Mol Breeding (2012) 29:601–608
DOI 10.1007/s11032-011-9576-8

# Expression of the *Arabidopsis AtMYB44* gene confers drought/salt-stress tolerance in transgenic soybean

**Jun Sung Seo · Hwang Bae Sohn · Kaeyoung Noh · Choonkyun Jung · Ju Hee An · Christopher M. Donovan · David A. Somers · Dae In Kim · Soon-Chun Jeong · Chang-Gi Kim · Hwan Mook Kim · Suk-Ha Lee · Yang Do Choi · Tae Wha Moon · Chung Ho Kim · Jong-Joo Cheong**

Received: 14 February 2011 / Accepted: 1 April 2011 / Published online: 19 April 2011


**Abstract** AtMYB44, a member of the subgroup 22 R2R3 MYB transcription factors, positively regulates abscisic acid signaling to induce stomatal closure, thus conferring drought/salt-stress tolerance in *Arabidopsis thaliana*. In this study, *AtMYB44* was transformed into soybean [*Glycine max* (L.) Merrill] using the cotyledonary-node method. The resulting homozygous lines were shorter than the non-transgenic controls (Bert) throughout the growth period when grown in a greenhouse. The transgenic soybeans exhibited significantly enhanced drought/salt-stress tolerance, as observed in *Arabidopsis*. In field cultivation studies, the transgenic soybean plants showed reduced growth, but much higher yields upon seed harvest, demonstrating improved environmental stress tolerance. The amino acid and fatty acid compositions were not significantly altered in seeds harvested from the transgenic lines. These results suggest that the interaction of AtMYB44 with specific sequences in target gene promoters and/or specific proteins activates a tolerance mechanism that is conserved in *Arabidopsis* and soybean.

**Keywords** *Arabidopsis* · AtMYB44 · Soybean · Drought · Salt stress · Transcription factor

C. M. Donovan, retired.

J. S. Seo · H. B. Sohn · K. Noh · C. Jung · Y. D. Choi · T. W. Moon · J.-J. Cheong (✉)
Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea
e-mail: cheongjj@snu.ac.kr

J. H. An · C. H. Kim (✉)
Department of Food and Nutrition, Seowon University, Chongju 361-742, Korea
e-mail: chkim@seowon.ac.kr

C. M. Donovan · D. A. Somers
Department of Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN 55108, USA

D. I. Kim · S.-C. Jeong · C.-G. Kim · H. M. Kim
Bio-Evaluation Center, Korea Research Institute of Bioscience and Biotechnology, Cheongwon, Chungbuk 363-883, Korea

S.-H. Lee
Department of Plant Science and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Korea

*Present Address:*
D. A. Somers
Monsanto Co., 700 Chesterfield Parkway West, Mail Code CC5A, Office CC5221, Chesterfield, MO 63017, USA

## Introduction

Abiotic stresses, such as drought and salinity, adversely affect crop productivity. Biotechnological transformation of genes that could control the cellular processes of stress tolerance is among the strategies for improving crop productivity. A category of attractive genes to be transformed comprises transcription factors that regulate RNA polymerase activity for the expression of target genes. An important prerequisite for inter-species transformation of transcription factor genes is that the two species should contain homologous molecular compartments leading to the activation of the same specific cellular mechanism, such as transcription factor(s) with identical roles and the same *cis*-elements on the target gene promoters.

A transcription factor is composed of at least two discrete domains, a DNA-binding domain and a catalytic (activation or repression) domain. MYB transcription factors contain a conserved DNA-binding domain structurally and functionally related to those of the retrovirus oncogene *v-myb* and its cellular homologue *c-myb* (Peters et al. 1987). The MYB DNA-binding domain consists of two or three imperfect repeats of 50–53 amino acid residues (R1, R2 and R3) that form helix–turn–helix motifs. The repeats are well conserved between MYB proteins of yeast, animals and plants (Rosinsky and Atchley 1998).

In plants, two-repeat (R2R3) MYB family members predominate. In total, 126 R2R3 MYB-encoding genes have been identified in the *Arabidopsis* genome, making it one of the largest transcription factor groups in this plant (Yanhui et al. 2006). Roles of individual R2R3 MYB proteins have been explored in diverse plant processes, including hormonal signaling, cell-cycle control, stress response, secondary metabolism, cellular morphogenesis and meristem formation (Martin and Paz-Ares 1997; Jin and Martin 1999). Soybean also contains more than 100 R2R3 MYB genes in its genome. For example, *GmMYB101* was reported to be involved in the regulation of nitrogen fixation (Miyake et al. 2003). *GmMYB76*, *GmMYB92* and *GmMYB177* genes confer stress tolerance when expressed in transgenic *Arabidopsis* (Liao et al. 2008).

The *Arabidopsis* R2R3-type MYB transcription factors have been categorized into subgroups on the basis of conserved amino acid sequence motifs present on the carboxy-terminal side of the MYB domain. Subgroup 22 includes AtMYB44, AtMYB70, AtMYB73 and AtMYB77, and shares two conserved motifs: TGLYMSPxSP and GxFMxVVQEMIxxEVRSYM (Kranz et al. 1998; Romero et al. 1998; Stracke et al. 2001). The genes encoding subgroup 22 proteins have similar expression patterns and are associated with stress responses. *AtMYB44*, *AtMYB73* and *AtMYB77* are induced by wounding (Cheong et al. 2002) and white light treatment (Ma et al. 2005). These genes are transiently upregulated by cold stress (Fowler and Thomashow 2002). Microarray analysis revealed that these genes are upregulated together by salt stress in *sos2* (*salt overly sensitive 2*) mutants (Kamei et al. 2005). In addition, *AtMYB44* and *AtMYB77* expression levels are reduced in *fus3*, *lec1* and *abi3* mutants that are defective in dormancy development and desiccation tolerance during late embryogenesis and seed maturation (Kirik et al. 1998).

We reported previously that AtMYB44 plays a role in the abscisic acid (ABA)-mediated signaling pathway conferring abiotic stress tolerance via the enhancement of stomatal closure (Jung et al. 2008). Transgenic *Arabidopsis* overexpressing this gene exhibited enhanced drought/salt-stress tolerance by suppressing the expression of genes encoding a group of Ser/Thr protein phosphatase 2Cs (PP2Cs) that have been described as negative regulators of ABA signaling.

In this study, we transformed soybean with *AtMYB44*. The resulting transgenic lines exhibited enhanced drought/salt-stress tolerance without significant alteration of the seed chemical composition. Our data suggest that a mechanism conferring abiotic stress tolerance is conserved in *Arabidopsis* and soybean.

## Materials and methods

### Plant growth conditions

Soybeans were grown at 24°C and 50–60% relative humidity under a 16/8-h light/dark photoperiod in a growth chamber. A portion of the soybean plantlets grown for 4–5 weeks in the growth chamber were transplanted into a pot and grown in a greenhouse under natural light supplemented with 1,000-W high-pressure sodium lamps. For field testing, soybean seeds were sown (on 26 May 2010) and harvested (on

18 September 2010) at a field in the Bio-Evaluation Center (Cheongwon, Korea). Plants were spaced at 15-cm intervals in consecutive hill rows separated by 1 m, as described by Jeong et al. (2011).

### Generation of transgenic soybean

The full-length *AtMYB44* (At5g67300) cDNA (EST 119B8) was obtained from TAIR (The Arabidopsis Information Resource). The cDNA was fused with the CaMV 35S promoter gene as previously described (Jung et al. 2008) and inserted into pCAMBIA1305.1 (Cambia, Brisbane, QLD, Australia) at the *Pst*I and *Eco*RI sites. The DNA construct was transformed via *Agrobacterium tumefaciens* NTL4 into soybean (cultivar Bert) cotyledonary nodes, as previously described (Olhoft et al. 2003).

### Molecular characterization

For Southern blots, 5 μg of genomic DNA was digested with restriction enzymes, separated on 0.8% agarose gels and transferred onto nylon membranes. Northern blot analysis was performed with total RNA extracted from frozen, ground samples using the phenol/SDS/LiCl method (Carpenter and Simon 1998). Total RNA (5 μg) was separated on 1.3% agarose formaldehyde gels and transferred to GeneScreen Plus hybridization transfer membranes (PerkinElmer, Waltham, MA, USA). The cDNA probes used in the blot analyses were amplified by polymerase chain reaction (PCR) using two primers, 5′-TAC GACCATCGGGGTTAC-3′ and 5′-CTACTCGATTC TCCCAAC-3′, designed based on the AtMYB44-specific sequence: the nucleotide numbers 328–918 (GenBank accession number AY519648) corresponding to the amino acid residue numbers 109–306 (translation stop codon).

### Stress tolerance tests

For the drought-tolerance test, plants were soil-grown for 4 weeks in the growth chamber, and then watering was halted for 10 days. For the salt-tolerance test, soil-grown 4-week-old plants were treated with 100 mM NaCl for 3 days and then with 200 mM NaCl for 5 days.

### Chemical composition analyses

The moisture, crude ash, crude protein and crude lipid content of soybean seed powder were determined by oven-drying, furnace-combustion, Kjeldahl and ether-extraction methods, respectively (AOAC 1980).

Amino acid composition analysis was carried out using the High Speed Amino Acid Analyzer L-8900 (Hitachi, Tokyo, Japan) following the manufacturer's instructions. Acid hydrolysis of the soybean seed powder was performed at 110°C for 24 h with 6 N HCl containing 0.05% (w/v) 2-mercaptoethanol.

Fatty acid composition was analyzed by gas chromatography. For methylesterification, soybean seed powder was treated with 0.5 N NaOH (in methanol) and a 14% $BF_3$-methanol solution. Resultant fatty acid methyl-esters (FAMEs) were extracted with isooctane containing 0.5 mg/mL $C_{11:0}$ triundecanoic acid methyl ester as an internal standard. FAME analysis was performed with a gas chromatograph (6,890 N; Agilent, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) using a HP-FFAP (polyethylene glycol-terephthalic acid; 25 m × 0.32 mm × 0.5 μm) column (Hewlett-Packard, Palo Alto, CA, USA). The oven temperature was kept at 150°C for 1 min, raised to 200°C at a rate of 4°C/min and then held for 10 min. The injector temperature was kept at 250°C, while the detector temperature was 260°C. Hydrogen was used as the carrier gas at a flow rate of 30 mL/min.

## Results

### Transgenic soybean

We transformed the *35S:AtMYB44* construct into soybean (cultivar Bert) following the cotyledonary-node method (Olhoft et al. 2003). Seeds of each line were segregated over three generations and stable integration of the transgene was examined by PCR amplification (data not shown). Three lines of homozygous $T_3$ plants, termed L-2–L-4, were identified as homozygotes. Southern blotting showed that $T_3$ plants of these lines contained a single copy of the transgene (Fig. 1). $T_3$ plants of the L-1 line appeared to contain multiple copies of the transgene (not shown). Northern blotting revealed that *AtMYB44*

(approximately 0.9 kb) was expressed in the transgenic soybean lines (Fig. 2).

The transgenic lines L-2 and L-4 (Fig. 3a) had higher survival rates than wild-type plants during 10 days of water deprivation (Fig. 3b). Re-watering of wild-type plants did not rescue the wilted soybean leaves. The transgenic plants also showed significantly enhanced salt-stress tolerance. On watering with increasing concentrations of NaCl, up to 200 mM, the transgenic plants (L-2 and L-4) grew relatively well, whereas wild-type plants became wilted and chlorotic (Fig. 3c).

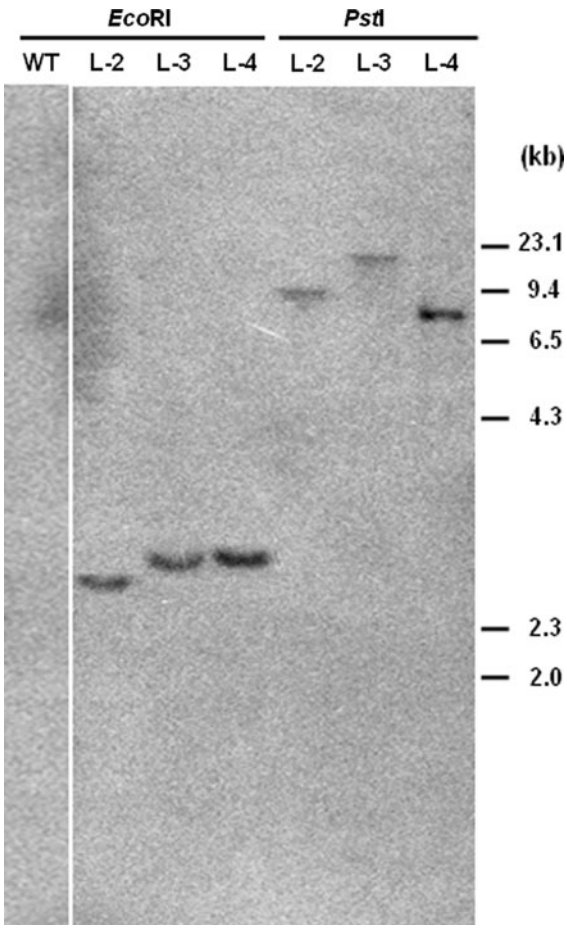


**Fig. 1** Southern blot analysis of transgenic soybeans. *AtMYB44* cDNA was fused to the cauliflower mosaic virus 35S (CaMV 35S) promoter and transformed into soybean (cultivar Bert). Genomic DNA isolated from $T_3$ plants was digested with *Eco*RI or *Pst*I, separated on an agarose gel, and transferred to nylon membranes. The blots were probed with an AtMYB44-specific DNA fragment. A region of the L-1 line was excised from the blot for clarification, leaving a gap in the gel between the non-transformed wild-type plants (*WT*) and other transgenic lines (*L-2*, *L-3*, and *L-4*)

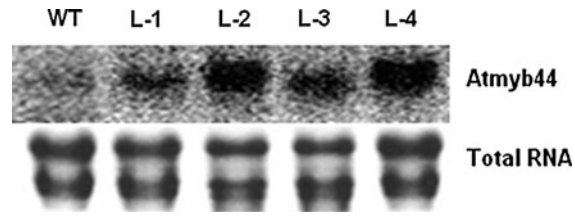


**Fig. 2** Northern blot analysis of transgenic soybeans. Total RNA was separated on agarose formaldehyde gels and transferred to GeneScreen Plus Hybridization Transfer Membranes. The blots were probed with an AtMYB44-specific DNA fragment. Equal RNA loading was confirmed using an ethidium bromide-stained gel. Our results demonstrate the constitutive expression of *AtMYB44* (approximately 0.9 kb) in the transgenic plants

## Growth of transgenic soybeans in a field

All of the transgenic soybean lines were shorter than the non-transgenic controls (Bert) throughout the growth period when grown in a greenhouse (data not shown) or in a field (Table 1). In a practical growth test in a field, none of the transgenic soybean lines showed a difference in developmental progression compared with wild-type plants. The appearance of cotyledons at the soil surface was observed approximately 2 weeks after the seeds were sown, budding flowers were seen after 3 weeks, fully opened flowers were observed after 5 weeks, and seed formation was noted after 7 weeks. In all plants, growth ceased approximately 6–7 weeks after the seeds were sown.

When compared with the cultivar Williams 82 (68 cm on average), the control (Bert; 80 cm) was taller, but its seed yield (average of 15 g/plant) was less than half, indicating that Bert was a poor choice for cultivation in this field. However, the transgenic Bert lines showed significantly improved seed yields of 23–44 g/plant, comparable to that of Williams 82. The difference in yield was dependent on the number of pods, rather than the size or weight of individual seeds.

## Chemical composition of transgenic soybean seeds

The transgenic lines showed little, if any, difference in general chemical composition, amino acid content, or fatty acid composition (Table 2). Approximately

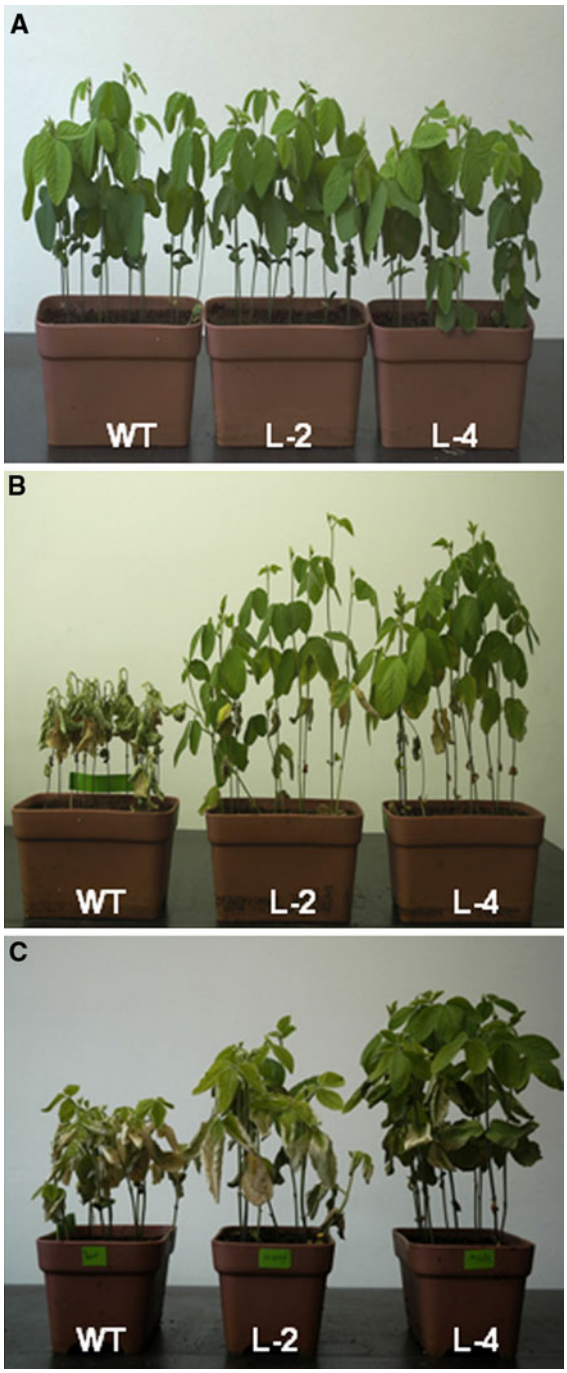


**Fig. 3** Abiotic stress tolerance tests of transgenic soybean lines. **a** Plants grown for 4 weeks in the growth chamber. **b** Drought tolerance test. Plants were grown for 4 weeks, and then watering was halted for 10 days. **c** Salt tolerance test. Four-week-old plants were treated with 100 mM NaCl for 3 days, and then with 200 mM NaCl for 5 days. *WT* represents the non-transgenic wild-type plants; *L-2* and *L-4* indicate the transgenic lines. Images represent a result from three independent experiments, which yielded reproducible results

35% (w/w) of the total protein and 18% of the total lipid were contained in soybean seeds harvested from the wild-type or transgenic plants. Among the types of fatty acids, palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$) were the most abundant in both the wild-type and transgenic plants. The amino acid content was not altered in the transgenic lines compared with that in the non-transformed wild-type plants. For amino acid composition analysis, soybean seed powder was acid-digested; thus, the amounts of tryptophan, asparagine, and glutamine were not determined in this experiment.

## Discussion

Transgenic soybean constitutively expressing *AtMYB44* (Fig. 1 and 2) exhibited significantly enhanced drought/salt-stress tolerance (Fig. 3), as observed in transgenic *Arabidopsis* (Jung et al. 2008). In the field cultivation study, whereby plants encountered changeable weather such as temporary water shortage and sudden low temperature, the transgenic soybean plants showed a much higher yield of seeds at harvest, implying improved stress tolerance. Transgenic soybeans were shorter throughout the growth period. Similar phenotypes have been observed in *Arabidopsis* lines that overexpress well-known ABA-dependent, drought-response genes, such as *DREB1A*/*CBF3* (Kasuga et al. 1999; Gilmour et al. 2000), *DREB2A* (Sakuma et al. 2006), *ABF3* (Kang et al. 2002) and *ABF4* (Kang et al. 2002).

Sequence-specific DNA binding has been demonstrated for many R2R3 proteins, allowing classification of their binding sites into three types (Romero et al. 1998). Type I sites, which have the sequence pAACnG (where p indicates T or C, and n indicates A, G, C, or T), are predominant in animal R2R3-MYB proteins (Howe and Watson 1991; Stober-Grässer et al. 1992). It has been reported that *Arabidopsis* subgroup 22 MYB transcription factors, including AtMYB44, AtMYB70, AtMYB73, and AtMYB77, belong to the Type I R2R3 MYB transcription factor family. As demonstrated by structural analysis of the R2R3 domain using heteronuclear multidimensional NMR, R2 and R3 each contain three helices, while the third helix in each is a recognition helix. R2 and R3 are closely packed in the major groove, so that the two recognition helices

**Table 1** Field test results

| | No. of seedlings[a] | Plant height (cm)[b] | Seed yield (g/plant)[c] | No. of pods/plant[c] |
|---|---|---|---|---|
| L-2 | 34 | 55.7 ± 8.2 | 30.65 | 49.8 |
| L-3 | 24 | 71.8 ± 10.8 | 38.70 | 62.2 |
| L-4 | 62 | 70.3 ± 4.1 | 44.41 | 63.5 |
| WT (Bert) | 98 | 80.2 ± 2.5 | 15.34 | 27.9 |
| Williams82 | 84 | 68.3 ± 3.8 | 37.21 | 66.3 |

[a] All plant lines had 100% germination rates

[b] Height of aerial part measured at 7 weeks after sowing the seeds

[c] For all plants in each line, the total values were measured three times and divided by the number of initial seedlings to calculate average values

contact each other directly to cooperatively bind the specific sequence AACnG (Ogata et al. 1994).

Thus, AtMYB44 may contact its specific binding sites on the promoters of target genes to regulate their expression. In the transgenic soybean lines produced in this study, the amino acid and fatty acid compositions of the seeds were not significantly altered (Table 2). This suggests that AtMYB44 acts on a limited number of target genes in the soybean genome, and not on all genes having Type I sequence-containing promoters.

Alternatively, or in addition, overexpressed AtMYB44 may interact with specific proteins, activating a mechanism for drought/salt-stress tolerance. Although R2R3 MYB transcription factors share high similarity in their R2R3 DNA-binding domains, individual genes play unique roles in diverse plant processes. For example, as revealed by a random oligonucleotide binding assay, AtMYB77 also showed a preference for binding to Type I sequences (Romero et al. 1998), yet AtMYB44 and AtMYB77 appear to have quite different biological roles. AtMYB77 is involved in auxin responses to control lateral root growth and development under changing environmental conditions (Shin et al. 2007). Double-knockout mutations in MYB44 and MYB77 (*myb44myb77*) did not change the auxin-responsive phenotype of *myb77*. Indeed, AtMYB77 interacts with auxin response factors (ARFs) in vitro (Shin et al. 2007).

AtMYB44 may work cooperatively with other regulators conserved in *Arabidopsis* and soybean, and this is currently under investigation. Additional

**Table 2** Chemical composition of soybean seeds

| | WT (Bert) | L-2 | L-4 |
|---|---|---|---|
| *General composition (g/100 g)* | | | |
| Moisture | 8.1 | 10.9 | 7.3 |
| Lipid | 18.1 | 17.8 | 18.3 |
| Proteins | 35.1 | 34.9 | 36.1 |
| Ash | 6.0 | 5.9 | 5.9 |
| *Amino acid composition (mg/100 g)* | | | |
| Aspartic acid | 3,601.1 | 3,515.0 | 3,724.2 |
| Threonine | 1,162.7 | 1,128.0 | 1,179.7 |
| Serine | 1,528.1 | 1,490.9 | 1,573.1 |
| Glutamic acid | 5,934.9 | 5,898.0 | 6,166.4 |
| Proline | 1,486.2 | 1,441.3 | 1,503.2 |
| Glycine | 1,291.5 | 1,256.9 | 1,328.8 |
| Alanine | 1,343.9 | 1,310.5 | 1,362.6 |
| Cystine | 211.1 | 215.9 | 219.8 |
| Valine | 1,534.5 | 1,520.1 | 1,566.1 |
| Methionine | 393.2 | 388.5 | 403.9 |
| Isoleucine | 1,341.3 | 1,325.7 | 1,372.4 |
| Leucine | 2,400.8 | 2,354.8 | 2,461.7 |
| Tyrosine | 1,001.1 | 981.6 | 1,032.2 |
| Phenylanine | 1,526.7 | 1,496.2 | 1,587.5 |
| Lysine | 1,913.1 | 1,885.3 | 1,963.2 |
| Histidine | 793.2 | 775.4 | 805.4 |
| Arginine | 2,213.4 | 2,179.8 | 2,308.4 |
| Total | 29,677.1 | 29,163.3 | 30,558.6 |
| *Fatty acid composition (%)* | | | |
| Saturated fatty acid | | | |
| C14:0 | 0.1 | 0.1 | 0.1 |
| C16:0 | 10.8 | 10.4 | 10.3 |
| C18:0 | 3.4 | 3.6 | 3.5 |
| C20:0 | 0.3 | 0.3 | 0.3 |
| C22:0 | 0.4 | 0.4 | 0.4 |
| C24:0 | 0.2 | 0.2 | 0.2 |
| Total | 15.2 | 15.0 | 14.8 |
| Unsaturated fatty acid | | | |
| C16:1 | 0.1 | 0.1 | 0.1 |
| C18:1 | 29.6 | 33.7 | 34.7 |
| C18:2 | 49.1 | 45.7 | 44.9 |
| C18:3 | 5.8 | 5.2 | 5.1 |
| C20:1 | 0.2 | 0.2 | 0.2 |
| Total | 84.8 | 84.9 | 85.0 |
| Unknown | 0.0 | 0.1 | 0.2 |
| Total | 100.0 | 100.0 | 100.0 |

Each value represents the average of two replicates which were almost identical

studies to identify the target genes, promoter binding sites, and interacting proteins are needed to define the biological role of AtMYB44 in the abiotic stress tolerance response.

**Acknowledgments** We thank Drs. Suk Hoo Yoon and Jin-Bong Hwang of the Korea Food Research Institute for technical advice on the chemical composition analyses. This research was supported by the Korean Ministry of Education, Science and Technology through a grant from the Crop Functional Genomics Center (CG2142) and the Basic Science Research Program of the National Research Foundation of Korea (NRF, grant number 2010-0025636). Graduate fellowships through the Brain Korea 21 Project are also acknowledged.

## References

AOAC (1980) Official methods of analysis, 13th edn. Association of Official Analytical Chemists, Washington, DC

Carpenter CD, Simon AE (1998) Preparation of RNA. Methods Mol Biol 82:85–89

Cheong YH, Chang HS, Gupta R, Wang X, Zhu T, Luan S (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. Plant Physiol 129:661–677

Fowler S, Thomashow MF (2002) Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell 14:1675–1690

Gilmour SJ, Sebolt AM, Salazar MP, Everard JD, Thomashow MF (2000) Overexpression of the Arabidopsis *CBF3* transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol 124:1854–1865

Howe KM, Watson RJ (1991) Nucleotide preferences in sequence-specific recognition of DNA by c-myc protein. Nucleic Acids Res 19:3913–3919

Jeong N, Moon J-K, Kim HS, Kim C-G, Jeong S-C (2011) Fine genetic mapping of the genomic region controlling leaflet shape and number of seeds per pod in the soybean. Theor Appl Genet 122:865–874

Jin H, Martin C (1999) Multifunctionality and diversity within the plant MYB-gene family. Plant Mol Biol 41:577–585

Jung C, Seo JS, Han SW, Koo YJ, Kim CH, Song SI, Nahm BH, Choi YD, Cheong J-J (2008) Overexpression of *AtMYB44* enhances stomata closure to confer abiotic stress tolerance in transgenic Arabidopsis. Plant Physiol 146:623–635

Kamei A, Seki M, Umezawa T, Ishida J, Satou M, Akiyama K, Zhu J-K, Shinozaki K (2005) Analysis of gene expression profiles in Arabidopsis salt overly sensitive mutants sos2–1 and sos3–1. Plant Cell Environ 28:1267–1275

Kang J-Y, Choi H-I, Im M-Y, Kim SY (2002) Arabidopsis basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell 14:343–357

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol 17:287–291

Kirik V, Kölle K, Miséra S, Bäumlein H (1998) Two novel *MYB* homologues with changed expression in late embryogenesis-defective *Arabidopsis* mutants. Plant Mol Biol 37:819–827

Kranz HD, Denekamp M, Greco R, Jin H, Leyva A, Meissner RC, Petroni K, Urzainqui A, Bevan M, Martin C, Smeekens S, Tonelli C, Paz-Ares J, Weisshaar B (1998) Towards functional characterization of the members of the *R2R3-MYB* gene family from *Arabidopsis thaliana*. Plant J 16:263–276

Liao Y, Zou H-F, Wang H-W, Zhang W-K, Ma B, Zhang J-S, Chen S-Y (2008) Soybean *GmMYB76*, *GmMYB92*, and *GmMYB177* genes confer stress tolerance in transgenic Arabidopsis plants. Cell Res 18:1047–1060

Ma L, Sun N, Liu X, Jiao Y, Zhao H, Deng XW (2005) Organ-specific expression of Arabidopsis genome during development. Plant Physiol 138:80–91

Martin C, Paz-Ares J (1997) MYB transcription factors in plants. Trends Genet 13:67–73

Miyake K, Ito T, Senda M, Ishikawa R, Harada T, Niizeki M, Akada S (2003) Isolation of a subfamily of genes for R2R3-MYB transcription factors showing up-regulated expression under nitrogen nutrient-limited conditions. Plant Mol Biol 53:237–245

Ogata K, Morikawa S, Nakamura H, Sekikawa A, Inoue T, Kanai H, Sarai A, Ishii S, Nishimura Y (1994) Solution structure of a specific DNA complex of the Myb DNA-binding domain with cooperative recognition helices. Cell 79:639–648

Olhoft PM, Flagel LE, Donovan CM, Somers DA (2003) Efficient soybean transformation using hygromycin B selection in the cotyledonary-node method. Planta 216:723–735

Peters CWB, Sippel AE, Vingron M, Klempnauer K-H (1987) *Drosophila* and vertebrate *myb* proteins share two conserved regions, one of which functions as a DNA-binding domain. EMBO J 6:3085–3090

Romero I, Fuertes A, Benito MJ, Malpica JM, Leyva A, Paz-Ares J (1998) More than 80 *R2R3-MYB* regulatory genes in the genome of *Arabidopsis thaliana*. Plant J 14:273–284

Rosinsky JA, Atchley WR (1998) Molecular evolution of the Myb family of transcription factors: evidence for polyphyletic origin. J Mol Evol 46:74–83

Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. Plant Cell 18:1292–1309

Shin R, Burch AY, Huppert KA, Tiwari SB, Murphy AS, Guilfoyle TJ, Schachtman DP (2007) The *Arabidopsis* transcription factor MYB77 modulates auxin signal transduction. Plant Cell 19:2440–2453

Stober-Grässer U, Brydolf B, Bin X, Grässer F, Firtel RA, Lipsick JS (1992) The Myb DNA-binding domain is highly conserved in *Dictyostelium discoideum*. Oncogene 7:589–596

Stracke R, Werber M, Weisshaar B (2001) The *R2R3-MYB* gene family in *Arabidopsis thaliana*. Curr Opin Plant Biol 4:447–456

Yanhui C, Xiaoyuan Y, Kun H, Meihua L, Jigang L, Zhaofeng G, Zhiqiang L, Yunfei Z, Xiaoxiao W, Xiaoming Q, Yunping S, Li Z, Xiaohui D, Jingchu L, Xing-Wang D, Zhangliang C, Hongya G, Li-Jia Q (2006) The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant Mol Biol 60:107–124