Over-expression of BvMTSH, a fusion gene for maltooligosyltrehalose synthase and maltooligosyltrehalose trehalohydrolase, enhances drought tolerance in transgenic rice

Joungsu Joo1, Hae Jong Choi1, Youn Hab Lee1, Sarah Lee2, Choong Hwan Lee2, Chung Ho Kim3, Jong-Joo Cheong4, Yang Do Choi5 & Sang Ik Song1*

1Division of Bioscience and Bioinformatics, Myongji University, Yongin 449-728, 2Division of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, 3Department of Food and Nutrition, Seowon University, Cheongju 361-742, 4Center for Food and Bioconvergence, Seoul National University, Seoul 151-921, 5Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea

INTRODUCTION

Plant abiotic stress tolerance has been modulated by engineering the trehalose synthesis pathway. However, many stress-tolerant plants that have been genetically engineered for the trehalose synthesis pathway also show abnormal development. The metabolic intermediate trehalose 6-phosphate has the potential to cause aberrations in growth. To avoid growth inhibition by trehalose 6-phosphate, we used a gene that encodes a bifunctional in-frame fusion (BvMTSH) of maltooligosyltrehalose synthase (BvMTH) and maltooligosyltrehalose trehalohydrolase (BvMTH) from the nonpathogenic bacterium Brevibacterium helvolum. BvMTH converts maltoligosaccharides into maltooligosyltrehalose and BvMTH releases trehalose. Transgenic rice plants that over-express BvMTSH under the control of the constitutive rice cytochrome c promoter (101MTSH) or the ABA-inducible Ai promoter (105MTSH) show enhanced drought tolerance without growth inhibition. Moreover, 101MTSH and 105MTSH showed an ABA-hyposensitive phenotype in the roots. Our results suggest that over-expression of BvMTSH enhances drought-stress tolerance without any abnormal growth and shows ABA hyposensitive phenotype in the roots. [BMB Reports 2014; 47(1): 27-32]
transgenic rice over-expressing BvMTSH show improve drought tolerance without affecting growth.

RESULTS

Transformation of rice with the recombinant fusion gene BvMTSH

To introduce the trehalose biosynthesis pathway without producing T6P as an intermediate in plants, we used a gene that encodes for a bifunctional in-frame fusion (BvMTSH) of the BvMTS and BvMTH genes from the nonpathogenic bacterium Brevibacterium helvolum (Fig. 1) (15). The BvMTSH coding sequences were expressed under the control of the constitutive rice cytochrome c promoter (101MTSH) (16) or the ABA-inducible Ai promoter (105MTSH) (17). Transgene copy numbers were determined using Southern blotting analysis (data not shown), which revealed that each insertion was independent. Transgene expression levels in 101MTSH and 105MTSH plants were examined using RT-PCR analyses (Fig. 1C). Trehalose contents in 101MTSH and 105MTSH seedlings were measured by gas chromatography–mass spectrometry (GC-MS) (Fig. S1). Trehalose contents of 101MTSH and 105MTSH plants were increased approximately two-fold compared with NT controls. All of the transgenic lines grew without growth inhibition during the vegetative growth stage; T1 to T4 seeds were collected from individual transgenic plants, and three independent homozygous T4 lines for each construct were used for further analysis.

Expression of the BvMTSH gene improved drought-stress tolerance

To evaluate the response of 101MTSH and 105MTSH transgenic plants to a water deficit, 4-week-old nontransgenic (NT) control plants and T4 transgenic seedlings were subjected to drought stress for 2 to 3 days followed by re-watering (Fig. 2A).

During re-watering, 101MTSH and 105MTSH plants showed better recovery from the drought-stress test and more growth compared to the severely injured NT plants. In 105MTSH plants, the ability to recover from drought stress was the greatest in line 4, which is consistent with the level of BvMTSH expression in the 105MTSH lines (Fig. 1C). After re-watering, 101MTSH showed the highest survival rate (Fig. 2A). The survival rates of the transgenic seedlings were 92-100% for the 101MTSH plants and 63-93% for the 105MTSH plants. These results indicate that over-expression of the BvMTSH fusion gene can improve the drought tolerance of transgenic rice.

The drought-stressed plants exhibited visual symptoms with a concomitant loss of chlorophyll. The \( F_{V}/F_{M} \) is a parameter...
widely used to indicate the maximum fluorescence after dark adaptation, which represents the maximum quantum yield of PSII. Healthy plants typically achieve a maximum Fv/Fm value of approximately 0.80, and values lower are observed in plants exposed to abiotic stress factors (18). To further verify the stress tolerance of 101MTSH and 105MTSH transgenic plants, we measured variations in the chlorophyll fluorescence ratio (Fv/Fm) after drought-stress treatments. For the stress treatments, the leaf discs of 3-week-old transgenic and NT seedlings were exposed to drought stress, and the reductions in the Fv/Fm values were measured (Fig. 2B). The values for Fv/Fm were higher by approximately 75% in the 101MTSH and 105MTSH plants compared to the NT control plants under the drought-stress conditions. The results of the stress test experiments confirmed that the 101MTSH and 105MTSH transgenic rice plants presented increased tolerance to drought stress during the vegetative stage. Therefore, the bifunctional in-frame fusion gene BvMTSH is useful in stress-tolerant plants that have been genetically engineered to contain the trehalose synthesis pathway.

ABA sensitivity of transgenic plants

Trehalose biosynthesis genes are reported to involve ABA and stress response (19-21). To test this, we analyzed the expression levels of a PP2C family gene (Abi2), a SnRK family gene (SAPK10), and several ABA/stress induced genes (LEA3, Rab16, Wsi18, SalT, Dip1, ASR1) in the transgenic plants under normal condition (Fig. S2). Only the expression level of ASR1 and SalT were commonly increased. We also analyzed the expression levels of ABA biosynthesis genes (ABA1, ABA2, ABA4, OsNCED1) (Fig. S3A). The expression level of ABA2 was down-regulated in the 101MTSH plants. To known the effect of MTSH overexpression on rice trehalose biosynthesis genes, the transcript levels of OsTPS1 and several OsTPP genes were analyzed (Fig. S3B). The expression levels of OsTPP3 and OsTPP5 were commonly decreased in the MTSH overexpressed plants. To study the effect of the 101MTSH and 105MTSH constructs on shoot and root growth under ABA conditions, the shoots and roots of the transgenic plants were studied. Seedlings of NT as well as 101MTSH and 105MTSH transgenic plants were grown on half-strength MS solid medium that contained 3 μM of ABA for 7 days (Fig. 3). Under normal conditions, 105MTSH line 4 exhibited shorter shoot lengths and 101MTSH line 4 showed longer root lengths. The other seedlings of the 101MTSH and 105MTSH lines grew at similar rates compared to NT control plants (Fig. 3A and B). In contrast, the root lengths of the 101MTSH and 105MTSH plants were significantly longer than the NT controls under ABA treatment (Fig. 3C and D). These results suggest that 101MTSH and 105MTSH transgenic plants are ABA hyposensitive in the roots.

DISCUSSION

Trehalose accumulates under abiotic stress in the resurrection plants and in many other plants. Trehalose accumulation under stress is related to the transcriptional activation of the trehalose biosynthesis genes (4). Abiotic stress tolerance has been modulated by engineering the trehalose synthesis pathway in many plants. Unfortunately, many stress-tolerant plants that contain the genetically engineered trehalose synthesis pathway also show abnormal development, such as growth inhibition (8-12). Furthermore, exogenous application of trehalose caused a decrease in NaCl accumulation and growth inhibition in rice (7). In Arabidopsis, adding exogenous trehalose induced the expression of genes involved in detoxification, the stress response, and growth inhibition (22). Previously, the mechanism of the undesired side effects of trehalose had been unknown. T6P is believed to be involved in these effects (23). There are several reports that support T6P as a potential agent. T6P accumulation exhibited growth inhibition in Arabidopsis (24). T6P has been reported to be a signaling metabolite that is involved in carbon utilization as well as growth and development (13, 23). A model has been proposed where T6P inhibition of SnRK1 is part of a growth-regulating loop in young and metabolically active heterotrophic plant tissues (23).

We hypothesized that trehalose biosynthesis that excludes T6P as an intermediate could lead to improved abiotic stress tolerance in transgenic plants without abnormal growth. There are at least five known biosynthetic pathways for trehalose synthesis (25). To examine this, we used the TreY/TreZ pathway. In this pathway, the biosynthesis of trehalose is mediated by TreY and TreZ without T6P as an intermediate. TreY converts α-1,4-glycosidic linkages at the reducing ends of maltooligosaccharides into α-1,1 linkages, which produces maltooligosyltrehalose. TreZ hydrolyzes the second α-1,4-glycosidic linkage of the intermediate to release trehalose (25). The non-

Fig. 3. The effect of the 101MTSH and 105MTSH constructs on growth. The phenotypes of 101MTSH and 105MTSH plants grown under normal (A) and ABA conditions (C). The shoot lengths (B) and root lengths (D) were analyzed under normal and ABA conditions.
pathogenic bacterium *Brevibacterium helvolum* contains these two enzymes, *BvMTS* (TreY) and *BvMTH* (TreZ) (14). In this study, we used the *BvMTS* gene (15) that encodes a bifunctional in-frame fusion of the *BvMTS* and *BvMTH* genes of *Brevibacterium helvolum* (Fig. 1). The fusion enzymes has some advantages, such as simple expression of a single re-combinant gene and faster rates of sequential enzyme re-actions by facilitating transfer of reaction intermediates to the catalytic sites of the next enzymes. In addition, the recombi-nant enzymes can produce trehalose from soluble starch with-out α-amylase (15). We produced 101MTSH and 105MTSH transgenic plants over-expressed *BvMTS* under the control of the constitutive rice cytochrome c promoter or the ABA-inducible *Ai* promoter, respectively.

101MTSH and 105MTSH plants displayed significantly en-hanced drought-stress tolerance (Fig. 2), which is in agreement with previous reports where trehalose synthesis was en-gineered into plants. For example, drought tolerance was ob-tained by over-expression of the yeast ScTPS1 gene in tobacco (8), the AtTPS1 gene in Arabidopsis (26), and the plastid TPS1 gene in tobacco (27). Over-expression of the bifunctional fu-sion genes OtsA-OtsB and ScTPS-ScTPP exhibited high trehalose accumulation and improved abiotic stress tolerance in rice and tobacco, respectively (27, 28). The trehalose level in creased three to four-fold after drought stress in tobacco (27). In Arabidopsis, heat and cold stress lead to two- and eight-fold increase of the trehalose level, respectively (29). Moreover, the trehalose content in OsTPS1 over-expressed transgenic rice which exhibit enhancement of abiotic stress tolerance was in creased 1.45 to 2.01-fold than wild-type (21). GC-MS analysis showed that trehalose contents of 101MTSH and 105MTSH plants were increased approximately two-fold under compared with NT controls. These results suggest that over-expressing *BvMTSH* enhanced the tolerance of rice seedling to drought by increasing trehalose levels. Furthermore, 101MTSH and 105MTSH plants exhibited no abnormal plant development or visible phenotypic alterations during vegetative growth. Under normal conditions, 105MTSH line 4 exhibited shorter shoot lengths and 101MTSH line 4 showed longer root lengths (Fig. 3). Even though, these lines showed higher trehalose content than other lines, the difference is about 25%. Moreover, 105MTSH line 4 and 101MTSH line 4 showed phenotype in different tissues, shoot and root, respectively. Therefore, we think that these variations could be resulted from transformation effect, such as somatic variation or transgene position effect. Moreover, 101MTSH and 105MTSH plants also grew normally and matured in the paddy field (data not shown). Our results suggest that the *BvMTSH* bifunctional in-frame fusion gene is useful in the production of stress-tolerant plants that show no growth inhibition when they have been genetically engineered to contain the trehalose synthesis pathway.

ABA is an important plant hormone in the abiotic stress response. Abiotic stresses, such as drought, trigger the ABA signaling pathway. Many molecular and cellular responses, including the expression of stress-related genes, are initiated by the ABA signaling pathway. Trehalose biosynthesis genes are reported to be involved in ABA signaling pathway. Trehalose biosynthesis genes are results in an ABA insensitive phenotype in Arabidopsis (26). In rice, two TPS genes are transiently induced by drought and exogenous ABA application in seedling roots and shoots (30). To investigate the relationship between trehalose-induced drought-stress tolerance and ABA signaling, 101MTSH and 105MTSH transgenic seedlings were grown under 3 μM ABA conditions. In response to ABA treatment, the shoot lengths of 101MTSH and 105MTSH seedlings showed no significant difference, except 101MTSH line 3. In contrast, the root lengths of 101MTSH and 105MTSH seedlings showed an ABA hyposensitive phenotype (Fig. 3B). This result indicates that trehalose is involved in ABA response in the roots, and over-expression of *BvMTSH* reduced root growth inhibition by ABA in rice.

In general, stress-inducible promoters have been considered the better promoters for transgenic plants enhanced stress tol-erance than constitutive promoters. Even though, 101MTSH showed higher survival rate than 105MTSH (Fig. 2A), stress in-ducible promoter showed low changes to growth alteration. These results suggest that ABA-inducible promoter (*Ai* pro-moter) is more suitable for the development of drought re-sistant transgenic rice using *BvMTSH* gene than constitutive promoter (cytochrome c promoter). This study indicates that the over-expression of *BvMTSH* enhanced drought-stress toler-ance without any abnormal growth. Moreover, trehalose is in-volved in ABA response in the roots. Our results suggest that the *BvMTSH* bifunctional in-frame fusion gene is useful in the production of stress-tolerant plants genetically engineered for the trehalose synthesis pathway.

**MATERIALS AND METHODS**

**Plasmid construction and transformation of rice**

Transgenic and non-transgenic (NT) rice plants with an *Oryza sativa* subsp. *japonica* cv. Nakdong background were used. The complete fusion gene *BvMTSH* was used. The attB-PCR products of *BvMTSH* using the primers MTSH-ATG (5'-AA AAAGCAGGGCTCATGAAAGACTCCGTCCTCAG) and MTSH-TGA (5'-AGAAAGCTGGGTGCCGGATCAAGCTTCAGGACT) were inserted into pMj101 and pMj105 through BP- and LR-recombination reactions performed according to the manufacture-r's instructions (Invitrogen). The over-expression plasmids pMj101 and pMj105 contained the constitutive rice cytochrome c promoter (101MTSH) or ABA-inducible *Ai* promoter (105MTSH), respectively. The plasmids were introduced into Agrobacterium tumefaciens LBA4404 by triparental mating (31).

**Semiquantitative reverse transcription (RT)-PCR**

Total RNA was isolated from the rice tissue samples using the
TRI REAGENT® (Molecular Research Center) according to the manufacturer’s instructions. For RT-PCR, the first strand of cDNA was synthesized from 5 μg of total RNA as a template using oligo (dT)18 primers according to the manufacturer’s instructions (RevertAid™ First Strand cDNA Synthesis Kit, Fermentas). A one-third dilution of the cDNA synthesis reaction mixture was prepared, and 1 μl of the diluted cDNA mixture was used as a template (32). The primers MTSH-H-ATG and MTSH+in1R (5’-ACGTCAGCCAGTGCGTGTA) were used for the PCR. PCR was performed at 95°C for 10 min, followed by 22 to 32 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s. Amplified products were resolved on a 1.5% (w/v) agarose gel. Tubulin was used as reference gene (tubulin-F, 5’-CATCGACATCAAGTTCGACC; tubulin-R, 5’-TC ACCATCGTGCAATCCGGA). The same results were obtained in three independent experiments. However, only the result from one experiment is presented.

Drought stress, ABA treatment and chlorophyll fluorescence measurements

For ABA treatment, independent homozygous T4 lines of 101MTSH and 105MTSH transgenic and NT control seeds were germinated on MS solid medium for 2 days. After germination, germinants of equal size were selected and transferred to MS solid medium containing 3 μM of ABA. The germinated seedlings were incubated with 16 h light/ 8 h dark cycles at 28°C in a grow chamber. To test drought-stress resistance, four-week-old NT and transgenic plants grown on soil were subjected to 2-3 days without water followed by watering in a greenhouse (33). The chlorophyll fluorescence of three-week-old NT and transgenic plants was measured using a pulse modulation fluorometer (mini-PAM, Walz, Germany). For the leaf disc test, the green portions of approximately 10 seedlings were cut using scissors prior to stress treatments in vitro. Under continuous light at 150 μmol m⁻² s⁻¹, the leaf discs were air-dried for 2 h (to induce drought stress). After drought-stress treatment, the leaf discs were dark-adapted for 10 minutes, and the minimal fluorescence level (F₀) was measured; a saturating light pulse was applied, and the maximal fluorescence level (Fₚₚ) was measured. The ratio of F₁ to Fₚₚ (F₁/Fₚₚ = Fₚₚ/F₀/Fₚₚ) represents the activity of photosystem II, which was used to assess the functional damage to the plants (34). The statistical significance of differences between groups was assessed using Student’s t-test.

Acknowledgements

We thank Baek Hie Nahm and Ju-Kon Kim at Myongji University for their helpful advice. This work was supported by the Technology Development Program for Life Industry through the Korea Institute of Planning and Evaluation for Technology of Food, Agriculture, Forestry and Fisheries (grant number 111076-5).

REFERENCES

1. Elbein, A. D. (1974) The metabolism of alpha,alphatrehalose. Adv. Carbohydr. Chem. Biochem. 30, 227-256.
2. Strom, A. R. and Kaasen, I. (1993) Trehalose metabolism in Escherichia coli: stress protection and stress regulation of gene expression. Mol. Microbiol. 8, 205-210.
3. Eleutherio, E. C., Araujo, P. S. and Panek, A. D. (1993) Protective role of trehalose during heat stress in Saccharomycyes cerevisiae. Cryobiology 30, 591-596.
4. Fernandez, O., Bethencourt, L., Quero, A., Sangwan, R. S. and Clement, C. (2010) Trehalose and plant stress responses: friend or foe? Trends Plant. Sci. 15, 409-417.
5. Paul, M. J., Primavesi, L. F., Jhurreea, D. and Zhang, Y. (2008) Trehalose metabolism and signaling. Annu. Rev. Plant. Biol. 59, 417-441.
6. Goddijn, O. J. M. and van Dun, K. (1999) Trehalose metabolism in plants. Trends. Plant. Sci. 4, 315-319.
7. Garcia, A. B., Engler, J., Iyer, S., Gerats, T., Van Montagu, M. and Caplan, A. B. (1997) Effects of osmoprotectants upon NaCl stress in rice. Plant. Physiol. 115, 159-169.
8. Romero, C., Belles, J. M., Vaya, J. L., Serrano, R. and Culiánez-Macia, F. A. (1997) Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. Planta. 201, 293-297.
9. Miranda, J. A., Avonce, N., Suarez, R., Thevelein, J. M., Van Dijck, P. and Iturriaga, G. (2007) A bifunctional TPS-TPP enzyme from yeast confers tolerance to multiple and extreme abiotic-stress conditions in transgenic Arabidopsis. Planta. 226, 1411-1421.
10. Goddijn, O. J., Verwoerd, T. C., Voogd, E., Krutwagen, R. W., de Graaf, P. T., van Dun, K., Poels, J., Ponstein, A. S., Damm, B. and Pen, J. (1997) Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol. 113, 181-190.
11. Holmstrom, K.-O., Mantyla, E., Welin, B., Mandal, A., Tunnela, O. E., Lendesborough, J. and Palva, E. T. (1996) Drought tolerance in tobacco. Nature 379, 683-684.
12. Pilon-Smits, E. A. H., Terry, N., Sears, T., Kim, H., Zayed, A., Hwang, S., van Dun, K., Voogd, E., Verwoerd, T. C., Krutwagen, R. W. H. H. and Goddijn, O. J. M. (1998) Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. J. Plant. Physiol. 152, 525-532.
13. Ponnu, J., Wahl, V. and Schmid, M. (2011) Trehalose-6-phosphate: connecting plant metabolism and development. Front. Plant. Sci. 2, 70.
14. Maruta, K., Nakada, T., Kubota, M., Chaen, H., Sugimoto, T., Kurimoto, M. and Tsujisaka, Y. (1995) Formation of trehalose from maltooligosaccharides by a novel enzymatic system. Biosci. Biotechnol. Biochem. 59, 2059-2064.
15. Kim, Y. H., Kwon, T. K., Park, S., Seo, H. S., Cheong, J. J., Kim, C. H., Kim, J. K., Lee, J. S. and Choi, Y. D. (2000) Trehalose synthesis by sequential reactions of recombiant maltooligosyltrehalose synthase and maltooligosyltrehalose trehalohydrolase from Brevibacterium helvolum. Appl. Environ. Microbiol. 66, 4620-4624.
16. Jang, I. C., Choi, W. B., Lee, K. H., Song, S. I., Nahm, B. H. and Kim, J. K. (2002) High-level and ubiquitous ex-
Over-expression of BvMTSH confers drought tolerance in rice

Joungsu Joo, et al.

15. Liu, A. L., Zou, J., Liu, C. F., Zhou, X. Y., Zhang, X. W., Luo, G. Y. and Chen, X. B. (2013) Over-expression of OsHsfA7 enhanced salt and drought tolerance in transgenic rice. BMB Rep. 46, 31-36.

16. Joo, J., Choi, H. J., Lee, Y. H., Kim, Y. K. and Song, S. I. (2013) A transcriptional repressor of the ERF family confers drought tolerance to rice and regulates genes preferentially located on chromosome 11. Planta. 238, 155-170.

17. Jang, I. C., Pakh, Y. M., Song, S. I., Kwon, H. J., Nahm, B. H. and Kim, J. K. (2003) Structure and expression of the rice class-I type histone deacetylase genes OsHDAC1-3: OsHDAC1 overexpression in transgenic plants leads to increased growth rate and altered architecture. Plant J. 33, 531-541.

18. Artus, N. N., Uemura, M., Steponkus, P. L., Gilmour, S. J., Lin, C. and Thomashow, M. F. (1996) Constitutive expression of the cold-regulated Arabidopsis thaliana COR15a gene affects both chloroplast and proplastid freezing tolerance. Proc. Natl. Acad. Sci. U. S. A. 93, 13404-13409.

19. Schluepmann, H., Pellny, T., van Dijken, A., Smeekens, S. and Paul, M. (2003) Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in Arabidopsis thaliana. Proc. Natl. Acad. Sci. U. S. A. 100, 6849-6854.

20. Ge, L. F., Chao, D. Y., Shi, M., Zhu, M. Z., Gao, J. P. and Lin, H. X. (2008) Overexpression of the trehalose-6-phosphate phosphatase gene OsTPP1 confers stress tolerance in rice and results in the activation of stress responsive genes. Planta. 228, 191-201.

21. Li, H. W., Zang, B. S., Deng, X. W. and Wang, X. P. (2011) Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. Physiol. Plant. 125, 114-126.

22. Schluepmann, H., Berke, L. and Sanchez-Perez, G. F. (2012) Metabolism control over growth: a case for trehalose-6-phosphate in plants. J. Exp. Bot. 63, 3379-3390.

23. Schluepmann, H., van Dijken, A., Aghdasi, M., Wobbes, B., Paul, M. and Smeekens, S. (2004) Trehalose mediated growth inhibition of Arabidopsis seedlings is due to trehalose-6-phosphate accumulation. Plant Physiol. 135, 879-890.

24. Avonce, N., Mendoza-Vargas, A., Morett, E. and Iturriaga, G. (2006) Insights on the evolution of trehalose biosynthesis. BMC Evol. Biol. 6, 109.

25. Avonce, N., Leyman, B., Mascoro-Gallardo, J. O., Van Dijck, P., Thevelein, J. M. and Iturriaga, G. (2004) The Arabidopsis trehalose-6-P synthase ATTPS1 gene is a regulator of glucose, abscisic acid, and stress signaling. Plant Physiol. 136, 3649-3659.

26. Jang, I. C., Pakh, Y. M., Song, S. I., Kwon, H. J., Nahm, B. H. and Kim, J. K. (2003) Structure and expression of the rice class-I type histone deacetylase genes OsHDAC1-3: OsHDAC1 overexpression in transgenic plants leads to increased growth rate and altered architecture. Plant J. 33, 1473-1481.

27. Liu, A. L., Zou, J., Liu, C. F., Zhou, X. Y., Zhang, X. W., Luo, G. Y. and Chen, X. B. (2013) Over-expression of OsHsfA7 enhanced salt and drought tolerance in transgenic rice. BMB Rep. 46, 31-36.

28. Joo, J., Choi, H. J., Lee, Y. H., Kim, Y. K. and Song, S. I. (2013) A transcriptional repressor of the ERF family confers drought tolerance to rice and regulates genes preferentially located on chromosome 11. Planta. 238, 155-170.