNA was estimated using NanoDrop-1000 spectrophotometer (NanoDrop Technologies, Inc. USA), and stored in a -80°C freezer. Total RNAs were cleaned using *DNase*I, and first-strand cDNA synthesis was performed by reverse transcription of mRNA using Oligo (dT)₂₀ primer and SuperScriptTM III Reverse Transcriptase.

The nucleotide sequence of each of the randomly selected clones was used to design primer pairs (Table 3). The specific sequences of the primer pairs were used in a semi-quantitative reverse transcription PCR (RT-PCR). Actin primers were used as loading controls and used as internal controls for normalization of the quantitative RT-PCR reaction.

Statistical analysis

Data requiring statistical analysis were computed using the Statistix version 8 (www.statistix.com). Significant P values were analyzed by using the two-sided Dunnett's multiple comparison, with the wild-type Gopum as control by using the same software. The frequency distribution of various agronomic traits and sequences was determined using Microsoft Excel 2007.

Nucleotide sequence analysis

All the inserted sequences were checked using the BLAST program in NCBI sequence database and another program in the GenBank/EMBL/DDBJ sequence database. The open reading frame (ORF) and conserved domain were

Table 3. Primers sequences used in the RT-PCR analysis and annotation of gene function by the NCBI database.

Gene	Forward Primer	Reverse Primer	Gene Function
BR 2	5'-GTGTTGACGACGTTGTGAC-3'	5'-CCGGGTCTAGATCTGTTCCA-3'	Cysteine protease 1
BR 4	5'-TGCCGTTATGCCATTATGA-3'	5'-ATCAAGACCCATCTCGGTTG-3'	Actin binding/depolymerizing factor 2
BR 7	5'-GCATTTCCTAAAGCCACCAA-3'	5'-CCAGGAGTAGGCTCGATCTG-3'	DEAD BOX RNA helicase RH15 - like protein
BR 11	5'-AACGAGAGAGGAGGGATGAT-3'	5'-CCAGAGGAGCTTCAGGGTTG-3'	Alcohol dehydrogenase 1
BR 15	5'-GCTTTGCGTGCCCTTCTTATC-3'	5'-GAAGAACCCACTTGGCAAAA-3'	UDP-glucose 4-epimerase 1
BR 22	5'-ACTTGCGAGCTTGACTCAT-3'	5'-GAGCATTTCTCCTGCGTTTC-3'	Hydrogen transporting ATPase
BR 23	5'-AATGCACGGAATAGAGG-3'	5'-CAGGTCCTCATTACCGGTGT-3'	Endoplasmic reticulum auxin-binding protein 2 mRNA
BR 37	5'-ATCGGAGGCAGCAAGTCAAGCG-3'	5'-CTCCATCGTAGCCTCCGTCAT-3'	CBL-interacting protein kinase 1
BR 39	5'-CAGGAAAAGAAGCGGATGAG-3'	5'-AGTGTGCCTGTCATCGTGAG-3'	Carbon-monoxide oxygenase (ACYB-1)
BR 43	5'-AACTCCAAGCGTTGGATATG-3'	5'-TCTCCAGGTTGCTTGTCT-3'	Hypothetical protein
BR 47	5'-CTAATGCTGACGGTGGGTTT-3'	5'-ACGAAAAGTTCGAAAGCTGA-3'	Unknown gene function
BR 61	5'-CCTCTGATCCCACCACACTT-3'	5'-TTAGGAGCAGCACACACTGG-3'	Rubredoxin family protein
BR 64	5'-AGAAGCTGATGGCTCGGTTA-3'	5'-CCTCTTCTCGGCTGAACAAC-3'	Beta-Ig-H3 domain-containing protein (FLA18)
BR 68	5'-CCTTACCGCCGCCACCATGTTT-3'	5'-AGGTGTCAAAAGTCGGGTTG-3'	Unknown gene function
BR 72	5'-ACCAAACACTCACCGGAGTC-3'	5'-ATCACCACCCGCAAGTAAAG-3'	Unknown protein function
BR 74	5'-CGATGTTTCGATGTCCTCCTT-3'	5'-GGCTCGATCATCGTCATTCT-3'	Haloacid dehalogenase-like hydrolase family protein
BR 77	5'-CATCTACCGAGCAACAGCAA-3'	5'-AATGCCCAATCCATACCAAA-3'	Unknown (<i>Zeamays</i> clone 92871)
BR 91	5'-TGGAAGCTCTCTCGTGGAA-3'	5'-CTCGTCGGACATAAATGGT-3'	Unknown protein
BR 119	5'-GTATCACTCTCGGGTTCCA-3'	5'-CCAAACAAATGGGACAATCC-3'	Unknown gene function
BR 129	5'-GTGGAGGGAGTCAACGATGT-3'	5'-CCGAGTCCCTGCGATATTTA-3'	Dimethylmenaquinone methyltransferase family protein
BR 192	5'-CATAAGGTACGCGACGGTTT-3'	5'-ACTAAAGCAGCTTCGGTCCA-3'	Glycosyltransferase family protein
BR 195	5'-CACTGGAGTTGACCCAAC-3'	5'-AATGCTAAGGGACCAACAC-3'	Aspartate aminotransferase 2
BR 289	5'-TGATCAGATGGAGTGGTGGA-3'	5'-CTCCTCGCTTGTGGAAGAC-3'	Plastid-lipid associated protein PAP / fibrillin family protein

generated using the BLASTN program (<http://www.ncbi.nlm.nih.gov/>). Sequence alignment, ORF translation, molecular weight calculation of the predicted proteins, and structural analysis of the deduced proteins were carried out by DNASTar's Lasergene sequence analysis software (www.dnastar.com).

RESULTS

Characterization of selected clones from FOX hunting system

Agrobacterium clones containing FL-cDNAs genes from *Brassica rapa* were subjected to genomic PCR using

forward and reverse primers (*pBigs_SfiI*-Fw and *pBigs_sfiI*-Rv) derived from the flanking sequences. Of the 933 clones analyzed, 80% showed single bands, while 4% showed multiple fragments (Fig. 2A). The remaining clones (16%) did not undergo fragment amplification. All the amplified single PCR fragments bearing the integrated FL-cDNA(s) from *B. rapa* clones were used to generate their respective sequences. The sizes of the introduced FL-cDNAs were variable, ranging from 100 to 2,400 bp (Fig. 2B). About 5% of the clones were smaller than 500 bp, whereas 95% ranged from 600 to 2,400 bp.

The Basic Alignment System Tool (BLAST) provided by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) was used to determine the functional

annotation of each clone. The frequency distribution based on function is presented in Fig. 2C. Variable gene function was observed from the viewpoint of molecular as well as biological importance. Most clones (32.15%) were characterized as having an unknown biological/molecular gene function. About 0.64% of clones appeared to possess functions related to catalytic activity, and 5.04% appeared to exhibit some binding activity. Transcription- and translation-related functions accounted for 2.36% of the clones. Moreover, 1.39% of the clones had homology with zinc finger-related proteins. A large number of other functions, each with a lower frequency than those reported above, collectively accounted for another 35.16% of the clones (Fig. 2C). These genes provide important resources for studying their function in plants.

Generation of transgenic rice lines

Using the protocol described by Lee et al. (2011) and

modified on the basis of that described by Toki et al. (2006), transgenic rice plantlets were regenerated within a month starting from the aseptic culture of the mature seeds (Fig. 3A). The plantlets were subjected to PCR analysis to verify the integration of FL-cDNAs. The genomic DNA PCR analysis is represented in Fig. 3B. Most of the regenerated plants (T_0) (73%) contained the expected band sizes (Fig. 3C), indicating the high efficiency of the transformation method. About 20% of the transgenic plants had *hpt* gene, whereas 2% had only FL-cDNAs gene.

The confirmed FOX rice lines were advanced in the succeeding planting season from 2009 to 2011. So far, we have propagated transgenic rice up to the fourth generation (T_4) (Table 4). Most of the transgenic rice lines were in the T_1 generation that constituted 470 independent rice lines in 137 independent clones. In the T_2 generation, 408 lines with 88 gene inserts were produced. In the T_3 generation, 46 lines were generated. In summary, a total of 250

Fig. 2. PCR amplification and BLAST analyses of 933 clones. (A) Pattern and numbers of cDNA-containing fragments. (B) frequency of gene according to the length (bp) of FOX cDNA library. (C) NCBI database annotation based on gene function.

Fig. 3. Development, PCR analysis and transformation efficiency in the generation of transgenic rice. (A) Development of selected 250 clones from FL-cDNA through *Agrobacterium*-mediated transformation (A) callus formation; (B) inoculation; (C) callus selection using hygromycin (D), (E) and (F) shoot formation and elongation; (G) and (H) root formation and elongation; (I) acclimatization; (B) Genomic PCR confirmation of inserted genes using T1 plants by pBigs SfiI (a) and HPT (b) primers in 1% agarose gel. L= Ladder, 1 = BR2, 2=BR4, 3=BR7, 4=BR23, 5=BR47, 6=BR61, 7=BR74, 8=BR91, 9=BR129, 10=BR192, 11=BR195, 12=BR174, 13=BR15, 14=BR37, WT= wild type; (C) The efficiency of *Agrobacterium*-mediated transformation based on the genomic PCR analysis of 1,072 T0 generation rice lines using hpt and pBigs_SfiI primers.

Table 4. Number of transgenic rice lines produced, confirmed gene inserts and its respective generation.

Generation*	Confirmed no. of gene inserts	No. of lines
T ₁	137	470
T ₂	88	408
T ₃	14	46
T ₄	11	226
Total	250	1,150

independent clones were inserted into the rice genome, leading to the generation of 1,150 independent transgenic lines (Table 4). These transgenic rice lines are a valuable resource for future analyses designed to determine the functions of each gene.

Gene copy number

To examine the gene copy number of 31 randomly selected lines, we used the digoxigenin (DIG) system method (Roche Molecular Biochemicals, USA). Because of variability in the gene sequences, we used *hpt* gene as probe. Our results showed that most of the transgenic lines

have a single copy number (Fig. 4A). However, in BR 37, which encodes CBL-interacting protein kinase 1, two gene copies were present in each of 4 lines (numbers 3, 4, 6, 7). Similarly, in BR 15, which encodes UDP-glucose-4-epimerase 1, two lines had two copies of the gene (numbers 12 and 13). Furthermore, in clone BR 4, two copies of the introduced gene (actin-depolymerizing factor 2) were observed. The rest of the lines harbored transgenes at single copy. The variability in copy number has been reported in previous studies (Schrider and Hahn 2010). This may be

due to the positional effect of the gene during transformation (Gelvin 2003).

Expression analysis of transgenes

To examine whether the expression of transgenes was elevated at the molecular level, 24 transgenic lines encoding different gene inserts were randomly selected. Expression of these 24 genes was examined by semi-quantitative RT-PCR analysis with individual gene-specific primers (as shown in Table 3) by using the young shoots of

Fig. 4. Southern blot analysis and expression pattern of randomly selected FOX rice lines. (A) hpt gene was used as probe using a non-isotope method (dioxigenin). BR 37 (BrCIPK1, CBL-interacting protein kinase 1); BR15 (BrUGE1, UDP-glucose-4-epimerase 1); BR2 (BrCP1, Cysteine protease 1); BR4 (Actin depolymerizing factor 2); BR119 (Unknown function); BR23 (ER auxin-binding protein 2); BR29 (Phospholipase C). (B) RT-PCR of selected rice lines using their respective primer pairs (Supplemental Table 2. Rice actin was used as loading adjustments (data not shown). Gopum was the wild type.

each rice seedling. mRNA expression was observed in all 24 genes with varying band intensities (Fig. 4B). Strong mRNA expression was observed in BR 2 (cysteine protease 1), BR 7 (DEAD box RNA helicase RH15), BR 37 (CBL-interacting protein kinase 1), BR 47 (unknown gene function), BR 74 (haloacid dehalogenase-like hydrolase), BR 192 (glycosyltransferase), BR 15 (UDP-glucose-4-epimerase 1), BR 68 (unknown function), and BR 22 (hydrogen-transporting ATPase). Moreover, weak mRNA expression was observed in BR 4 and BR 23 lines encoding an actin-binding/depolymerizing factor 2 and endoplasmic reticulum auxin-binding protein 2 mRNA, respectively.

Response of transgenic rice lines against cold and salinity

To determine the response of transgenic rice lines to cold, 840 independent lines comprising 250 genes were evaluated at the Chuncheon Experimental Station of the

Rural Development Administration, Korea. Most of the transgenic rice lines (94%) were moderately tolerant (seedlings were light yellow) to cold and about 5% were moderately susceptible (seedlings were turning brown) (Fig. 5A). Interestingly, 9 (1%) of the transgenic lines were tolerant (seedlings were light green) (Table 1). Of the 9 tolerant lines, 4 encode genes with an unknown function (BR 5, BR 119, BR 522, BR 503). Other tolerant lines encoded UDP-galactose transporter 3 (BR 457), DEAD box RNA helicase RH 15 (BR 7), Arv1-like protein (BR 115), carbon-monooxide oxygenase (ACYB-1) (BR 39) and light harvesting complex PSII (BR 541). Conversely, we found 6 transgenic lines that were susceptible to cold stress, 4 of which encode an unknown gene function. The rest of the transgenic lines responded to the cold in a manner indistinguishable from wild-type Gopum.

In the salinity test, 85% of the transgenic rice lines were found to be highly susceptible (almost all plants were dead

Fig. 5. Response of FOX rice lines against cold and salinity (see materials and methods for details).

or dying) (Fig. 5B). However, we found 7 tolerant transgenic lines (nearly normal growth and tillering) (Table 1). These lines were BR 7 (unknown gene function), BR 37 (CBL-interacting protein kinase 1), BR 40 (Phosphatase 2C), BR 74 (Haloacid dehalogenase-like hydrolase), BR 91 (unknown gene function), BR 115 (BREB subfamily A-4 ERF/AP2 transcription factor), and BR 119 (unknown

gene function). Moreover, 15 lines were shown to be moderately tolerant to salinity stress (their growth was nearly normal, but plants had some rolled leaves). These tolerant lines are an important resource for studying the mechanisms of resistance, and may eventually facilitate crop improvement in the future.

Fig. 6. Morpho-agronomic variability of 1,150 transgenic rice lines. Each transgenic rice line was compared to the wild type Gopum to generate significant differences (positive/negative) by two-pair wise Dunnett's.

Phenotypic characteristics of the FOX rice lines

To determine the phenotype of the FOX rice lines, all developed lines were evaluated in the field arranged in an incomplete block design. As shown in Fig. 6, the variability in the number of transgenic lines was significant in each of the agronomic traits measured. Indeed, 25.1% matured significantly earlier and 22% contained significantly higher chlorophyll content. Furthermore, 18% of the transgenic rice lines had lower chlorophyll contents. Other agronomic traits such as filled grains, number of tiller, panicle length, and culm length and plant height showed relatively low

variability among lines. All morpho- agronomic traits are recorded. For example, the BR 4 line, which encodes actin-depolymerizing factor 2, exhibited a higher number of tillers. Similarly, BR 119, which encodes a gene with unknown function, gave rise to shorter plants.

The altered agronomic traits observed in the FOX lines may be caused by each gene. Further functional analysis is necessary to determine the function of each gene. Regardless of this, these results provide the basic information required to accelerate future functional studies.

Fig. 7. The field evaluation of transgenic rice. A to C, the field plots of the transgenic lines in the vegetative stage arranged in incomplete block design; D, high senescence observed in BR 73; E, mild senescence in BR61; F, observed dwarf in BR122; G, partial sterility observed in BR 452.

DISCUSSION

A total of 1,150 transgenic rice lines with FL-length cDNA genes from Chinese cabbage were generated; these lines contained 250 *Brassica rapa* FL-cDNAs (Table 4). The size distribution pattern and the average size of FL-cDNAs inserted into the genomes of 250 FOX-rice lines was 900 to 2,100 bp (Fig. 2B). Analyses of individual gene inserts revealed that genes associated with tolerance to cold and salinity were functionally diverse, both at the molecular and biological levels (Fig. 2C). Approximately 1/3 of the gene inserts have no known ascribed function. Furthermore, most of the randomly selected transgenic lines were present as single gene copies (Fig. 3). We overexpressed a variety of transgenes in transgenic rice (Fig. 4). We suggest that our approach will be useful for systematic genome-wide gain-of-function analyses of the *Brassica rapa* genes that have been inserted into rice.

Phenotypic databases of rice properties have been developed by several groups. These include the International Rice Information System (IRIS) (Wu et al. 2005), Oryzabase (Kurata and Yamazaki 2006), Rice Mutant Database (RMD; Zhang et al. 2006), Rice Tos17 Insertion Mutant Database (Miyao et al. 2007), and Gramene (<http://www.gramene.org/>). In addition to these useful databases, the transgenic rice lines harboring FL-cDNAs gene from *Brassica* spp. are expected to provide valuable information for researchers to uncover functions of *Brassica* genes. Appropriate biological or molecular methodologies are needed to reveal the function of each gene.

We found that 7.9% of the observed transgenic rice plants showed morpho-agronomically altered phenotypes (Fig. 6). Among the 1,150 transgenic lines, 224 lines (26.4%) had a significantly altered number of days to heading. Similarly, 40% had a significant change in the chlorophyll content. Examples of these altered phenotypes are presented in Fig. 7. This rate was much higher than that determined by using activation tagging of rice populations (Jeong et al. 2002). However, for culm length, plant height, panicle length, number of tillers/hill, and percent filled spikelet/panicle, only 3.7% of transgenic lines were different compared to the wild type. During *Agrobacterium*

transformation, minimal weak growth of the regenerants was observed. This is consistent with a previous report (Nakamura et al. 2007) and underscores the notion that transgenic regenerants often suffer from the effects of environmental factors such as humidity, temperature, macro- and micro-nutrient deficiencies or excesses, and microbes (pathogens), especially in the initial growth stage after transfer to soil. Alternatively, during tissue culture for selection and regeneration of transgenic plants, a variety of both epigenetic (e.g., DNA methylation, gene silencing, activation of retrotransposons and transposable elements) and genetic (e.g., nucleotide substitutions, deletions, insertions, rearrangements) alterations may occur in the plant genome (Kaepler et al. 2000; Cheng et al. 2006; Noro et al. 2007). These factors, i.e., abiotic and biotic factors and tissue culture-induced variations, may influence the growth conditions to some degree, and consequently, alter the phenotypes of transgenic plants. This may explain the increased rate of phenotype alteration in our transgenic lines (Nakamura et al. 2007).

The response of transgenic rice lines to salinity and cold was tested on 900 independent lines. Eight hundred forty six (94%) of the lines were moderately tolerant to the cold stress (Fig. 5A), and 9 lines (1%) were tolerant. This is not surprising since wild-type Gopum is moderately tolerant to cold stress (Choi et al. 2007). However, most of the transgenic lines (85%) were highly susceptible to salinity stress (Fig. 5B). Only 5% were moderately tolerant, and 2% were tolerant in the transgenic rice lines. In any case, our results have identified possibly agronomically important genes that should be characterized in future studies. Furthermore, manipulation of these genes may lead to improvements in the tolerance parameters of this economically important crop.

The development of full-length cDNA collections is an effective strategy for increasing the catalog of gene transcripts. These data serve as a valuable resource for describing gene expression profiles, and ultimately classify genes into families based on their functions (Abe et al. 2011). Full-length cDNA can also serve as a powerful tool to facilitate genomic or other research (Seki and Shinozaki 2009). Several techniques have been established to prepare enriched full-length cDNA libraries (Seki et al. 1998). The

utility of full-length cDNAs has been confirmed in various plants, including *Arabidopsis* (Yang et al. 2006; Alexandrov et al. 2006), rice (Ralph et al. 2008), poplar (Jia et al. 2006), maize (Alexandrov et al. 2009), tomato (Town et al. 2006), and soybean (Umezawa et al. 2008). A major advantage of this approach is that most of the clones contain the complete coding sequence, in addition to the 5'- and 3'-untranslated regions.

In this study, we have described the phenotypes and abiotic reactions of individual transgenic rice lines that carry gene inserts isolated from Chinese cabbage. Our results have thus provided a list of potentially economically important genes that can be further studied during future abiotic studies and functional analyses.

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REFERENCES

- Abe H, Narusaka Y, Sasaki I, Hatakeyama K, Shin IS, Narusaka M, Fukami-Kobayashi F, Matsumoto S and Kobayashi M. 2011. Development of full-length cDNAs from chinese cabbage (*Brassica rapa* Subsp. *pekinensis*) and identification of marker genes for defence. *DNA Research* 18:277-289.
- Alexandrov NN, Brover VV, Freidin S, Troukhan ME, Tatarinova TV, Zhang H, Swaller TJ, Lu YP, Bouck J, Flavell RB and Feldmann KA. 2009. Insights into corn genes derived from large-scale cDNA sequencing. *Plant Mol. Biol.* 69:179-194.
- Alexandrov NN, Troukhan ME, Brover VV, Tatarinova T, Flavell RB and Feldmann KA. 2006. Features of *Arabidopsis* genes and genome discovered using full-length cDNAs. *Plant Mol. Biol.* 60:69-85.
- An G, Jeong DH, Jung KH and Lee S. 2005. Reverse genetic approaches for functional genomics of rice. *Plant Mol. Biol.* 59:111-123.
- Cheng C, Daigen M and Hirochika H. 2006. Epigenetic regulation of the rice retrotransposon Tos17. *Mol. Genet. Genomics* 276:378-390.
- Cho YG, Kang HJ, Lee JS, Lee YT, Lim SJ, Gauch H, Eun MY and McCouch SR. 2007. Identification of quantitative trait loci in rice for yield, yield components, and agronomic traits across years and locations. *Crop Sci.* 47:2403-2417.
- Choi IS, Kim YG, Cho YC, Hong HC and Baek MK. 2007. A medium-maturing, multi-disease resistant and good eating-quality rice variety "Gopum". *Kor. J. of Plant Breeding Sci.* 39:586-587.
- Fagard M and Vaucheret H. 2000. (Trans)gene silencing in plants: how many mechanisms? *Plant Mol. Biol.* 51:167-174.
- Gelvin SB. 2003. *Agrobacterium*-mediated plant transformation: The biology behind the "gene-jockeying" tool. *Microbiol. Mol. Biol. Rev.* 67:16-37.
- Hirochika H, Guiderdoni E, An G, Hsing YI, Eun MY, Han C, Upadhyaya N, Ramachandran S, Zhang Q, Pereira A, Sundaresan V and Leung H. 2004. Rice mutant resources for gene discovery. *Plant Mol. Biol.* 54:325-334.
- Ichikawa T, Nakazawa M, Kawashima M, Iizumi H, Kuroda H, Kondou Y, Tsuchida Y, Suzuki K, Ishikawa A, Seki M, Fujita M, Motohashi R, Nagata N, Takagi T, Shinozaki K and Matsui M. 2006. The FOX hunting system: an alternative gain-of-function gene hunting technique. *Plant J.* 48:974-985.
- International Rice Genome Sequencing Project (IRGSP). 2005. The map-based sequence of the rice genome. *Nature* 436:793-800.
- International Rice Research Institute (IRRI). 2007. Standard evaluation system for rice. IRRI, Los Baños, Laguna Philippines. p.56
- Jeong DH, An S, Kang HG, Moon S, Han JJ, Park S, Lee HS, An K and An G. 2002. T-DNA insertional mutagenesis for activation tagging in rice. *Plant Physiol.* 130:1636-1644.
- Jia J, Fu J, Zheng J, Zhou X, Huai J, Wang J, Wang M, Zhang Y, Chen X, Zhang J, Zhao J, Su Z, Lv Y and Wang G. 2006. Annotation and expression profile analysis of 2073 full-length cDNAs from stress-induced maize. *Zea mays* L. seedlings. *Plant J.* 48:710-727.
- Kaepler SM, Kaepler HF and Rhee Y. 2000. Epigenetic aspects of somaclonal variation in plants. *Plant Mol. Biol.* 43:179-188.
- Karlowski WM, Schoof H, Janakiraman V, Stuempflen V and

- Mayer KF. 2003. MOsDB: an integrated information resource for rice genomics. *Nucleic Acids Res.* 31:190-192.
- Kurata N and Yamazaki Y. 2006. Oryzabase: an integrated biological and genome information database for rice. *Plant Physiol.* 140:12-17.
- Lee HJ, Abdula SE, Jee MG, Jang DW and Cho YG. 2011. High-efficiency and Rapid *Agrobacterium*-mediated genetic transformation method using germinating rice seeds. *J. Plant Biotechnol.* 38:251-257.
- Manigbas NL, Park DS, Park SK, Han SI, Park HM, Hwang WH, Kim SM, Wang HJ, Kang HW, Kim DH, Yun DJ and Yi GW. 2011. Enhanced tolerance of transgenic rice overexpressing *Arabidopsis thaliana nucleoside diphosphate kinase (AtNDPK2)* against various environmental stresses. *Philipp. Agric. Scientist* 94:29-37.
- Marathe R, Anandalakshmi R, Smith TH, Pruss GJ and Vance VB. 2000. RNA viruses as inducers, suppressors and targets of post-transcriptional gene silencing. *Plant Mol. Biol.* 43:295-306.
- Miyao A, Iwasaki Y, Kitano H, Itoh J, Maekawa M, Murata K, Yatou O, Nagato Y and Hirochika H. 2007. A large-scale collection of phenotypic data describing an insertional mutant population to facilitate functional analysis of rice genes. *Plant Mol. Biol.* 63:625-635.
- Nakamura H, Hakata M, Amano K, Miyao A, Toki N, Kajikawa M, Pang J, Higashi N, Ando S, Toki S, Fujita M, Enju A, Seki M, Nakazawa M, Ichikawa T, Shinozaki K, Matsui M, Nagamura Y, Hirochika H and Ichikawa H. 2007. A genome-wide gain-of-function analysis of rice genes using the FOX-hunting system. *Plant Mol. Biol.* 65:357-371.
- Noro Y, Takano-Shimizu T, Syono K, Kishima Y and Sano Y. 2007. Genetic variations in rice in vitro cultures at the EPSPs-RPS20 region. *Theor. Appl. Genet.* 114: 705-711.
- Ohyanagi H, Tanaka T, Sakai H, Shigemoto Y, Yamaguchi K, Habara T, Fujii Y, Antonio BA, Nagamura Y, Imanishi T, Ikeo K, Itoh T, Gojobori T and Sasaki T. 2006. The rice annotation project database (RAP-DB): hub for *Oryza sativa* ssp. japonica genome information. *Nucleic Acids Res.* 34:741-744.
- Ouyang S, Zhu W, Hamilton J, Lin H, Campbell M, Childs K, Thibaud-Nissen F, Malek RL, Lee Y, Zheng L, Orvis J, Haas B, Wortman J and Buell CR. 2007. The TIGR rice genome annotation resource: improvements and new features. *Nucleic Acids Res.* 35:883-887.
- Ralph SG, Chun HJE, Cooper D, Kirkpatrick R, Kolosova N, Gunter L, Tuskan GA, Douglas CJ, Holt RA, Jones SJM, Marra MA and Bohlmann J. 2008. Analysis of 4,664 high-quality sequence-finished poplar full length cDNA clones and their utility for the discovery of genes responding to insect feeding. *BMC Genomics* 9:57.
- Rice Annotation Project. 2007. Curated genome annotation of *Oryza sativa* ssp. japonica and comparative genome analysis with *Arabidopsis thaliana*. *Genome Res.* 17: 175-183.
- Sasaki T, Matsumoto T, Antonio BA and Nagamura Y. 2005. From mapping to sequencing, post-sequencing and beyond. *Plant Cell Physiol.* 46:3-13.
- Schrider DR and Hahn MW. 2010. Gene copy-number polymorphism in nature. *Proc. Biol. Sci.* 277:3213-3221.
- Seki M, Narusaka M, Kamiya A, Ishida J, Satou M, Sakurai T, Nakajima M, Enju A, Akiyama K, Oono Y, Muramatsu M, Hayashizaki Y, Kawai J, Carninci P, Itoh M, Ishii Y, Arakawa T, Shibata K, Shinagawa A and Shinozaki K. 2002. Functional annotation of a full-length *Arabidopsis* cDNA collection. *Science* 296:141-145.
- Seki M, Carninci P, Nishiyama Y, Hayashizaki Y and Shinozaki K. 1998. High-efficiency cloning of *Arabidopsis* full-length cDNA by biotinylated CAP trapper. *Plant J.* 15:707-720.
- Seki M and Shinozaki K. 2009. Functional genomics using RIKEN *Arabidopsis thaliana* full-length cDNAs. *J. Plant Res.* 122:355-366.
- Sun MM, Abdula SE, Lee HY, Cho YC, Han LZ, Koh HJ and Cho YG. 2011. Molecular aspect of good eating quality formation in japonica rice. *PLoS ONE* 6(4): e18385. doi:10.1371/journal.pone.0018385.
- Toki S, Hara N, Ono K, Onodera H, Tagiri A, Oka S and Tanaka H. 2006. Early infection of scutellum tissue with *Agrobacterium* allows high-speed transformation of rice. *Plant J.* 47:969-976.
- Town CD, Cheung F, Maiti R, Crabtree J, Haas BJ, Wortman JR, Hine EE, Althoff R, Arbogast TS, Tallon LJ, Vigouroux M, Trick M and Bancroft I. 2006. Comparative genomics of *Brassica oleracea* and *Arabidopsis thaliana* reveal gene loss, fragmentation, and dispersal after polyploidy. *Plant Cell* 18:1348-1359.
- Umezawa T, Sakurai T and Totoki Y. 2008. Sequencing and analysis of approximately 40,000 soybean cDNA clones from a full-length-enriched cDNA library. *DNA Res.* 15:333-346.
- Vaucheret H and Fagard M. 2001. Transcriptional gene silencing in plants: targets, inducers and regulators. *TRENDS in Genetics* 17:29-34.

- Wu JL, Wu C, Lei C, Baraoidan M, Bordeos A, Madamba MR, Ramos-Pamplona M, Mauleon R, Portugal A, Ulat VJ, Bruskiewich R, Wang G, Leach J, Khush G and Leung H. 2005. Chemical and irradiation-induced mutants of indica rice IR64 for forward and reverse genetics. *Plant Mol. Biol.* 59:85-97.
- Yang TJ, Kim JS, Kwon SJ, Lim KB, Choi BS, Kim JA and Jin M. 2006. Sequence-level analysis of the diploidization process in the triplicated FLOWERING LOCUS C region of *Brassica rapa*. *Plant Cell* 18: 1339-1347.
- Zhang J, Li C, Wu C, Xiong L, Chen G, Zhang Q and Wang S. 2006. RMD: a rice mutant database for functional analysis of the rice genome. *Nucleic Acids Res.* 34: 745-748.
- Zhao W, Wang J, He X, Huang X, Jiao Y, Dai M, Wei S, Fu J, Chen Y, Ren X, Zhang Y, Ni P, Zhang J, Li S, Wang J, Wong GK, Zhao H, Yu J, Yang H and Wang J. 2004. BGI-RIS: an integrated information resource and comparative analysis workbench for rice genomics. *Nucleic Acids Res.* 32:377-382.

Appendix Table 1. List of transgenic rice with full-length cDNA from *B. rapa*, its annotation function and their morpho-agronomic traits.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 2	cysteine protease 1	5.7	9.0	more green leaves
BR 4	ADF2 (Actin depolymerizing factor 2)	5.0	9.0	more number of tiller
BR 5	PsbQ2 mRNA for oxygen-evolving enhancer protein 3-2	3.0	9.0	more green leaves
BR 6	Zea mays clone 93807 mRNA sequence	5.0	9.0	more green leaves
BR 7	Dead box RNA helicase RH15 - like protein	3.0	3.0	same phenotype
BR 9	PEC-1 mRNA for Lipid transfer protein	5.0	9.0	more green leaves
BR 12	Rac GTPase activator	5.0	9.0	same phenotype
BR 14	CBL-interacting protein kinase 3	5.2	5.5	less green leaves
BR 17	mRNA for aluminum-induced	5.0	9.0	less green leaves
BR 23	endoplasmic reticulum auxin-binding protein 2	9.0	7.0	less green leaves
BR 26	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH004A06, complete sequence	5.3	9.0	same phenotype
BR 29	Phospholipase C	5.2	7.0	less green leaves
BR 33	ABC transporter-like protein	9.0	9.0	less green leaves
BR 37	CBL-interacting protein kinase 1	5.4	3.0	less green leaves, less plant height
BR 39	carbohydrate binding	4.3	9.0	less green leaves
BR 40	protein phosphatase 2C, putative / PP2C,	4.7	3.0	less green leaves, shorter culm length
BR 46	plasma membrane intrinsic protein 2 (<i>PIP2</i>)	5.0	9.0	less green leaves, more taller
BR 48	senescence-associated cysteine protease (CP1)	4.8	5.0	more number of tiller
BR 49	polypeptide of photosystem II reaction center	9.0	9.0	less green leaves
BR 50	chlorophyll a/b binding protein		9.0	less green leaves
BR 51	methyl-CpG-binding domain 4 (MBD4)	9.0	9.0	less green leaves, longer panicle length
BR 57	putative protein transport protein SEC61 alpha subunit	5.0	7.0	less green leaves
BR 60	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB042D24, complete sequence	5.0	9.0	less green leaves
BR 61	rubredoxin family protein	6.0	9.0	less green leaves
BR 64	GDSL esterase/lipase APG	5.0	9.0	less green leaves, longer panicle length, Shorter culm length
BR 72	WSCP1 mRNA for water-soluble chlorophyll protein precursor	5.0	9.0	same phenotype
BR 73	unknown protein	6.0	9.0	less green leaves
BR 74	haloacid dehalogenase-like hydrolase family protein	5.0	3.0	less green leaves, more number of tiller
BR81	regulatory subunit of protein kinase CK2	5.0	7.0	less green leaves, shorter culm length
BR 84	ATCTIMC (Cytosolic triose phosphate isomerase)	5.0	9.0	shorter culm length
BR 89	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH004P05, complete sequence	5.7	9.0	same phenotype

Appendix Table 1. Continued.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 91	unknown protein	5.2	3.0	less green leaves, longer culm length
BR 92	5-enolpyruvylshikimate-3-phosphate synthase (EPSP)	5.0	9.0	same phenotype
BR 97	G6PD1 (glucose-6-phosphate dehydrogenase 1)	5.1	9.0	same phenotype
BR 99	40S ribosomal protein S7 (RPS7C)	5.0	9.0	same phenotype
BR 104	protein binding / zinc ion binding	5.0	9.0	same phenotype
BR 105	Lhcb6 protein mRNA	5.0	9.0	more number of tiller
BR 111	protein kinase family protein	5.0	9.0	same phenotype
BR 112	<i>Arabidopsis thaliana</i> AT5g47540/MNJ7_13 mRNA, complete cds	5.0	9.0	same phenotype
BR 113	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB052L10, complete sequence	5.8	9.0	same phenotype
BR 114	<i>Zea mays</i> clone 13119 mRNA sequence	5.0	9.0	less plant height
BR 115	<i>Arabidopsis thaliana</i> At5g25810 mRNA, complete cds	3.0	9.0	same phenotype
BR 116	Unknown	5.0	9.0	same phenotype
BR 118	<i>Vitis vinifera</i> , whole genome shotgun sequence, contig VV78X164547.5, clone ENTAV 115	6.0	9.0	same phenotype
BR 119	unknown protein	3.0	3.0	shorter plant height
BR 120	<i>Zea mays</i> clone 14950 mRNA sequence	5.0	6.2	same phenotype
BR 122	DNAJ heat shock N-terminal domain-containing protein	5.0	9.0	same phenotype
BR 124	putative thioredoxin reductase	4.8	9.0	same phenotype
BR 125	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrS004A14, complete sequence	5.0	9.0	less plant height
BR 128	<i>Zea mays</i> clone 9139 mRNA sequence	5.0	9.0	same phenotype
BR 129	dimethylmenaquinone methyltransferase family protein	5.0	9.0	same phenotype
BR133	prephenate dehydratase family protein	5.0	9.0	same phenotype
BR139	231 CLA1-like protein mRNA	5.1	9.0	same phenotype
BR147	231 CLA1-like protein mRNA	5.0	9.0	same phenotype
BR148	protein kinase family protein / C-type lectin domain-containing protein	5.0	5.2	same phenotype
BR 149	XTR4 (xyloglucan endotransglycolase 4)	5.0	9.0	same phenotype
BR 151	clathrin adaptor complexes medium subunit-related	5.0	9.0	same phenotype
BR 152	unknown mRNA	5.0	9.0	same phenotype
BR 158	unknown protein (AT1G34010) mRNA	5.0	9.0	same phenotype
BR 160	SCL5; transcription factor (SCL5)	5.0	5.8	same phenotype
BR162	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB005N03, complete sequence	5.0	9.0	same phenotype
BR 165	<i>Arabidopsis thaliana</i> genomic DNA, chromosome 5, P1 clone:MZN1	5.0	9.0	more number of tiller
BR 171	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH004A18, complete sequence	5.5	9.0	more number of tiller
BR 173	BSD domain-containing protein	4.7	9.0	same phenotype

Appendix Table 1. Continued.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 174	SEC14 cytosolic factor family protein / phosphoglyceride transfer family protein	5.5	9.0	same phenotype
BR 178	unknown protein	4.0	9.0	same phenotype
BR 179	<i>Zea mays</i> clone 8974 mRNA sequence	5.5	5.6	same phenotype
BR 192	glycosyltransferase family protein	5.0	9.0	same phenotype
BR 195	ASP2 (aspartate aminotransferase 2 (<i>ASP2</i>))	5.7	9.0	same phenotype
BR 225	unknown	5.0	9.0	same phenotype
BR 240	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH080C09, complete sequence	5.0	9.0	same phenotype
BR 246	CTR1 (constitutive triple response 1	5.0	9.0	same phenotype
BR 250	unknown protein	5.0	9.0	same phenotype
BR 255	Indole-3-glycerol phosphate synthase	5.5	9.0	same phenotype
BR 256	leucine-rich repeat transmembrane protein kinase	5.0	9.0	same phenotype
BR 257	Unknown	5.0	9.0	same phenotype
BR 261	<i>Arabidopsis thaliana</i> Full-length cDNA Complete sequence from clone GSLTSIL12ZB10 of Silique of strain col-0 of <i>Arabidopsis thaliana</i> (thale cress)	5.0	9.0	same phenotype
BR 262	Glycosyl hydrolase family 17 protein	4.8	9.0	same phenotype
BR 263	CA2 (beta carbonic anhydrase 2); carbonate dehydratase/ zinc ion binding (CA2)	5.0	9.0	longer panicle length
BR 264	Eukaryotic translation initiation factor 1A	5.0	9.0	same phenotype
BR 265	<i>Zea mays</i> clone 11869 mRNA sequence	5.0	9.0	shorter plant height
BR 271	rhodanese-like domain-containing protein	5.0	9.0	same phenotype
BR 273	WD-40 repeat family protein	5.0	9.0	same phenotype
BR 274	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH006E24, complete sequence	5.0	9.0	same phenotype
BR 275	pentatricopeptide (PPR) repeat-containing protein	5.0	6.0	same phenotype
BR 293	<i>Zea mays</i> clone 11032 mRNA sequence	4.0	9.0	same phenotype
BR 303	nodulin family protein	5.0	9.0	same phenotype
BR 305	Unknown protein	5.0	9.0	same phenotype
BR 308	<i>Zea mays</i> clone 15053 mRNA sequence	4.9	9.0	same phenotype
BR 311	Actin (Act1) mRNA	5.0	9.0	same phenotype
BR 318	unknown protein	5.4	9.0	same phenotype
BR 319	aldehyde dehydrogenase	5.0	9.0	same phenotype
BR 322	unknown protein	5.0	9.0	same phenotype
BR 336	unknown protein	5.0	9.0	same phenotype
BR 323	unknown protein	5.0	9.0	same phenotype
BR 330	unknown protein	5.0	9.0	same phenotype
BR 336	unknown protein	5.0	9.0	shorter plant height
BR 339	<i>HDA6</i> (histone deacetylase 6)	5.0	9.0	shorter plant height
BR 343	<i>ATNUDT2</i> (<i>Arabidopsis thaliana</i> Nudix hydrolase homolog 2); ADP-ribose diphosphatase/ NAD binding / hydrolase (<i>ATNUDT2</i>) mRNA, complete cds	5.0	9.0	same phenotype

Appendix Table 1. Continued.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 348	<i>ELO1</i> (elongate 1) mRNA	5.0	9.0	more green leaves, shorter plant height
BR 349	unknown protein	5.0	6.0	same phenotype
BR 367	unknown gene function	4.8	9.0	same phenotype
BR 368	ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit	5.0	9.0	more green leaves
BR 369	60S ribosomal protein L10A (RPL10aC)	5.0	9.0	same phenotype
BR 370	tubulin-specific chaperone C-related	5.0	9.0	same phenotype
BR 374	ABC1 family protein	5.0	9.0	same phenotype
BR 375	<i>Zea mays</i> clone 13431 mRNA sequence	5.5	9.0	same phenotype
BR 376	transcriptional repressor	5.0	9.0	same phenotype
BR 377	<i>Arabidopsis thaliana</i> clone 37944 mRNA, complete sequence	5.0	9.0	same phenotype
BR 383	CLA1-like protein mRNA	5.0	9.0	same phenotype
BR 384	unknown protein	5.0	9.0	shorter plant height
BR 386	60S ribosomal protein L28 (RPL28C)	5.0	9.0	less green leaves, shorter plant height
BR 393	unknown protein	5.5	9.0	same phenotype
BR 418	WNK1 (WITH NO LYSINE (K) 1); kinase (WNK1) mRNA	5.2	9.0	same phenotype
BR 425	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH080C09, complete sequence	5.5	9.0	shorter plant height, longer panicle length
BR 436	putative nuclear transport factor 2 mRNA	5.0	9.0	same phenotype
BR 438	60S acidic ribosomal protein P0 (RPP0B)	5.0	9.0	same phenotype
BR 445	transporter-related	5.0	9.0	more green leaves
BR 449	FTSH8 (FtsH protease 8); ATP-dependent peptidase/ATPase/ metallopeptidase/ zinc ion binding (FTSH8) mRNA	5.0	6.9	same phenotype
BR 450	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrS015K01, complete sequence	5.0	9.0	more green leaves
BR 452	nuclear transport factor 2 mRNA	5.2	9.0	more green leaves
BR 499	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH003N18, complete sequence	5.0	7.0	same phenotype
BR467	<i>Arabidopsis thaliana</i> PTAC16 (PLASTID TRANSCRIPTIONALLY ACTIVE 16); binding / catalytic (PTAC16) mRNA, complete cds	5.0	9.0	same phenotype
BR 468	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB034N10, complete sequence	5.0	9.0	more green leaves
BR 471	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i> hypothetical protein, mRNA	5.0	9.0	same phenotype
BR 470	B-box type zinc finger protein (Bbox1) mRNA	9.0	9.0	more number of tiller
BR 472	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB028I01, complete sequence	5.0	6.4	same phenotype
BR 473	<i>Zea mays</i> clone 17728 mRNA sequence	5.0	9.0	shorter culm length
BR 474	ACC oxidase (ACC0x2)	5.0	9.0	same phenotype

Appendix Table 1. Continued.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 475	<i>Zea mays</i> clone 17728 mRNA sequence	5.0	9.0	more taller plant height
BR 478	unknown protein	5.0	9.0	same phenotype
BR 479	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB021M22, complete sequence	5.0	9.0	same phenotype
BR 480	<i>Arabidopsis thaliana</i> unknown protein (AT3G18050) mRNA	5.0	9.0	same phenotype
BR 481	AL5 (ALFIN-LIKE 5); DNA binding / methylated histone residue binding (AL5)	5.0	9.0	same phenotype
BR 482	<i>Arabidopsis thaliana</i> LHCB3 (LIGHT-HARVESTING CHLOROPHYLL B-BINDING PROTEIN 3); structural molecule (LHCB3) mRNA, complete cds	5.0	9.0	more green leaves, longer panicle length
BR 482	unknown protein	5.0	9.0	more green leaves
BR 484	<i>Zea mays</i> clone 12745 mRNA sequence	5.0	9.0	same phenotype
BR 486	<i>Arabidopsis thaliana</i> LHCB3 (LIGHT-HARVESTING CHLOROPHYLL B-BINDING PROTEIN 3); structural molecule (LHCB3) mRNA, complete cds	5.0	9.0	same phenotype
BR 487	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB021M22, complete sequence	5.0	9.0	same phenotype
BR 488	unknown gene function	5.7	9.0	more green leaves
BR 489	<i>Arabidopsis thaliana</i> 60S ribosomal protein L13 (RPL13D) (AT5G23900) mRNA, complete cds	5.0	9.0	more green leaves
BR 490	short-chain dehydrogenase/reductase (SDR) family protein	5.0	9.0	more green leaves
BR 491	transducin family protein / WD-40 repeat family protein (AT2G16405) mRNA	5.0	9.0	same phenotype
BR 492	Phagocytosis and cell motility protein ELMO1-related (AT3G03610) mRNA	5.0	9.0	same phenotype
BR 494	HD2C (HISTONE DEACETYLASE 2C)	5.0	9.0	same phenotype
BR 495	unknown protein	5.1	9.0	same phenotype
BR 496	unknown gene function	5.0	9.0	shorter culm length
BR 497	unknown gene function	5.0	9.0	same phenotype
BR 498	unknown protein	5.0	9.0	same phenotype
BR 499	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH003N18, complete sequence	5.0	9.0	same phenotype
BR 485	<i>Arabidopsis thaliana</i> unknown protein (At1g15180) mRNA, complete cds / MATE efflux family protein [<i>Arabidopsis thaliana</i>]	5.0	9.0	shorter culm length
BR 500	<i>Arabidopsis lyrata</i> subsp. <i>lyrata</i> hypothetical protein, mRNA	5.1	9.0	same phenotype
BR 501	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB030F12, complete sequence	5.0	9.0	same phenotype
BR 502	<i>Brassica oleracea</i> var. <i>botrytis</i> tonoplast intrinsic protein bobTIP26-1 mRNA, complete cds	5.0	9.0	same phenotype
BR 503	metal tolerance protein 5 (MTP5)	5.0	9.0	same phenotype
BR 504	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrH003N18, complete sequence	5.0	9.0	same phenotype

Appendix Table 1. Continued.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 505	<i>Brassica napus</i> 1433-3 mRNA, complete cds	5.0	9.0	same phenotype
BR 506	<i>Brassica oleracea</i> var. botrytis tonoplast intrinsic protein bobTIP26-1 mRNA, complete cds	5.0	9.0	shorter culm length
BR 507	<i>Arabidopsis thaliana</i> 60S ribosomal protein L21 (RPL21C) (AT1G09690) mRNA, complete cds	5.0	9.0	shorter culm length
BR 508	<i>Arabidopsis thaliana</i> BOU (A BOUT DE SOUFFLE); binding / transporter (BOU) mRNA, complete cds	5.0	9.0	same phenotype
BR 509	<i>Arabidopsis thaliana</i> ING1 (INHIBITOR OF GROWTH 1); DNA binding / methylated histone residue binding (ING1) mRNA, complete cds	5.0	9.0	same phenotype
BR 510	hypothetical protein	5.0	9.0	shorter culm length
BR 511	VHS domain-containing protein / GAT domain-containing protein	5.3	9.0	same phenotype
BR 512	RsGGT2 mRNA for gamma-glutamyl transferase	5.0	9.0	same phenotype
BR 513	hypothetical protein	4.5	5.0	same phenotype
BR 514	<i>Arabidopsis thaliana</i> clone 25991 mRNA, complete sequence	5.0	9.0	same phenotype
BR 515	Prephenate dehydrogenase family protein	5.0	9.0	same phenotype
BR 516	<i>Brassica rapa</i> subsp. pekinensis clone KBrB028I01, complete sequence	5.0	9.0	same phenotype
BR 517	ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein tyrosine kinase (NAK)	5.0	9.0	same phenotype
BR 518	<i>Arabidopsis thaliana</i> ATS1; glycerol-3-phosphate O-acyltransferase (ATS1) mRNA, complete cds	3.0	9.0	same phenotype
BR 519	<i>Arabidopsis thaliana</i> HVA22J (HVA22-LIKE PROTEIN J)	5.0	9.0	same phenotype
BR 520	<i>Arabidopsis thaliana</i> COX6B (CYTOCHROME C OXIDASE 6B); cytochrome-c oxidase (COX6B) mRNA, complete cds	5.0	9.0	same phenotype
BR 521	<i>Zea mays</i> clone 10882 mRNA sequence	5.0	9.0	same phenotype
BR 522	hypothetical protein, mRNA	3.0	9.0	same phenotype
BR 523	<i>Arabidopsis thaliana</i> AtMAPR2 (<i>Arabidopsis thaliana</i> membrane-associated progesterone binding protein 2); heme binding (AtMAPR2) mRNA, complete cds	5.0	9.0	same phenotype
BR 524	inorganic pyrophosphatase family protein, mRNA	5.0	9.0	same phenotype
BR 525	unknown gene function	5.5	9.0	same phenotype
BR 526	PsbQ2 mRNA for oxygen-evolving enhancer protein 3-2	5.0	9.0	same phenotype
BR 527	<i>Brassica rapa</i> clone 231 CLA1-like protein mRNA, complete cd	5.0	9.0	same phenotype
BR 528	NAK; ATP binding / kinase/ protein kinase/ protein serine/threonine kinase/ protein tyrosine kinase (NAK) mRNA	5.0	9.0	same phenotype
BR 529	unknown gene function	6.0	9.0	same phenotype
BR 530	Myb family transcription factor	5.2	9.0	same phenotype
BR 531	<i>Zea mays</i> clone 14041 mRNA sequence	5.0	9.0	same phenotype

Appendix Table 1. Continued.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 532	Sterol 4-alpha-methyl-oxidase 2 (SMO2-2)	5.0	9.0	same phenotype
BR 533	hypothetical protein	5.0	9.0	more taller plant height
BR 534	hypothetical protein	4.5	9.0	same phenotype
BR 535	mRNA for ACC oxidase (ACC0x2)	5.0	9.0	same phenotype
BR 536	hypothetical protein	5.0	9.0	same phenotype
BR 537	<i>Brassica rapa</i> subsp. pekinensis clone KBrB021M22, complete sequence	5.0	9.0	same phenotype
BR 538	hypothetical protein	5.0	9.0	same phenotype
BR 539	<i>Raphanus sativus</i> SAUR gene	5.0	9.0	same phenotype
BR 541	LHCB4.2 (light harvesting complex PSII)	3.0	9.0	more taller plant height, longer panicle length
BR 542	<i>Brassica rapa</i> subsp. pekinensis clone KBrB048F07, complete sequence	5.0	9.0	same phenotype
BR 544	<i>Brassica rapa</i> subsp. pekinensis clone KBrH003N18, complete sequence	5.0	9.0	more taller plant height
BR 545	<i>Arabidopsis thaliana</i> mRNA for 50S ribosomal protein L3, complete cds, clone: RAFL04-10-N10	5.0	9.0	more taller plant height
BR 546	<i>Arabidopsis thaliana</i> ATNUDX19 (ARABIDOPSIS THALIANA NUDIX HYDROLASE HOMOLOG 19); hydrolase/ metal ion binding (ATNUDX19) mRNA, complete cds	4.0	9.0	more taller plant height
BR 547	UDP-galactose transporter 3 (<i>ATUTR3</i>)	3.0	5.0	same phenotype
BR 548	<i>A. lyrata</i> subsp. lyrata inorganic pyrophosphatase family protein, mRNA	5.0	9.0	more taller plant height
BR 549	BcRUBP mRNA for ribulose 1,5-bisphosphate carboxylase/oxygenase	5.0	9.0	more taller plant height
BR 550	<i>Arabidopsis thaliana</i> ATRDH2 (ARABIDOPSIS THALIANA RHODANESE HOMOLOGUE 2); 3-mercaptopyruvate sulfurtransferase/ thiosulfate sulfurtransferase (ATRDH2) mRNA, complete cds	5.0	9.0	shorter plant height
BR 551	<i>Arabidopsis thaliana</i> RNA binding (AT4G39040) mRNA, complete cds	5.0	5.0	same phenotype
BR 552	ribosomal protein S6 family protein	5.0	9.0	same phenotype
BR 553	<i>Brassica rapa</i> PsbQ2 mRNA for oxygen-evolving enhancer protein 3-2, complete cds	5.0	9.0	same phenotype
BR 554	transducin family protein, mRNA	5.0	9.0	same phenotype
BR 555	<i>Brassica rapa</i> subsp. pekinensis clone KBrB034A02, complete sequence	5.0	9.0	same phenotype
BR 556	Hypothetical protein, mRNA	5.0	9.0	more taller plant height
BR 557	BnD21 mRNA sequence	5.0	5.0	more taller plant height
BR 558	unknown gene function	5.0	9.0	more taller plant height
BR 560	<i>B. oleracea</i> mRNA for ACC oxidase (ACC0x2)	5.0	9.0	same phenotype
BR 561	<i>Arabidopsis thaliana</i> COX6B (CYTOCHROME C OXIDASE 6B); cytochrome-c oxidase (COX6B) mRNA, complete cds	5.0	5.8	longer culm length

Appendix Table 1. Continued.

Code	Gene Function	Cold stress score ^a	Salinity stress score ^b	Observed phenotype ^c
BR 562	<i>Arabidopsis thaliana</i> protein binding / zinc ion binding (AT5G45100) mRNA, complete cds	5.0	9.0	same phenotype
BR 563	<i>Brassica rapa</i> subsp. <i>pekinensis</i> clone KBrB036G18, complete sequence	5.0	9.0	same phenotype
BR 566	<i>Arabidopsis thaliana</i> ALB3 (ALBINO 3); P-P-bond-hydrolysis-driven protein transmembrane transporter (ALB3) mRNA, complete cds	5.0	9.0	same phenotype
BR 567	<i>Zea mays</i> clone 12993 mRNA sequence	9.0	9.0	same phenotype
BR 569	<i>Zea mays</i> clone 14041 mRNA sequence	5.0	9.0	same phenotype
BR 571	<i>Arabidopsis thaliana</i> mRNA for putative mrp protein, complete cds, clone: RAFL05-19-J04	5.0	9.0	same phenotype
BR 572	<i>Brassica rapa</i> clone 231 CLA1-like protein mRNA, complete cds	5.0	9.0	same phenotype
BR 579	<i>Zea mays</i> clone 20053 mRNA sequence	5.0	9.0	same phenotype
BR 734	<i>Arabidopsis thaliana</i> PDCB1 (PLASMODESMATA CALLOSE-BINDING PROTEIN 1); callose binding / polysaccharide binding (PDCB1) mRNA, complete cds	5.0	9.0	same phenotype
BR 582	isoflavone reductase family protein	5.0	9.0	same phenotype
BR 583	hypothetical protein	5.0	9.0	same phenotype
BR 525	disease resistance response protein	5.0	6.8	same phenotype
BR 633	ribosomal protein L9 family protein	5.0	9.0	same phenotype
BR 640	TMS membrane family protein / tumour differentially expressed (TDE) family protein	5.0	9.0	same phenotype
BR 660	translational inhibitor protein like	5.0	9.0	same phenotype
BR 670	RPL9 (RIBOSOMAL PROTEIN L9); structural constituent of ribosome (RPL9)	5.0	9.0	same phenotype
BR 700	tonoplast intrinsic protein bobTIP26-1	5.0	9.0	same phenotype
BR 708	<i>ATPHB2</i> (PROHIBITIN 2) (<i>ATPHB2</i>)	5.0	9.0	same phenotype
BR 721	unknown protein	5.0	5.3	same phenotype
BR 725	40S ribosomal protein S4 (RPS4A)	5.0	9.0	same phenotype
BR 735	SPP2 (SUCROSE-6F-PHOSPHATE PHOSPHOHYDROLASE 2); catalytic/ magnesium ion binding / phosphatase/ sucrose-phosphatase (SPP2)	5.0	9.0	same phenotype
BR 743	mitochondrial pyruvate dehydrogenase kinase (PDK)	5.0	7.0	same phenotype

^a Cold stress score: 1= seedling dark green, 3=seedling light green, 5=seedling yellow, 7=seedling brown, 9=seedling dead. The cold screening was conducted at Rural Development Administration, Chuncheon, Korea

^b Salinity stress score was based on the following at seedling stage: 1=Growth nearly normal, 3= Growth nearly normal but some leaves rolled (sal), 5= Growth reduced and most leaves rolled (salt; only a few elongating, 7= Growth completely ceases; most leaves dry; some plants dying, 9= Almost all plants dead or dying

^c Observed phenotype was based on significant differences between the wilt type Gopum using pair-wise Dunnett's multiple comparison. The same phenotype means no significant differences in the agro-morphological character such as plant height, culm length, chlorophyll contents, panicle length, number of tillers.