Genetics and Molecular Research

Overexpression of soybean R2R3-MYB transcription factor, *GmMYB12B2*, and tolerance to UV radiation and salt stress in transgenic *Arabidopsis*

X.W. Li[1,2]*, Y. Wang[1]*, F. Yan[1], J.W. Li[1], Y. Zhao[3], X. Zhao[4], Y. Zhai[3] and Q.Y. Wang[1]

[1]College of Plant Science, Jilin University, Changchun, China
[2]College of Life Science, Engineering Research Center of the Chinese Ministry of Education for Bioreactor and Pharmaceutical Development, Jilin Agricultural University, Changchun, Jilin, China
[3]College of Life Science and Agroforestry, Qiqihaer University, Qiqihaer, China
[4]Jilin Province Institute of Product Quality Supervision and Inspection, Changchun, China

*These authors contributed equally to this study.
Corresponding author: Q.Y. Wang
E-mail: qywang@jlu.edu.cn

Genet. Mol. Res. 15 (2): gmr.15026573
Received October 26, 2015
Accepted December 9, 2015
Published May 25, 2016
DOI http://dx.doi.org/10.4238/gmr.15026573

ABSTRACT. MYB, v-myb avian myeloblastosis viral oncogene homolog, proteins play central roles in plant stress response. Previously, we identified a novel R2R3-MYB transcription factor, *GmMYB12B2*, which affected the expression levels of some key enzyme genes involved in flavonoid biosynthesis in transgenic *Arabidopsis*. In the present study, we analyzed the expression levels of *GmMYB12B2* under salt, low temperature, drought, abscisic acid (ABA), and ultraviolet (UV) radiation treatments in soybean using semi-quantitative reverse transcription polymerase chain reaction. The expression of

GmMYB12B2 was drastically induced by UV irradiation and salt treatment, but no response was detected under low temperature, drought, and ABA stresses. A detailed characterization of the *GmMYB12B2* overexpression lines revealed that *GmMYB12B2* might be involved in response of plants to UV radiation and salt stresses. Transgenic *Arabidopsis* lines constitutively expressing *GmMYB12B2* showed an increased tolerance to salt and UV radiation treatment compared with wild-type plants. The expression levels of certain salt stress-responsive genes, such as *DREB2A* and *RD17*, were found to be elevated in the transgenic plants. These results indicate that *GmMYB12B2* acts as a regulator in the plant stress response.

Key words: NaCl treatment; Germination; Plant height; Proline

INTRODUCTION

Plant growth and agricultural production are greatly constrained by environmental stresses, such as salinity, drought, extreme temperatures, ultraviolet (UV) irradiation, and pathogen attacks. Plants, as sessile organisms, have evolved appropriate regulatory mechanisms that act at the cellular, molecular, physiological, and biochemical levels to sense and rapidly adapt to stress conditions. Various stress-inducible genes play important roles in these processes (Ahuja et al., 2010; Hirayama and Shinozaki, 2010). The products of stress-inducible genes can be classified as either functional or regulatory proteins (Kreps et al., 2002; Seki et al., 2002). Transcription factors are defined as important regulatory proteins that control the early transcription course of various functional genes by specifically binding to the cis-acting elements of target genes. It has been demonstrated that a number of large families of plant transcription factors, such as AP2/EREBP, WRKY, bZIP, and MYB (v-myb avian myeloblastosis viral oncogene homolog), as well as some zinc finger proteins are involved in the plant stress response.

MYB proteins constitute a large family in plants. This protein family is characterized by the presence of a structurally conserved DNA binding domain, the MYB domain, and members of the family are classified into four types on the basis of the number of repeat(s) in the MYB domain; 4R-MYBs have four repeats, 3R-MYBs (R1R2R3-MYB) have three repeats, R2R3-MYBs have two repeats, and MYB-related proteins usually, but not always, have a single repeat (Rosinski and Atchley, 1998; Jin and Martin, 1999; Dubos et al., 2010). The R2R3-MYB genes constitute a large gene family with the largest number of members in plants. There are 126 R2R3-MYBs in *Arabidopsis* (Riechmann et al., 2000; Dubos et al., 2010), 192 in poplar (Wilkins et al., 2009), and 118 in grape (Matus et al., 2008). Members of the MYB gene family were found to be involved in a number of physiological and biochemical processes, including cellular morphogenesis, organ formation (Penfield et al., 2001; Schmitz et al., 2002; Suo et al., 2003; Steiner-Lange et al., 2003; Murray et al., 2003), secondary metabolism, responses to diseases and hormones, and modulation of cell cycle (Stracke et al., 2001; Du et al., 2009; Dubos et al., 2010; Feller et al., 2011).

Soybean (*Glycine max*) is a chief source of edible vegetable oil and is the dominant source of high-quality protein for livestock and humans. There are 244 typical R2R3-MYB proteins in a primary soybean genome data set (Du et al., 2012). However, only 12 members

of the MYB family have been functionally characterized, thus far, in soybean (Miyake et al., 2003; Yang, 2007; Liao et al., 2008; Du et al., 2008; Libault et al., 2009; Yi et al., 2010; Gillman et al., 2011; Takahashi et al., 2011). The functions of most plant MYB genes are unknown. Previously, we identified a novel R2R3-MYB transcription factor, *GmMYB12B2*, which affected the expression levels of some key enzyme genes involved in flavonoid biosynthesis in transgenic *Arabidopsis*. The aims of the present study were to 1) investigate the expression patterns of the gene in response to salt, drought, low temperature, abscisic acid (ABA), and UV radiation treatments in soybean and 2) demonstrate the regulation of *GmMYB12B2* in transgenic *Arabidopsis* during the salt and UV radiation stress response.

MATERIAL AND METHODS

Plant materials and stress treatments

Seedlings of the soybean cultivar 'Jilin 32' were grown hydroponically in a greenhouse at 25°C for 14 days prior to being subjected to stress treatments. For the stress treatments, Hoagland solution was supplemented with NaCl, ABA, and polyethylene glycol 8000 to final concentrations of 150 mM, 100 μM and 10%, respectively. Cold treatment involved placing seedlings into a 4°C growth chamber. The UV radiation treatment involved exposing the seedlings to UV irradiation for the indicated times. After each treatment, the leaves of soybean seedlings were harvested at 1, 3, 6, 9, or 12 h and quickly frozen in liquid nitrogen prior to storage at -80°C for the extraction of total RNA. *Arabidopsis* plants were grown in a growth chamber at 22°/18°C with a light/dark cycle of 16 h light and 8 h darkness.

Real-time quantitative reverse transcription polymerase chain reaction (RT-PCR) and semi-quantitative RT-PCR

Total RNA was extracted from plants using the RNA Plant Plus Reagent (Tiangen, Changchun, China) according to the manufacturer protocol and cDNA was reverse transcribed using M-MLV reverse transcriptase (TaKaRa, Changchun, China). Quantitative RT-PCR analysis was performed using SYBR Green I dye (TaKaRa, Changchun, China) and a real-time PCR machine (Applied Biosystems 7500, Foster City, CA, USA). The *Arabidopsis AtActin* gene (GenBank accession No. AK230311) was chosen as an internal control. For semi-quantitative RT-PCR analyses, all PCRs were performed with 2X Taq PCR MasterMix (Tiangen, Changchun, China), a pair of primers (0.2 μM each), and cDNA in a final volume of 25μL. The PCR protocol was as follows: 94°C for 8 min and 26-34 cycles of 94°C for 30 s, 51°/60°C for 30 s, 72°C for 1 min followed by a final extension at 72°C for 8 min. The *β-Actin* gene (AK285936) was chosen as an internal control. The gene-speciðc primer pairs used for RT-PCR were as follows: *GmMYB12B2-SqPCR*: 5'-CCAACGCTCAAGCACACAGT-3' and 5'-CCCAAGTTTGTTGTCGGAGG-3'; *AtActin*: 5'-ACTGTGCCAATCTACGAGGGT-3' and 5'-TCTTACAATTTCCCGCTCTGC-3'; *DREB2A*: 5'-TGGAGAATGGTGCGGAAGA-3' and 5'-AGCGAATCCTGCTGTTGTT-3'; *RD17*: 5'-GAAACCTCAAGAGACAACGAC-3' and 5'-AGCTTTTCGATGACACTAGGC-3'; *GmActin*: 5'-CGTCTGCGATAATGGAACTG-3' and 5'-TCTGGGTCATCTTCTCACGA-3'; *GmMYB12B2-qPCR*: 5'-CTATTGGAGAACGAG AGTGGT-3' and 5'-CATTTCCTGCTATGTCCGAGT-3'.

Arabidopsis transformation

The coding region of *GmMYB12B2* was cloned into a binary vector, pCAMBIA1301, under the control of *CaMV 35S* promoter. The pCAMBIA1301-*GmMYB12B2* plasmid, thus formed, was introduced into *Arabidopsis* (*Arabidopsis thaliana* L. cv. Columbia) using *Agrobacterium*-mediated transformation following the floral dip method (Clough and Bent, 1998). *Arabidopsis* plants were grown in a growth chamber at 22°C with a light/dark cycle of 16 h light and 8 h darkness. Seeds from the transformed plants (T_0) were harvested and sowed on MS medium containing 25 mg/L hygromycin. The T_1 and T_2 transformants were screened and verified by PCR. Positive T_3 transformants were used for further analyses.

Stress tolerance analyses of transgenic *Arabidopsis*

For NaCl treatment, seeds (>100) of the wild-type and transgenic *Arabidopsis* plants were planted in triplicate on filter papers saturated with different concentrations of NaCl and incubated at 4°C for 4 days before incubation at 22°C under a photoperiod of 16 h/8 h (light/dark). Germination (emergence of radicals) was scored after 3 days. The experiment was repeated three times, and the results were consistent. The statistical program SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. The results from one set of experiments are shown.

For high salinity treatment, 14-day-old seedlings (WT and transgenic) grown on MS agar media were transferred to potted soil and grown for 7 days. The plants were watered with 150 mM NaCl. After 10 days, the heights of 10 plants from one line were measured. The experiments were repeated three times independently, and the results were consistent. The results from one set of experiments are shown.

For UV treatment, 14-day-old seedlings (WT and transgenic) grown on MS agar media were transferred to potted soil and grown for 7 days. Then, the plants were exposed in a UV chamber equipped with two UV lamps (40 W/lamp, BSC-1360-LIIB2, HADONGLIAN, Harbin, China) for 30 min every day. The distance between the UV lamps and plants was 42 cm. After 7 days, the survival of the plants was examined and photographs were taken. The experiments were repeated independently, and the results were consistent.

Measurement of proline

WT and transgenic seedlings (14 days old) grown under normal conditions were used to determine proline content. Plant samples (100 mg) were homogenized in 1 mL 3% sulfosalicylic acid using a mortar and a pestle; the homogenate was mixed and centrifuged at 12,000 *g* for 15 min at 4°C. The supernatant (200 µL) was transferred to a new tube, and 200 µL each of acid ninhydrin and acetic acid were added. The reaction mixture was boiled in a water bath at 100°C for 1 h and subsequently incubated at 4°C for 30 min. After the addition of 800 µL toluene, the mixtures were vortexed for 15 s, and 700 µL of the toluene phase was removed for measurement of absorbance at 520 nm, using a spectrophotometer. The results from five samples were averaged. Vertical bars represent the standard deviation for N = 5.

RESULTS

Expression pattern of *GmMYB12B2* under stress conditions

To determine whether the expression of *GmMYB12B2* is responsive to abiotic stresses, its expression pattern in soybean was examined. Semi-quantitative RT-PCR revealed that *GmMYB12B2* was responsive to salt and UV radiation treatments, but no response was detected under low temperature, drought or ABA stresses (Figure 1). The expression of *GmMYB12B2* under salt and UV irradiation showed a similar pattern; the expression was induced dramatically after 6 h of either salt or UV treatment and reached its peak at 12 h post-treatment.

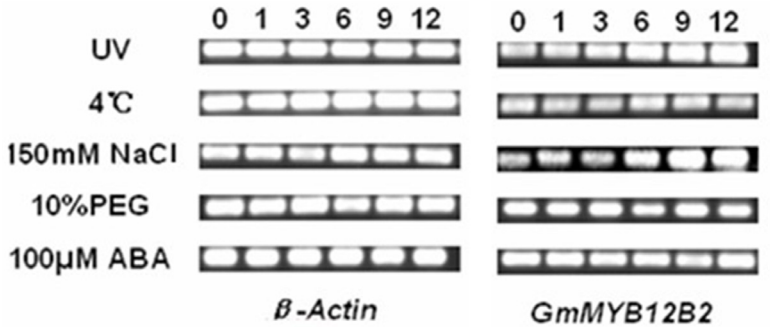

Figure 1. Expression of *GmMYB12B2* transcripts. Samples were collected at 0, 1, 3, 6, 9 and 12 h after the initiation of treatments.

Overexpression of *GmMYB12B2* improves tolerance to salt stress and UV irradiation in transgenic *Arabidopsis*

The expression of *GmMYB12B2* was induced under salt stress. Thus, it may participate in salt tolerance in plants. *GmMYB12B2* transgenic seeds were germinated on NaCl medium, and their germination rates were compared. Figure 2 shows that at 100 mM NaCl, the germination rates of the *GmMYB12B2* transgenic plants were higher than those of WT plants and other transgenic plants. At 150 mM NaCl, only 5.94% of WT seeds were able to germinate, whereas approximately 15% of the *GmMYB12B2* transgenic seeds were able to germinate. The plate-grown seedlings were also transferred to soil and treated with 150 mM NaCl for 10 days. Figure 3 shows that, under this treatment, the *GmMYB12B2* transgenic plants grew better and were taller than the WT plants. In the absence of stress, none of the transgenic plants were significantly different from WT plants (data not shown). Taken together, these results indicated that the transgenic plants were more tolerant to salt stress than the WT plants.

In soybean plants, the expression of *GmMYB12B2* was responsive to UV irradiation. Therefore, we examined the effect of UV irradiation on the performance of the *GmMYB12B2* transgenic plants. WT and transgenic seedlings grown for 14 days on MS agar media were transferred to potted soil and grown for 7 days. Thereafter, the plants were exposed to UV irradiation for 30 min every day. After 7 days, the *GmMYB12B2* transgenic plants grew significantly better than the WT plants (Figure 4A2 and B2). These results implied that the *GmMYB12B2* gene might confer salt and UV irradiation tolerance in the transgenic *Arabidopsis* plants.

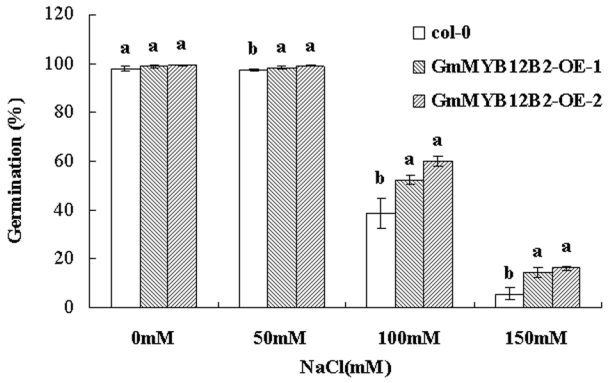

Figure 2. Comparison of the germination rates of wild type and transgenic *Arabidopsis* seeds under salt stress. Col-0, wild type; GmMYB12B2-OE, *GmMYB12B2* overexpressing plant. Experiments were performed in triplicate and the bars indicate SD. Significant differences among the treatments are indicated by different lowercase letters ($P < 0.05$).

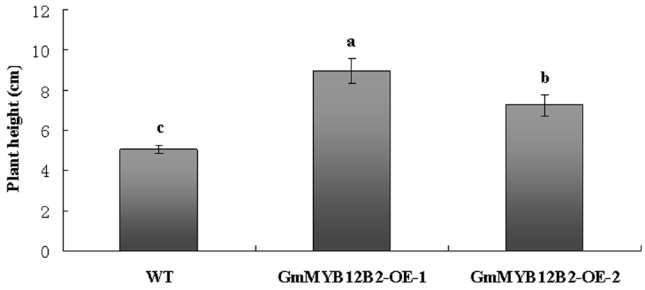

Figure 3. Effect of salt stress on the height of plants grown in soil. The height of plants treated with 150 mM NaCl was measured. WT, wild type; GmMYB12B2-OE, *GmMYB12B2* overexpression plant. Each data point represents the mean of 10 plants from one line. Bars indicate SD. Significant differences among the treatments are indicated by different lowercase letters ($P < 0.05$).

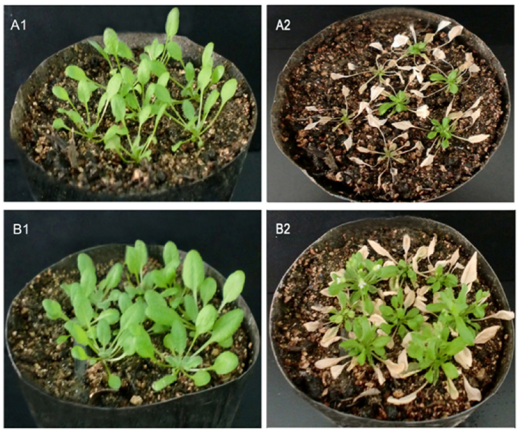

Figure 4. *GmMYB12B2* enhances UV stress tolerance in *Arabidopsis* plants. **A1.** and **A2.** Phenotypes of wild-type plants before and after treatment with UV radiation for 7 days. **B1.** and **B2.** Phenotypes of transgenic plants before and after treatment with UV radiation for 7 days.

Alterations in proline content, *DREB2A* expression, and *RD17* expression in *GmMYB12B2* transgenic plants

Proline levels were measured in 14-day-old WT and transgenic plants grown under normal conditions. Figure 5 shows that the level of free proline was significantly higher in *GmMYB12B2* transgenic plants compared with WT plants. The higher proline levels might have contributed to the salt tolerance of the transgenic plants.

Because overexpression of *GmMYB12B2* led to stress tolerance in transgenic *Arabidopsis* plants, we examined whether the typical salt stress-responsive genes *DREB2A* and *RD17* were altered in these plants. As shown in Figure 6, the expression levels of *DREB2A* and *RD17* were higher in *GmMYB12B2* transgenic plants than in WT plants. These results suggest that *GmMYB12B2* may be a regulator of the salt stress response.

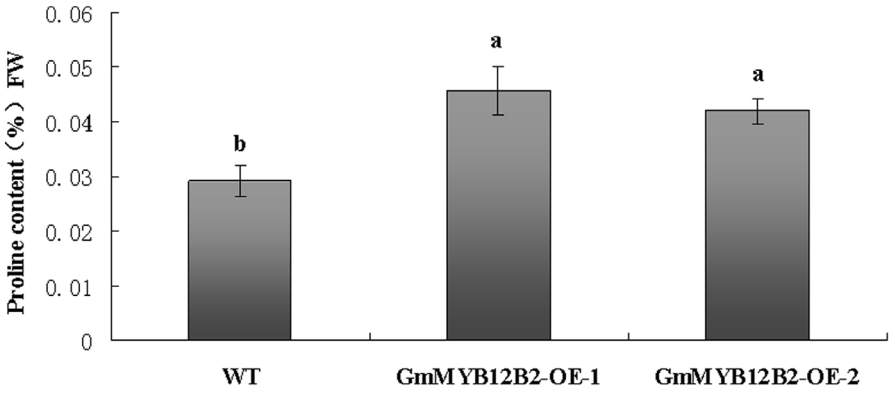

Figure 5. Proline content in WT and *GmMYB12B2* transgenic lines. WT, wild type; GmMYB12B2-OE, *GmMYB12B2* overexpressing plant. Each data point represents the mean of five replicates and the bars indicate SD. Significant differences among the treatments are indicated by different lowercase letters ($P < 0.05$).

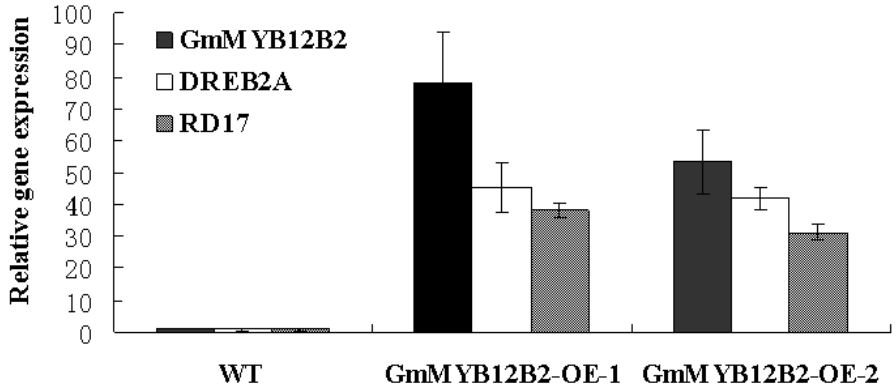

Figure 6. Quantitative real-time PCR analyses of *GmMYB12B2*, *DREB2A*, and *RD17* transcripts. Total RNAs were isolated from 2-week-old plants grown on MS plates and then subjected to RT-PCR analysis. WT, wild type; GmMYB12B2-OE, *GmMYB12B2* overexpressing plant. Bars indicate the SD of three replicates. Values were normalized against the results for *β-actin*.

DISCUSSION

The R2R3-MYB subfamily is involved in a variety of biological functions. Members of this family have been shown to play important roles in plant development and responses to hormones and environmental factors. In the soybean genome, there are 252 MYB-encoding genes, including 244 typical R2R3-MYB proteins, six R1R2R3-MYB proteins, and two 4R-like MYB proteins (Du et al., 2012). To date, the functions of several GmMYB proteins have been studied in different pathways. For example, GmMYBJ6 and GmMYBJ7 may play key roles in the signal transduction pathways related to ABA and NAA in soybean (Du et al., 2008). The expression of *GmMYBJ6* in transgenic tobacco could increase the total flavonoid levels and improve resistance to UV-B radiation and drought (Yang, 2007). *Arabidopsis* plants overexpressing *GmMYB76* or *GmMYB177* showed improved salt and freezing tolerance compared with wild-type plants. However, these transgenic plants exhibited reduced sensitivity to ABA treatment at the germination stage (Liao et al., 2008). An R1 MYB transcription factor, *GmMYB176*, regulates *CHS8* expression and isoflavonoid synthesis in soybean (Yi et al., 2010). A loss-of-function mutation in a specific R2R3 MYB transcription factor gene (Glyma09g36990) in soybean resulted in a brown hilum and brown seed coat (Gillman et al., 2011). *GmMYB-G20-1* is a candidate gene for the *W2* locus, which generates a purple-blue color and a high vacuolar pH in soybean flower petals (Takahashi et al., 2011; Takahashi et al., 2013). Taken together, these data indicate that GmMYB proteins in soybean are involved in many plant-specific processes such as secondary metabolism and responses to hormones and environmental factors.

As a typical R2R3-MYB transcription factor, *GmMYB12B2* was induced by salt and UV irradiation treatments but was unaltered under low temperature, drought, and ABA stresses in soybean (Figure 1). ABA is an abiotic stress response phytohormone, and the exogenous application of ABA to plants can mimic a stress condition (Ingram and Bartels, 1996; Jakab et al., 2005). Here, *GmMYB12B2* was not induced by exogenous ABA (Figure 1), indicating that it might be involved in the regulation of gene expression in response to stress through an ABA-independent pathway.

The overexpression of *GmMYB12B2* in *Arabidopsis* enhanced tolerance to salt and UV irradiation stresses (Figures 2-4). The *GmMYB12B2* gene may confer salt tolerance through upregulation of the downstream genes *DREB2A* and *RD17* (Figure 6). *DREB2A*, an AP2 domain transcription factor, is induced by dehydration and salt stress. Constitutive overexpression of *DREB2A* caused significant stress tolerance in transgenic plants. *DREB2A* can promote the expression of *RD17* (Sakuma et al., 2006). *RD17* contains a DRE or DRE-related motif in its promoter region and is induced by dehydration, salt, and cold (Gilmour et al., 1992; Kasuga et al., 1999). Therefore, *GmMYB12B2* might contribute to basal salt tolerance at least via the activation of the above two genes. Free proline is increased in plants in response to many stresses (Delauney and Verma, 1993), and its accumulation could contribute to the increase in salt tolerance (Khedr et al., 2003). Increased proline content in transgenic plants (Figure 5) might also potentially contribute to the improved performance of the *GmMYB12B2* transgenic plants during the salt treatment test (Figures 2 and 3). Transgenic *Arabidopsis* lines constitutively expressing *GmMYB12B2* showed increased flavonoid accumulation compared with the wild-type plants (Li et al., 2013). The accumulation of flavonoids may be one reason for the UV irradiation tolerance.

Further research should be performed to study the mechanisms by which the

GmMYB12B2 gene regulates plant tolerance to salt and UV irradiation stresses. However, the current results strongly suggest that *GmMYB12B2* is an ideal candidate gene for the genetic manipulation of soybean with the goal of abiotic stress tolerance breeding.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (#30971808) and the Major Science and Technology Sponsored Program for Transgenic Biological Breeding (#2008ZX08004-003).

REFERENCES

Ahuja I, de Vos RC, Bones AM and Hall RD (2010). Plant molecular stress responses face climate change. *Trends Plant Sci.* 15: 664-674. http://dx.doi.org/10.1016/j.tplants.2010.08.002

Clough SJ and Bent AF (1998). Floral dip: a simplified method for Agrobacterium- mediated transformation of *Arabidopsis thaliana. Plant J.* 16: 735-743. http://dx.doi.org/10.1046/j.1365-313x.1998.00343.x

Delauney AJ and Verma DPS (1993). Proline biosynthesis and osmoregulation in plants. *Plant J.* 4: 215-223. http://dx.doi.org/10.1046/j.1365-313X.1993.04020215.x

Du H, Tang XF, Liu L, Yang WJ, et al. (2008). Cloning and Functional Identification of Two MYB Transcription Factors *GmMYBJ6* and *GmMYBJ7* in Soybean. *Acta Agron. Sin.* 34: 1179-1187. http://dx.doi.org/10.1016/S1875-2780(08)60042-5

Du H, Zhang L, Liu L, Tang XF, et al. (2009). Biochemical and molecular characterization of plant MYB transcription factor family. *Biochemistry (Mosc.)* 74: 1-11. http://dx.doi.org/10.1134/S0006297909010015

Du H, Yang SS, Liang Z, Feng BR, et al. (2012). Genome-wide analysis of the MYB transcription factor superfamily in soybean. *BMC Plant Biol.* 12: 106. http://dx.doi.org/10.1186/1471-2229-12-106

Dubos C, Stracke R, Grotewold E, Weisshaar B, et al. (2010). MYB transcription factors in *Arabidopsis. Trends Plant Sci.* 15: 573-581. http://dx.doi.org/10.1016/j.tplants.2010.06.005

Feller A, Machemer K, Braun EL and Grotewold E (2011). Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. *Plant J.* 66: 94-116. http://dx.doi.org/10.1111/j.1365-313X.2010.04459.x

Gillman JD, Tetlow A, Lee JD, Shannon JG, et al. (2011). Loss-of-function mutations affecting a specific *Glycine max* R2R3 MYB transcription factor result in brown hilum and brown seed coats. *BMC Plant Biol.* 11: 155. http://dx.doi.org/10.1186/1471-2229-11-155

Gilmour SJ, Artus NN and Tomashow MF (1992). cDNA sequence analysis and expression of two cold-regulated genes *Arabidopsis thaliana. Plant Mol. Biol.* 18: 13-21. http://dx.doi.org/10.1007/BF00018452

Hirayama T and Shinozaki K (2010). Research on plant abiotic stress responses in the post-genome era: past, present and future. *Plant J.* 61: 1041-1052. http://dx.doi.org/10.1111/j.1365-313X.2010.04124.x

Ingram J and Bartels D (1996). The molecular basis of dehydration tolerance in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 47: 337-403. http://dx.doi.org/10.1146/annurev.arplant.47.1.377

Jakab G, Ton J, Flors V, Zimmerli L, et al. (2005). Enhancing *Arabidopsis* salt and drought stress tolerance by chemical priming for its abscisic acid responses. *Plant Physiol.* 139: 267-274. http://dx.doi.org/10.1104/pp.105.065698

Jin H and Martin C (1999). Multifunctionality and diversity within the plant MYB-gene family. *Plant Mol. Biol.* 41: 577-l585. http://dx.doi.org/10.1023/A:1006319732410

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, et al. (1999). Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat. Biotechnol.* 19: 287-291.

Khedr AH, Abbas MA, Wahid AA, Quick WP, et al. (2003). Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum L.* to salt-stress. *J. Exp. Bot.* 54: 2553-2562. http://dx.doi.org/10.1093/jxb/erg277

Kreps JA, Wu Y, Chang HS, Zhu T, et al. (2002). Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and

cold stress. *Plant Physiol.* 130: 2129-2141. http://dx.doi.org/10.1104/pp.008532

Li XW, Li JW, Zhai Y, Zhao Y, et al. (2013). A R2R3-MYB transcription factor, *GmMYB12B2*, affects the expression levels of flavonoid biosynthesis genes encoding key enzymes in transgenic *Arabidopsis* plants. *Gene* 532: 72-79. http://dx.doi.org/10.1016/j.gene.2013.09.015

Liao Y, Zou HF, Wang HW, Zhang WK, et al. (2008). Soybean *GmMYB76, GmMYB92*, and *GmMYB177* genes confer stress tolerance in transgenic *Arabidopsis* plants. *Cell Res.* 18: 1047-1060. http://dx.doi.org/10.1038/cr.2008.280

Libault M, Joshi T, Takahashi K, Hurley-Sommer A, et al. (2009). Large-Scale Analysis of Putative Soybean Regulatory Gene Expression Identifies a Myb Gene Involved in Soybean Nodule Development. *Plant Physiol.* 151: 1207-1220. http://dx.doi.org/10.1104/pp.109.144030

Matus JT, Aquea F and Arce-Johnson P (2008). Analysis of the grape MYB R2R3 subfamily reveals expanded wine quality-related clades and conserved gene structure organization across *Vitis* and *Arabidopsis* genomes. *BMC Plant Biol.* 8: 83-98. http://dx.doi.org/10.1186/1471-2229-8-83

Miyake K, Ito T, Senda M, Ishikawa R, et al. (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. http://dx.doi.org/10.1023/B:PLAN.0000009296.91149.34

Murray F, Kalla R, Jacobsen J and Gubler F (2003). A role for *HvGAMYB* in an ther development. *Plant J.* 33: 481-491. http://dx.doi.org/10.1046/j.1365-313X.2003.01641.x

Penfield S, Meissner RC, Shoue DA, Carpita NC, et al. (2001). *MYB61* is required for mucilage deposition and extrusion in the *Arabidopsis* seed coat. *Plant Cell* 13: 2777-2791. http://dx.doi.org/10.1105/tpc.13.12.2777

Riechmann JL, Heard J, Martin G, Reuber L, et al. (2000). *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290: 2105-2110. http://dx.doi.org/10.1126/science.290.5499.2105

Rosinski JA and Atchley WR (1998). Molecular evolution of the Myb family of transcription factors: evidence for polyphyletic origin. *J. Mol. Evol.* 46: 74-83. http://dx.doi.org/10.1007/PL00006285

Sakuma Y, Maruyama K, Osakabe Y, Qin F, et al. (2006). Functional analysis of an *Arabidopsis* transcription factor, *DREB2A*, involved in drought-responsive gene expression. *Plant Cell* 18: 1292-1309. http://dx.doi.org/10.1105/tpc.105.035881

Schmitz G, Tillmann E and Carriero F (2002). The tomato blind gene encodes a MYB transcription factor that controls the formation of lateral meristems. *Proc. Natl. Acad. Sci. USA* 99: 1064-1069. http://dx.doi.org/10.1073/pnas.022516199

Seki M, Narusaka M, Ishida J, Nanjo T, et al. (2002). Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. *Plant J.* 31: 279-292. http://dx.doi.org/10.1046/j.1365-313X.2002.01359.x

Steiner-Lange S, Unte US, Eckstein L, Yang C, et al. (2003). Disruption of *Arabidopsis thaliana MYB26* results in male sterility due to non-dehiscent anthers. *Plant J.* 34: 519-528. http://dx.doi.org/10.1046/j.1365-313X.2003.01745.x

Stracke R, Werber M and Weisshaar B (2001). The R2R3-MYB gene family in *Arabidopsis thaliana. Curr. Opin. Plant Biol.* 4: 447-456. http://dx.doi.org/10.1016/S1369-5266(00)00199-0

Suo J, Liang X, Pu L, Zhang Y, et al. (2003). Identification of *GhMYB109* encoding a R2R3 MYB transcription factor that expressed specifically in fiber initials and elongating fibers of cotton (*Gossypium hirsutum L.*). *Biochim. Biophys. Acta* 1630: 25-34. http://dx.doi.org/10.1016/j.bbaexp.2003.08.009

Takahashi R, Benitez ER, Oyoo ME, Khan NA, et al. (2011). Nonsense Mutation of an MYB Transcription Factor Is Associated with Purple-Blue Flower Color in Soybean. *J. Hered.* 102: 458-463. http://dx.doi.org/10.1093/jhered/esr028

Takahashi R, Yamagishi N and Yoshikawa N (2013). A MYB Transcription Factor Controls Flower Color in Soybean. *J. Hered.* 104: 149-153. http://dx.doi.org/10.1093/jhered/ess081

Wilkins O, Nahal H, Foong J, Provart NJ, et al. (2009). Expansion and diversification of the *Populus* R2R3-MYB family of transcription factors. *Plant Physiol.* 149: 981-993. http://dx.doi.org/10.1104/pp.108.132795

Yang WJ (2007). Cloning and charaeterization of MYB transcription factor genes from soybean. Doctoral thesis, Chengdu, Sichuan Agricultural University. (in Chinese with English abstract)

Yi JX, Derynck MR, Li XY, Telmer P, et al. (2010). A single-repeat MYB transcription factor, *GmMYB176*, regulates *CHS8* gene expression and affecs isoflavonoid biosynthesis in soybean. *Plant J.* 62: 1019-1034.