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Full Length Research Paper

Identification of salt-stress induced differentially expressed genes in barley leaves using the annealing-control-primer-based GeneFishing technique

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In the present study, we have used an annealing-control-primer (ACP)-based differentially display RT-PCR method to identify salt-stress-induced differentially expressed genes (DEGs) in barley leaves. Using 120 ACPs, a total of 11 up-regulated genes were identified and sequenced. Temporal expression patterns of some up-regulated DEGs in response to salt stress were further analyzed by Northern blot analysis. The possible roles of these identified genes are discussed within the context of their putative role in response to salt stress. Thus, the identification of some novel genes – such as SnRK1-type protein kinase; 17 kDa, class I, small heat shock protein; and RNase S-like protein precursor genes – may offer a new avenue for better understanding the salt stress response in plants, knowledge which might be helpful for developing future strategies.

Key words: Barley, GeneFishing technique, salt stress.

INTRODUCTION

Salinity in agricultural fields, a major environmental stress, is a severe constraint on crop growth and productivity in many regions, and the situation has become a global concern. It has been reported that around 5% of the cultivated land is affected by salinity (Askari et al. 2006). Moreover, it has been predicted that increasing salinization in agricultural fields will reduce the land available for cultivation by 30% within the next 25 years, and up to 50% by the year 2050 (Wang et al., 2003). Excess salt in soil or in solutions interferes with several physiological and biochemical processes, resulting in problems such as ion imbalance, mineral deficiency, osmotic stress, ion toxicity and oxidative stress; these conditions ultimately interact with several cellular components, including DNA, proteins, lipids, and pigments in plants (Zhu, 2002), impeding the growth and development of a vast majority of crops and vegetables. However, there are varying levels of sensitivity to salt stress among plants, including barley genotypes (Chen et al., 2007). The protection of crops against salinity-induced damage is, therefore, a global challenge for the near future. Plants can employ numerous physiological and biochemical strategies to cope with adverse conditions by altering the functioning of a number of genes. Therefore, the identification of genes involved in biotic or abiotic stress responses is a fundamental step in understanding the molecular mechanisms of stress responses and developing transgenic plants with enhanced tolerance to stress.

Recently, an improved, new technique for identifying differentially expressed genes (DEGs) was developed based on annealing control primers (ACPs) (Hwang et al., 2003; Kim et al., 2004). This method has been frequently used to identify DEGs in mammalian systems (Hwang et al., 2003; Kim et al., 2004; Kim et al., 2008). However, in the present study, for the first time, we have used this

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technique to identify salt-responsive genes in barley leaves. The main goal of this study was to identify novel genes that are differentially regulated upon exposure to salt stress and thus to provide new insight for the development of plants with enhanced tolerance to saline conditions.

MATERIALS AND METHODS

Plant material, salt treatment and sample collection

Barley (*Hordeum vulgare* L. cv. Yeongyang Bori) seeds were obtained from the National Institute of Animal Science, RDA, Republic of Korea. Surface-sterilized seeds were germinated in a plastic tray for 7 d in a control growth chamber at 24 ± 1℃ (day/night) with a 12 h photoperiod under an irradiance of 350 µmol m^{-2} s^{-1} and a relative humidity of 60 - 70%. Seedlings were treated with 200 mM NaCl for 6 h in a hydroponic culture system. Seedlings without any treatment were used as controls. Leaf samples (treated and untreated) were collected after the treatment and kept at -80℃ until analyzed.

Synthesis of first-strand cDNAs

Total RNA was isolated from the leaf tissues of treated and control plants using the Plant RNeasy mini kit (Qiagen, CA, USA) and used for reverse-transcriptase-catalyzed first-strand cDNA synthesis. The reverse transcription reaction was performed for 1.5 h at 42℃ in a final reaction volume of 20 µl containing 3 µg of the purified, total RNA; 4 µl of 5× reaction buffer (Promega, Madison, WI, USA); 5 µl of dNTPs (each 2 mM); 2 µl of 10 µM dT-ACP1 [5´-CTGTGAATGCTGCGACTACGATIIIIIT(18)-3´]; 0.5 µl of RNasin® RNase Inhibitor (40 U/µl; Promega); and 1 µl of Moloney murine leukemia virus reverse transcriptase (200 U/µl; Promega). After synthesis, first-strand cDNAs were diluted by the addition of 80 µl of ultra-purified water for the GeneFishing™ PCR.

ACP-based GeneFishing™ reverse transcription chain reaction, gene cloning and sequencing

Using the GeneFishing™ DEG kit (Seegene, Seoul, South Korea), DEGs were identified by an ACP-based PCR method as described by Kim et al. (2004). The amplified PCR products were separated on 2% agarose gels stained with ethidium bromide (EtBr), and DEGs were selected visually. Selected DEGs were extracted from the gel by using the GENCLEAN® II Kit (Q-BIO gene, Carlsbad, CA, USA) and directly cloned into a TOPO TA cloning vector (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The cloned plasmids were sequenced with an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the M13 forward primer (5'-CGCCAGGGTTTTCCCAGTCACGA-3') or M13 reverse primer (5'-AGCGGATAA CAATTTCACACAGGA-3'). Sequencing data were confirmed with the GenBank database through the BlastX program of NCBI (http://www.ncbi.nlm.nih.gov/BLAST/) and/or the barley germplasm database (http://shigen.lab.nig.ac.jp/barley/).

RNA extraction and Northern blot analysis

Northern blot analysis was carried out to investigate the temporal expression of some selected genes in response to salt stress. Leaf samples were harvested 1, 3, 6, 12 and 24 h after treatment. Total RNAs were isolated from the leaf tissues of treated and control plants using a Plant RNeasy mini kit (Qiagen, CA, USA). Ten micrograms of total RNA samples were separated on a 1.2% agarose gel containing formaldehyde. Gene-specific PCR products (DEGs 9, 13, 14, 20, 32, 39 and 49) were labeled with [α-^{32}P] dCTP using a random primer labeling kit (GE Healthcare). Northern blot analysis was performed as described previously (Ahsan et al. 2005).

RESULTS AND DISCUSSION

In order to identify the salt-responsive, differentially expressed genes, ACP-based GeneFishing PCR technology was used with a combination of 120 arbitrary primers. Among the 120 GeneFishing primers (GPs), a total of 19 GPs showed differentially expressed DNA bands, with 11 bands increased and 8 bands decreased in intensity in the treated sample compared to the control. The sizes of the bands varied from 150 to 1000 bp (Table 1). Among the DEGs, GPs 25, 39, 47, 63, 89, 98, 101, 104 and 119 showed significantly increased or newly synthesized bands in the treated sample. Figure 1A shows a typical agarose gel electropherogram for PCR products amplified using ACPs for control and treated samples, and 1B represents the close-up views of some increased DNA bands. In this study, these increased DNA bands were purified from the agarose gels and cloned into TOPO TA cloning vectors for further analysis. The clones were sequenced as described in the Materials and Methods. The sequence similarities of these DEGs are summarized in Table 1.

Along with previously reported salt-responsive genes and/or proteins, such as myo-inositol 1-phosphate synthases (MIPS), metallothioneine (MT), alcohol dehydrogenase (ADH) and lipoxygenase 2 (LOX2), some novel genes – such as SnRK1-type protein kinase; 17 kDa, class I, small heat shock protein; and RNase S-like protein precursor (rsh1) genes – were also identified. According to their putative physiological functions, these identified genes are involved in several processes and/or pathways including stress and detoxification, protein biosynthesis, signaling, and regulatory networks.

From all the identified DEGs, seven genes were selected to confirm the efficacy of the ACP system, as well as to investigate the temporal mRNA expression patterns of these genes in response to salt stress (Figure 2). The selected DEGs encoded SnRK1-type protein kinase (DEG-9); LOX2 (DEG-13); CHS (DEG-14); LHC II type I protein (DEG-20); a 17 kDa, class I, small heat shock protein (DEG-32); MIPS (DEG-39) and MT2 (DEG-49). Results of a northern blot analysis were consistent with the ACP-based RT-PCR results for all of the selected genes at the 6 h time point; however, some of the genes, such as those for the SnRK1-type protein kinase (DEG-9) and the LHC II type I protein (DEG-20), showed declining expression until the time point examined (Figure 2). On

Table 1. Salt-stress induced differentially expressed genes (DEGs) in barley leaves identified ACP-based differentially display RT-PCR method.

No.	GP	DEG No.	PCR products (bp)	Identity	Accession no.	Score	e-value
1	25	DEG 9	1021	SnRK1-type protein kinase, partial	AJ007990	32	0.99
2	39	DEG 13	419	Lipoxygenase 2 (lox2:Hv:3 gene)	AJ507213	34	0.1
3	47	DEG 14	523	CHS gene for chalcone synthase	X58339	1013	0.0
4	63	DEG 20	249	LHC II type I protein	X89023	100	1E-21
5	63	DEG 21	113	Alcohol dehydrogenase gene (Adh2)	X12733	30	0.38
6	89	DEG 31	722	LHC II type I protein	X89023	521	e-148
7	89	DEG 32	284	17 kDa class I small heat shock protein	Y07844	32	0.26
8	98	DEG 35	196	RNase S-like protein precursor (rsh1) gene, complete cds	AF182197	303	3E-83
9	101	DEG 39	357	Myo-inositol 1-phosphate synthase mRNA, complete cds	AF056325	34	0.084
10	104	DEG 43	571	LHC II type I protein	X89023	454	e-128
11	119	DEG 49	548	Metallothioneine type2 (mt2b gene).	AJ511346	367	e-102

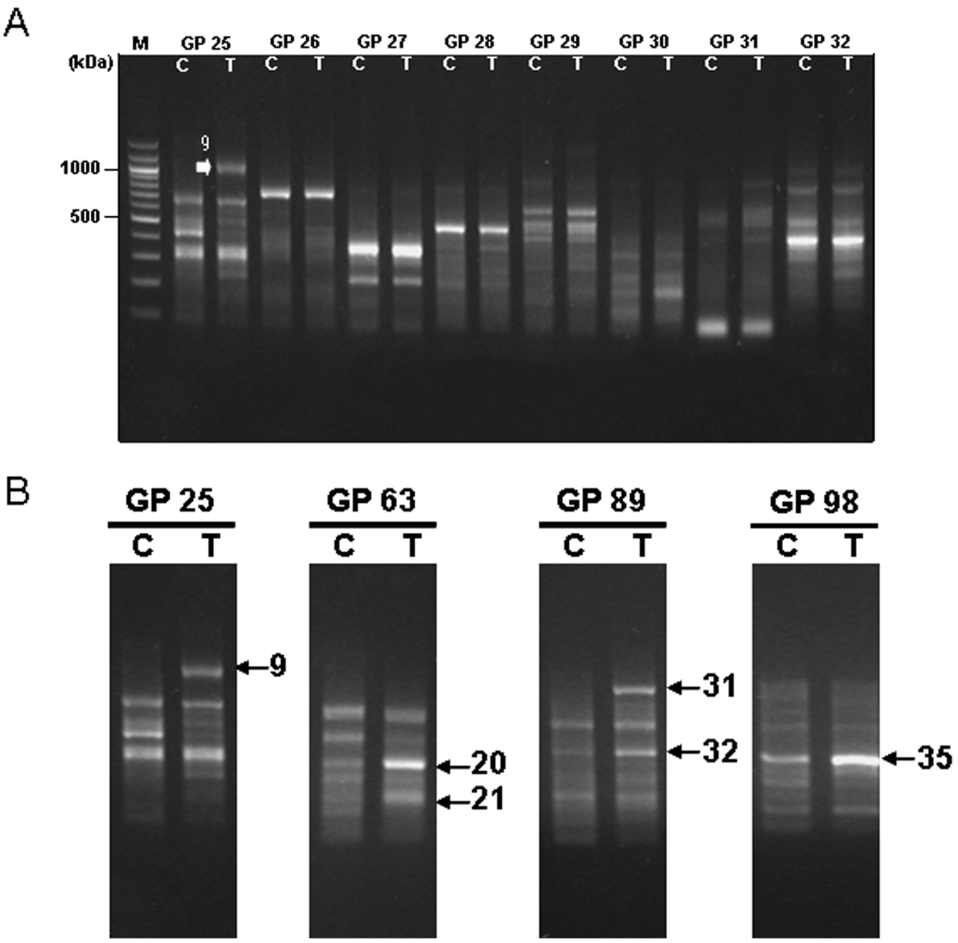

Figure 1. A typical agarose gel shows differentially expressed PCR bands (A). B, represents the close up views of some DEGS.

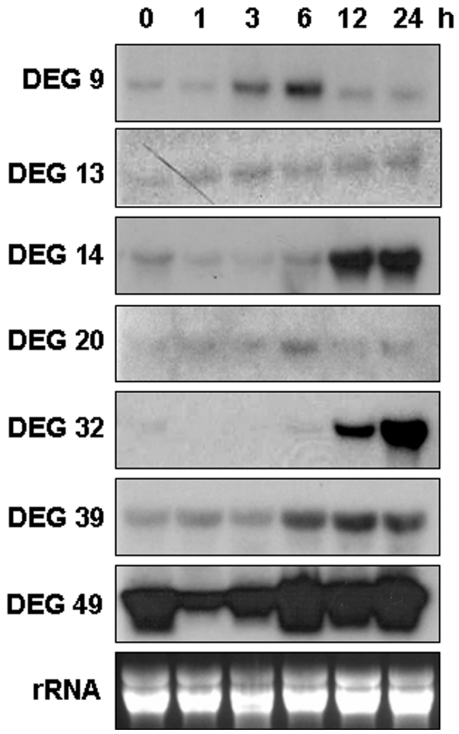

Figure 2. Northern blot analysis of some salt induced differentially expressed genes.

the other hand, LOX2 (DEG-13); CHS (DEG-14); the 17 kDa, class I, small heat shock protein (DEG-32); MIPS (DEG-39) and MT2 (DEG-49) increased temporally in response to salt stress. These results clearly indicate that the mRNA levels of salt-induced, differentially expressed genes vary in response to the length of salt treatment.

High salinity caused osmotic stress and ionic imbalance in addition to inducing oxidative stress in plants. Therefore, plants have evolved a complex mechanism including ion homeostasis, osmotic adjustment, ion extrusion, and compartmentalization in protecting cells against high saline condition (Zhu, 2002). Gene up-regulation under stress condition indicates their strong needs in cells in order to protect or adapt that adverse condition. In the present study we therefore showed our interest to the up-regulated genes and thereby identified successfully a total of 11 up-regulated genes in barley leaves in response to salt stress. According to their putative role these genes are mainly involved in stress and detoxification pathways, and regulatory networks. A discussion of the possible role of these genes in the adaptation of plants to salt-stress conditions follows.

DEG-9 was identified as encoding a SnRK1-type protein kinase, a homologue of SNF1 (sucrose non-fermenting 1) and/or AMPK (AMP-dependent protein kinase) of yeast and/or mammals, respectively; however, little is known about the physiological role of SnRK1 in plants (Polge and Thomas, 2007). Lovas et al. (2003) first reported the involvement of SnRK1 in the plant stress response when they noted the salt hypersensitivity of the antisense StubGAL83 transgenic plants, suggesting that SnRK1 might activate defense and/or adaptation mechanisms for highly saline conditions. Moreover, it has been suggested that the targets of SnRK1 kinases are probably numerous and might take part, directly or indirectly, in carbohydrate and/or energy homeostasis (Polge and Thomas, 2007).

Lipoxygenases (LOXs) are ubiquitous enzymes in eukaryotes, they have been found at relatively high levels in plants, and they are involved in the synthesis of compounds with growth regulator activity and signaling molecules such as jasmonic acid (Creelman and Mullet, 1997). In the present study, *LOX2* (DEG-13) was identified as a gene up-regulated in response to salt stress in barley leaves (Figure 1A). It has been reported that *LOX2:Hv:1* is chloroplast-localized, and it might be involved in jasmonate biosynthesis or in the degradation of chloroplastic membrane constituents during a senescence process (Voros et al. 1998). Moreover, *LOXs* are also responsible for membrane degradation because they catalyze the dioxygenation of polyunsaturated fatty acids, producing hydroperoxy fatty acids that are toxic to the cell (Macri et al., 1994). Thus, up-regulation of *LOX2* in response to salt stress indicates that *LOX* action leads to membrane degradation and related cell death in salt-induced oxidative conditions (Melillo et al., 1990) as a result of the synthesis of jasmonic acid, which is known to be involved in senescence, or other signaling pathways (Creelman and Mullet, 1997; Rusterucci et al., 1999).

Chalcone synthase (CHS) (DEG-14), which catalyzes the first committed step in flavonoid biosynthesis, showed a temporal up-regulation in response to salt stress (Figure 2). The enzymes of the flavonoid biosynthesis pathway, such as CHS, are activated in plants under various stress conditions (Bieza and Lois, 2001; Ahsan et al., 2008). In addition, flavonoids – a large group of phenolic secondary metabolites – are widespread among plants and have a protective role in plant tissues exposed to several external environmental stresses such as drought, cold, wounding, or UV light (Winkel-Shirley, 2002; Jaakola et al., 2004).

Three DEGs (DEG-20, 31 and 43) were identified as encoding light harvesting complex (LHC) II type I protein. Up-regulation of LHC II type I protein in response to salt stress suggests that this protein may protect the cells against salt-induced ROS. In support of this hypothesis, Johnson et al. (2007) have recently shown that protein of the LHC family bound with zeaxanthin enhances the resistance of *Arabidopsis* to photooxidative stress by a lipid-protective, antioxidant mechanism.

Alcohol dehydrogenase (*Adh*) genes are among the best characterized loci in higher plants, both genetically and at the molecular level; the best known of these genes

responds to hypoxic and/or anoxic conditions. DEG-21 was identified as *Adh*2 (Table 1). Transcriptional activation of the *Adh* gene has also been noticed in response to several environmental stresses including saline conditions (Matton et al., 1990; Christie et al., 1991; Baisakh et al. 2008). Consistent with the salt-stress response at the mRNA level, it has also been demonstrated that *Arabidopsis* mutants with defective *Adh* expression showed defective responses to cold and osmotic stresses (Conley et al., 1999). The role of *Adh*2 in response to salt stress is not clearly understood; however, the crosstalk between the alcohol fermentation pathway and one of the salt-tolerance mechanisms needs to be investigated (Baisakh et al., 2008).

Plants' small heat shock proteins (sHSPs) are encoded by nuclear genes in five multi-gene families and are localized to different cellular compartments including the cytosol (class I and II), chloroplast (class III), ER (class IV), and mitochondrion (class V) (Vierling 1991). sHSPs are usually undetectable in vegetative tissue under normal growth conditions but can be induced by developmental stimuli or by environmental stresses (Sun et al. 2002). Plant sHSPs also accumulate in response to a large number of stresses including salt stress (Sun et al., 2002; Kore-eda et al., 2004; Lee et al., 2007). In the present study, a 17 kDa, class I, small heat shock protein gene (DEG-32) was up-regulated in barley leaves by salt treatment (Figures 1 and 2), suggesting that increased activity of this sHSP may repair and aid in the renaturation of stress-damaged proteins and/or reestablishing normal protein conformations in the cytosol.

S-like RNase genes constitute an important family of RNA-degrading enzymes that have been found in all plants that have been examined for their presence. Gausing (2000) cloned the rsh1 (RNase S-like homologue) from barley and found that rhs1 is exclusively expressed in young leaf tissue and is substantially induced by light. Earlier studies have shown that S-like RNases function in several physiological processes including phosphate starvation, senescence, wounding, the cell death pathway, defense against pathogens and the light signaling pathway (Bariola and Green, 1997; Liang et al., 2002). In the present study, an RNase S-like protein precursor (rsh1) gene (DEG-35) was identified from the salt-stress-treated leaves (Figure 1). Up-regulation of this gene in response to salt stress suggests that the gene might be involved in salt-stress-induced senescence or nutrient deficiency processes; however, to clarify this hypothesis, further studies are needed.

Myo-inositol 1-phosphate synthase (MIPS) catalyses the conversion of D-glucose 6-phosphate to 1-myo-inositol-1-phosphate, the first and rate-limiting step in the biosynthesis of all inositol-containing compounds (Abreu and Aragao, 2007); its derivatives are commonly studied with respect to cell signaling and membrane biogenesis, but they also participate in responses to salinity in animals and plants (Nelson et al., 1998). In our investigation, MIPS (DEG-39) expression gradually increased in response to salt stress (Figure 2), which is very consistent with other published reports (Ishitani et al., 1996; Nelson et al., 1998; Chun et al., 2003; Baisakh et al., 2008). Das-Chatterje et al. (2006) provide evidence that overexpression of MIPS confers salt-tolerance to evolutionarily diverse organisms from prokaryotes to eukaryotes, including crop plants. Thus, these results suggest that MIPS could be used as a biomarker for salt stress.

Metallothioneins (MTs) are cysteine-rich, low-molecular-weight, metal-binding proteins that are commonly involved in metal detoxification and homeostasis in both prokaryotes and eukaryotes (Zhou and Goldsbrough, 1994; Jin et al. 2006; Zhigang et al., 2006; Nishiuchi et al., 2007). In agreement with other transcriptomic analyses of the salt response in plants, we also identified MT (DEG-49) as a gene up-regulated under salt stress (Figure 2). Although the ability of MTs to bind metal ions is their most notable feature, a few reports have shown that transcripts of MTs accumulated under several abiotic stress conditions, including salt stress (Jin et al., 2006; Nishiuchi et al., 2007). This observation suggests that, in addition to having metal-binding activities, MTs are certainly involved in other processes such as the scavenging of reactive oxygen species (ROS) (Akashi et al. 2004) and the protection of cells against salt stress or salt-induced ROS (Nishiuchi et al., 2007). In support of this hypothesis, there is now evidence that overexpression of plant MTs in prokaryotes provides enhanced tolerance to salt and/or oxidative stress (Jin et al., 2006; Nishiuchi et al., 2007).

Although there have been a few transcriptomic and/or proteomic analyses carried out to investigate the salt-stress response in barley, in the present study, we have used a new ACP-based, differential display, RT-PCR method to identify salt-stress-induced, differentially expressed genes in barley leaves. Together with previously identified salt-responsive genes, for the first time, we have identified some novel, salt-induced genes, including SnRK1-type protein kinase and RNase S-like protein precursor genes. Thus, identification of these genes may provide a more comprehensive knowledge of the complexity of the AMP-activated protein kinase (AMPK) cascade, as well as the role of RNA-degrading enzymes in salt stress. However, further studies are required to analyze these proteins' precise role in response to salt stress.

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REFERENCES

Abreu EF, Aragao FJ (2007). Isolation and characterization of a myo-inositol-1-phosphate synthase gene from yellow passion fruit (*Passiflora edulis* f. flavicarpa) expressed during seed development and environmental stress. Ann. Bot. 99: 285-292.

Ahsan N, Lee DG, Alam I, Kim PJ, Lee JJ, Ahn YO, Kwak S.S, Lee IJ, Bahk JD, Kang KY, Renaut J, Komatsu S, Lee BH (2008). Comparative proteomic study of arsenic-induced differentially expressed proteins in rice roots reveals glutathione plays a central role during As stress. Proteomics, 8: 3561-3576.

Ahsan N, Yoon HS, Jo J (2005). Molecular cloning of a BcPGIP cDNA from *Brassica campestris* and its expression to several stresses. Plant Sci. 169: 1081-1089.

Akashi K, Nishimura N, Ishida Y, Yokota A (2004). Potent hydroxyl radical-scavenging activity of drought-induced type-2 metallothionein in wild watermelon. Biochem. Biophy. Res. Commun. 323: 72-78.

Akashi K, Nishimura N, Ishida Y, Yokota A (2006). Effects of salinity levels on proteome of *Suaeda aegyptiaca* leaves. Proteomics, 6: 2542–2554.

Baisakh N, Subudhi PK, Varadwaj P (2008). Primary responses to salt stress in a halophyte, smooth cordgrass (*Spartina alterniflora* Loisel.). Funct. Integr. Genomics. Doi: 10.1007/s10142-008-0075-x.

Bariola PA, Green PJ (1997). Plant ribonucleases. In: D'Alessio G, Riordan JF (ed) Ribonucleases: Structures and Functions. Academic Press. NY., pp. 163-190.

Bieza K, Lois R (2001). An *Arabidopsis* mutant tolerant to lethal ultraviolet-B levels shows constitutively elevated accumulation of flavonoids and other phenolics. Plant Physiol. 126: 1105-1115.

Chen Z, Cuin TA, Zhou M, Twomey A, Naidu BP, Shabala S (2007). Compatible solute accumulation and stress-mitigating effects in barley genotypes contrasting in their salt tolerance. J. Exp. Bot. 58: 4245-4255.

Christie PJ, Hahn M, Walbot V (1991). Low-temperature accumulation of alcohol dehydrogenase-1 mRNA and protein activity in maize and rice seedlings. Plant Physiol. 95: 699-706.

Chun JA, Jin UH, Lee JW, Yi YB, Hyung NI, Kang MH, Pyee JH, Suh MC, Kang CW, Seo HY, Lee SW, Chung CH (2003). Isolation and characterization of a myo-inositol 1-phosphate synthase cDNA from developing sesame (*Sesamum indicum* L.) seeds: functional and differential expression, and salt-induced transcription during germination. Planta 216: 874-880.

Conley TR, Peng HP, Shih MC (1999). Mutations affecting induction of glycolytic and fermentative genes during germination and environmental stresses in *Arabidopsis*. Plant Physiol. 119: 599-608.

Creelman RA, Mullet JE (1997). Biosynthesis and action of jasmonates in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 48: 355-381.

Das-Chatterjee A, Goswami L, Maitra S, Dastidar KG, Ray S, Majumder AL (2006). Introgression of a novel salt-tolerant L-myo-inositol 1-phosphate synthase from *Porteresia coarctata* (Roxb.) Tateoka (PcINO1) confers salt tolerance to evolutionary diverse organisms. FEBS Lett. 580: 3980-3988.

Gausing K (2000). A barley gene (rsh1) encoding a ribonuclease S-like homologue specially expressed in young light-grown leaves. Planta. 210: 574-579.

Hwang IT, Kim YJ, Kim SH, Kwak CI, Gu YY, Chun JY (2003). Annealing control primer system for improving specificity of PCR amplification. BioTech. 35: 1180-1184.

Ishitani M, Majumder AL, Bornhouser A, Michalowski CB, Jensen RG, Bohnert HJ (1996). Coordinate transcriptional induction of myo-inositol metabolism during environmental stress. Plant J. 9: 537-548.

Jaakola L, Määttä-Riihinen K, Kärenlampi S, Hohtola A (2004). Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L) leaves. Planta 218: 721-728.

Jin S, Cheng Y, Guan Q, Liu D, Takano T, Liu S (2006). A metallothionein-like protein of rice (rgMT) functions in *E. coli* and its gene expression is induced by abiotic stresses. Biotechnol. Lett. 28: 1749-1753.

Johnson MP, Havaux M, Triantaphylidès C, Ksas B, Pascal AA, Robert B, Davison PA, Ruban, AV, Horton P (2007). Elevated zeaxanthin bound to oligomeric LHCII enhances the resistance of Arabidopsis to photooxidative stress by a lipid-protective, antioxidant mechanism. J. Biol. Chem. 282: 22605-22618.

Kim TW, Kim HJ, Lee C, Kim HY, Baek SH, Kim JH, Kwon KS, Kim JR (2008). Identification of replicative senescence-associated genes in human umbilical vein endothelial cells by an annealing control primer system. Exp. Geront. 43: 286-295.

Kim YJ, Kwak CI, Gu YY, Hwang IT, Chun JY (2004). Annealing control primer system for identification of differentially expressed genes on agarose gels. BioTechnology 36: 424-426.

Kore-eda S, Cushman MA, Akselrod I, Bufford D, Fredrickson M, Clark E, Cushman JC (2004). Transcript profiling of salinity stress responses by large-scale expressed sequence tag analysis in *Mesembryanthemum crystallinum*. Gene, 341: 83-92.

Lee DG, Ahsan N, Lee SH, Kang KY, Bahk JD, Lee IJ, Lee BH (2007). A proteomic approach in analyzing heat-responsive proteins in rice leaves. Proteomics, 7: 3369-3383.

Liang L, Lai Z, Ma W, Zhang Y, Xue Y (2002). AhSL28, a senescence- and phosphate starvation-induced S-like RNase gene in *Antirrhinum* Biochem Biophy. Acta. 1579: 64-71.

Lovas A, Szabo L, Banfalvi Z (2003). Antisense repression of StubGAL83 affects root and tuber development in potato. Plant J. 33: 139–147.

Macrì F, Braidot E, Petrussa E, Vianello A (1994). Lipoxygenase activity associated to isolated soybean plasmamembranes. Biochem. Biophy. Acta. 1215: 109-114.

Matton DP, Constabel P, Brisson N (1990). Alcohol dehydrogenase gene expression in potato following elicitor and stress treatment. Plant Mol. Biol. 14: 775-783.

Nelson DE, Rammesmayer G, Bohnert HJ (1998). Regulation of cell-specific inositol metabolism and transport in plant salinity tolerance. Plant Cell. 10: 753-764.

Nishiuchi S, Liu S, Takano T (2007). Isolation and characterization of a metallothionein-1 protein in *Chloris virgata* Swartz that enhances stress tolerances to oxidative, salinity and carbonate stress in *Saccharomyces cerevisiae*. Biotechnol. Lett. 29: 1301-1305.

Polge C, Thomas M (2007). SNF1/AMPK/SnRK1 kinases, global regulators at the heart of energy control. Trends Plant Sci. 12: 20-28.

Rustérucci C, Montillet JL, Agnel JP, Battesti C, Alonso B, Knoll A, Bessoule JJ, Etienne P, Suty L, Blein JP, Triantaphylidès C (1999) Involvement of lipoxygenase-dependent production of fatty acid hydroperoxides in the development of the hypersensitive cell death induced by cryptogein on tobacco leaves. J. Biol. Chem. 274: 36446-36455.

Sun W, Van Montagu M, Verbruggen N (2002). Small heat shock proteins and stress tolerance in plants. Biochem. Biophy. Acta. 1577: 1-9.

Vierling E (1991). The roles of heat shock proteins in plants, Ann. Rev. Plant Physiol. Plant Mol. Biol. 42: 579-620.

Vörös K, Feussner I, Kühn H, Lee J, Graner A, Löbler M, Parthier B, Wasternack C (1998). Characterization of a methyljasmonate-inducible lipoxygenase from barley (*Hordeum vulgare* cv. Salome) leaves. Eur. J Biochem. 251: 36-44.

Wang W, Vinocur B, Altman A (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta. 218: 1-14.

Winkel-Shirley B (2002). Biosynthesis of flavonoids and effects of stress, Curr. Opin. Plant Biol. 5: 218-223.

Zhigang A, Cuijie L, Yuangang Z, Yejie D, Wachter A, Gromes R, Rausch T (2006). Expression of BjMT2, a metallothionein 2 from *Brassica juncea*, increases copper and cadmium tolerance in *Escherichia coli* and *Arabidopsis thaliana*, but inhibits root elongation in Arabidopsis thaliana seedlings. J. Exp. Bot. 57: 3575-3582.

Zhou J (1994). Goldsborough, P.B., Functional homologs of fungal metallothionein genes from Arabidopsis. Plant Cell. 6: 875-884.

Zhu JK (2002). Salt and drought stress signal transduction in plants. Ann. Rev. Plant Biol. 53: 247-273.