Journal of Plant Physiology 169 (2012) 628–635

Contents lists available at SciVerse ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Overexpression of *OsHsp17.0* and *OsHsp23.7* enhances drought and salt tolerance in rice

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ARTICLE INFO

Article history:
Received 25 July 2011
Received in revised form
15 December 2011
Accepted 15 December 2011

Keywords:
Drought tolerance
Oryza sativa L
Salt resistance
Small heat shock protein
Transgenic plant

ABSTRACT

Heat shock proteins (Hsps) play an important role in plant stress tolerance. We previously reported that expression of *OsHsp17.0* and *OsHsp23.7* could be enhanced by heat shock treatment and/or other abiotic stresses. In this paper, stress tolerance assays of transgenic rice plants overexpressing *OsHsp17.0* and *OsHsp23.7* have been carried out. Both OsHsp17.0-OE and OsHsp23.7-OE transgenic lines demonstrated higher germination ability compared to wild-type (WT) plants when subjected to mannitol and NaCl. Phenotypic analysis showed that transgenic rice lines displayed a higher tolerance to drought and salt stress compared to WT plants. In addition, transgenic rice lines showed significantly lower REC, lower MDA content and higher free proline content than WT under drought and salt stresses. These results suggest that *OsHsp17.0* and *OsHsp23.7* play an important role in rice acclimation to salt and drought stresses and are useful for engineering drought and salt tolerance rice.

Introduction

Abiotic stresses such as drought, salinity, extreme temperature are frequently causing cellular damage and secondary stresses such as osmotic and oxidative stresses, resulting in disruption of osmotic and ionic homeostasis and damage to proteins and membranes (DaMatta and Ramalho, 2006; Wang et al., 2003). Abiotic stresses are the major impediments restraining plant growth and resulting in significant reductions in crop productivity (Wang et al., 2003). Plants respond to these adverse conditions by developing a series of physiological and biochemical strategies.

Heat shock proteins (Hsps) belong to a class of proteins that are conserved in prokaryotes and eukaryotes and are especially abundant in plants. Hsps are highly expressed in plants and other organisms after being stimulated by high temperature and other stresses (Vierling, 1991). According to the molecular weight, Hsps can be divided into five families: Hsp100s, Hsp90s, Hsp70s, Hsp60s, and a group of sHsps (small heat shock proteins) with a molecular mass ranging from 15 to 42 kDa (Trent, 1996). The sHsps are much more abundant in higher plants than in other organisms (Vierling, 1991). Induction of sHsp gene expression and protein accumulation upon environmental stresses indicated that these proteins play an important role in stress tolerance (Sun et al., 2002). Previous studies have shown that constitutive expression of certain sHsp enhanced tolerance of transgenic plants to various stresses. Sun et al. (2001) suggested that overexpression of *AtHsp17.6A* enhanced drought and salt tolerance of transgenic plants. Transgenic rice plants overexpressing sHsp17.7 protein exhibited significantly increased thermotolerance and greater resistance to UV-B stress than untransformed control plants (Murakami et al., 2004). Constitutive expression of *RcHsp17.8* in transgenic *Arabidopsis* confers higher thermotolerance and resistance to salt, drought and osmotic stresses (Jiang et al., 2009). Overexpression of *CaHsp26* in transgenic tobacco protects PSII and PSI during chilling stress under low irradiance (Guo et al., 2007).

Rice is the world's most important food crop and a primary source of food for more than half the world's population (Khush, 2005). We have previously demonstrated that the expression of *OsHsp17.0* and *OsHsp23.7* could be enhanced by heat shock treatment and/or other abiotic stresses (Zou et al., 2009). In the present study, transgenic rice plants overexpressing *OsHsp17.0* and *OsHsp23.7* were generated to further characterize their functions in abiotic stress tolerance. Our results demonstrated that overexpression of *OsHsp17.0* and *OsHsp23.7* enhanced tolerance of transgenic plants to salt and drought stresses.

Abbreviations: APX, ascorbate peroxidase; Hsps, heat shock proteins; MDA, malondialdehyde; PEG, polyethylene glycol; REC, relative electrical conductivity; ROS, reactive oxygen species; SOD, superoxidase dismutase.

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doi:10.1016/j.jplph.2011.12.014

Materials and methods

Plasmid construction and rice transformation

Full-length cDNA clones of *OsHsp17.0* (AK121025) and *OsHsp23.7* (AK060051) were obtained from the National Institute of Agrobiological Sciences (NIAS, Tsukuba, Japan). The complete ORFs of *OsHsp17.0* and *OsHsp23.7* were PCR-amplified with primers 5′-CGGGATCCCACCCAACTCTTCTTCTTCC-3′ and 5′-GCTCTAGACTTGACCTTGACAAACTCCC-3′ for *OsHsp17.0*, and with primers 5′-CGGGATCCATGAGCCTACTGCTGCT-3′ and 5′-AATCTAGATCGTACACCTGGATCAACA-3′ for *OsHsp23.7*. The full-length cDNA plasmids were used as templates. Then the PCR products were cloned in pCAMBIA1301-Multi (modified from pCAMBIA1301) under the control of the CaMV 35S promoter respectively. Both of the constructs were transformed into rice (*Oryza sativa* ssp. *japanica* var. *Nipponbare*) according to the rice genetic transformation method described by Toki et al. (2006).

RNA isolation and real-time qPCR analysis

For real-time quantitative PCR analysis, total RNA was isolated from rice shoots and germinating embryos dissected from seeds. After the RNA was digested with RNase-free DNase I (Fermentas), RNA quality and quantity were determined by electrophoresis and spectrophotometry (NanoDrop 2.5.1). Five hundred nanograms of total RNA from each sample was reverse transcribed with the ReverTra Ace®qPCR RT Kit (Toyobo). Real-time PCR was performed on an ABI 7300 real-time PCR system (Applied Biosystems) using SYBR® Premix Ex Taq™ II (Takara) under the following conditions: 95 °C for 30 s, followed by 40 cycles at 95 °C for 5 s, and 60 °C for 31 s. Rice Ubiquitin 5 gene *UBQ5* (AK061988) was used as an endogenous control. The following gene-specific primers were used:

OsHsp17.0: 5′-AAGGTGGACCAGGTGAAGG-3′ and 5′-GACCTTGACAAACTCCCGTT-3′;
OsHsp23.7: 5′-AGACCACCCACCATTGAGATT-3′ and 5′-GCCACCAACAAGGATGAACAT-3′;
UBQ5: 5′-GGAAGTAAGGAAGGAGGAGGAA-3′ and 5′-CAGAGGTGATGCTAAGGTGTTC-3′.

Data represent three biological replicates and three technical replicates. The relative changes in gene expression were quantified using the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). The data were expressed as mean ± standard error.

Evaluation of seed germination ability under osmotic and salt stresses

To determine the effect of osmotic and salinity stress on seed germination, 3 × 40 seeds of each material were germinated in 9 cm^2 Petri dishes containing solid 1/2 MS medium supplemented with different concentrations of mannitol (0, 50, 100 and 150 mM mannitol) and NaCl (0, 50, 100 and 150 mM NaCl), respectively. The germination was conducted in a growth chamber (30 °C and a 14 h light/10 h dark cycle). Five days later, the number of germinated seeds whose radicles protruded from the seed envelope by 2–3 mm was counted. And another five days later, the plant height and primary root length were measured. All experiments were repeated at least three times.

Tolerance assay of transgenic rice seedlings under drought and salt stresses

At one week following germination on 1/2 MS solid medium, the seedlings were transferred to 1/2 Hoagland solution and placed in a growth chamber (14-h-light/10-h-dark cycles) at 30 °C and 75% relative humidity. After two-week growth in the growth chamber, the seedlings were then used for abiotic stress treatments. For drought tolerance treatment, whole seedling plants were exposed to air for 9.5 h (25 ± 1 °C, 40 ± 5% relative humidity), then transferred back to 1/2 Hoagland solution for 10 d of recovery. For high-salt stress treatment, seedlings were treated in 200 mM NaCl solution for 24 h and then transferred back to 1/2 Hoagland solution for 10 d of recovery. The seedlings were evaluated for their survival percentage based on observations that actively growing seedlings as survivors and the non-growing and wilted seedlings were as non-survivors. All experiments were repeated at least three times.

Tolerance assay of transgenic rice seedlings under temperature stresses

Experiments were performed as described by Koh et al. (2007) with some modification. Seeds were germinated on 1/2 MS solid medium for 4 d, then placed in 1/2 Hoagland solution for another 4 d at 30 °C and 75% relative humidity in the growth chamber. For heat stress treatment, 8-day-old seedlings were exposed to 45 °C for 24 h. For low temperature treatment, 8-day-old seedlings were treated at 5 °C for 4 d. After temperature stress treatments, the seedlings were allowed to recover at 30 °C and 75% relative humidity in the growth chamber for 7 d. The seedlings were evaluated for their survival percentage based on observations that actively growing seedlings as survivors and the non-growing and wilted seedlings were as non-survivors. All experiments were repeated at least three times.

Measurements of relative electrical conductivity (REC), malondialdehyde (MDA) and proline content

Three-week-old seedlings were used for abiotic stress treatments and the assays of leaf REC, MDA and proline content. For drought treatment and salt treatment, plants were treated with 20% PEG and 200 mM NaCl for 3 d, respectively. For temperature treatments, plants were exposed to 45 °C and 5 °C for 30 h, respectively. The leaf REC was measured at the beginning and at the end of each stress treatment as the method described by Yu et al. (2006). Five seedling shoots were harvested at the beginning and at the end of each stress treatment and finely ground in liquid nitrogen using a mortar and pestle previously chilled with liquid nitrogen and the frozen powder was immediately used for MDA and proline assay. MDA content was measured according to Kuk et al. (2003). Free proline content was measured in acidic extracts and quantified spectrophotometrically using the acid–ninhydrin reagent with proline as a standard (Bates et al., 1973).

Results

Enhanced expression of OsHsp17.0 and OsHsp23.7 in transgenic plants

The expression of *OsHsp17.0* and *OsHsp23.7* in transgenic lines (T_2 generation) was checked by real-time qPCR. Three highly over-expressed lines were identified in the OsHsp17.0-OE and OsHsp23.7-OE lines respectively (Fig. 1A) and were used for later characterization.

Overexpression of OsHsp17.0 and OsHsp23.7 enhanced drought tolerance

Seed germination ability of transgenic lines and WT rice was evaluated after 5 d of germination on the medium containing 0, 50, 100 and 150 mM mannitol. No difference in germination