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Research paper

A gibberellin-stimulated transcript, *OsGASR1*, controls seedling growth and α-amylase expression in rice

Sang-Choon Lee[a,c], Soo-Jin Kim[a], Soon-Ki Han[a], Gynheung An[b], Seong-Ryong Kim[a,*]

[a] Department of Life Science, Sogang University, Seoul 121-742, Republic of Korea
[b] Crop Biotech Institute & Department of Plant Molecular Systems Biotechnology, Kyung Hee University, Yongin, 446-701, Republic of Korea
[c] Department of Plant Science, Plant Genomics and Breeding Institute, and Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, 151-921 Seoul, Republic of Korea

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ABSTRACT

From a T-DNA-tagging population in rice, we identified *OsGASR1* (LOC_Os03g55290), a member of the *GAST* (gibberellin (*GA*)-*S*timulated *T*ranscript) family that is induced by salt stress and ABA treatment. This gene was highly expressed in the regions of cell proliferation and panicle development, as revealed by a GUS assay of the mutant line. In the *osgasr1* mutants, the second leaf blades were much longer than those of the segregating wild type due to an increase in cell length. In addition, five α-amylase genes were up-regulated in the mutants, implying that OsGASR1 is a negative regulator of those genes. These results suggest that OsGASR1 plays important roles in seedling growth and α-amylase gene expression.

1. Introduction

Numerous members of the gibberellin (*GA*)-*S*timulated *T*ranscript (*GAST*) gene family have been identified in various species, such as *Lycopersicon esculentum*, *Petunia* x *hybrida*, *Arabidopsis thaliana*, and *Oryza sativa* (rice). These genes encode small proteins with distinct domains, including the signal peptide and GASA (GA-Stimulated in Arabidopsis) domain. The GASA domain is highly conserved among GAST proteins and consists of approximately 60 amino acids that contain 12 cysteine residues in the C-terminal region (Nahirñak et al., 2012). Most *GAST*s are responsive to GA and some are also responsive to abscisic acid (ABA) and auxin (Nahirñak et al., 2012). These genes are involved in various biological processes, including cell and shoot elongation, flowering, seed development, mediation of hormone pathways, and abiotic-stress responses (Shi et al., 1992; Ben-Nissan et al., 2004; Wigoda et al., 2006; Alonso-Ramírez et al., 2009; Wang et al., 2009; Rubinovich and Weiss, 2010; Sun et al., 2013; Lee et al., 2015). The GAST genes are parts of a multigene family. For example, the genomes of *A. thaliana*, *Zea mays*, and rice have 14, 10, and nine such genes, respectively (Roxrud et al., 2007; Zimmermann et al., 2010).

Among the rice GAST genes, only three have been functionally studied. *OsGASR1* (*Oryza sativa GA S*timulated *R*ice *1*) and *OsGASR2* were isolated through EST analysis and shown to be highly expressed in the apical meristems of shoots and roots, suggesting that they have roles in cell division and proliferation (Ikeda et al., 1997; Furukawa et al., 2006). Furthermore, *OsGASR1* is strongly responsive to salt and ABA treatments, and it confers increased tolerance to salt and reactive oxygen species when it is over-expressed in a plant (Lee et al., 2015). *OsGSR1* is antagonistically regulated by GA and brassinosteroid (BR) and thus it is thought to be involved in mediation between the GA- and BR-signaling pathways (Wang et al., 2009). In addition, *OsGASR7*, identical to *OsGSR1*, has been proposed as a candidate gene responsible for a rice trait for grain length, based on results from a genome-wide association study (Huang et al., 2011). Homologues of *OsGASR7* in *Triticum aestivum* and *T. urartu* are also significantly associated with traits for grain length and weight (Ling et al., 2013). Recently, a wheat OsGASR1-homologue, *TaGASR1*, was identified to be involved in tolerance to heat and oxidative stress in wheat (Zhang et al., 2017).

In plants, T-DNA insertion mutagenesis has been used to determine many previously unknown gene functions (Krysan et al., 1999; An et al., 2005). For example, a reporter gene trap harboring *GUS* indicates the time and location of endogenous expression, with the trap itself possibly causing a mutant phenotype that reflects the function of its corresponding gene (Jeon et al., 2000). By screening T-DNA tagging lines of rice, many research groups have uncovered genes responsive to various abiotic stresses (Kim et al., 2004; Lee et al., 2004; Koh et al., 2007; Park et al., 2010).

Although the GAST genes have common features and much effort

Abbreviations: ABA, abscisic acid; BR, brassinosteroid; GA, gibberellin; GUS, beta-glucuronidase; qRT-PCR, quantitative real-time PCR; RT-PCR, reverse transcription-PCR; WT, wild-type

* Corresponding author.
E-mail address: sungkim@sogang.ac.kr (S.-R. Kim).

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has been put forth to investigate their functions, they have still not been well-elucidated in plants. Therefore, we applied a gene trap system to isolate a salt- and ABA-responsive *OsGASR1* gene and its T-DNA tagging lines. We then further characterized the physiological and molecular functions of *OsGASR1* by evaluating knock-out mutants.

2. Materials and methods

2.1. Plant materials

For morphological examination of mature plants, seeds of rice (*O. sativa* ssp. *japonica*) obtained from wild-type (WT) cultivar Dongjin and transgenic lines were germinated in the dark at 30 °C and then transferred to soil in either a greenhouse or paddy field. For studies at the seedling stage, seeds were dehusked, surface-sterilized, and germinated at 30 °C in the dark for 5 d either on an agar medium containing distilled water or on a half-strength MS medium (Murashige and Skoog, 1962). The germinants were then transferred to continuous light (intensity of 50 μmol m^{-2} s^{-1}). For GA and ancymidol treatments, seeds were held for 5–10 d on MS agar media containing 10 μM of either gibberellic acid (GA_3) or ancymidol. For GUS assays and total RNA isolation, seedlings were hydroponically cultured in Yoshida solution, as described by Lee et al. (2005).

2.2. Screening of T-DNA tagging lines and GUS assays

After various stress treatments including cold (5 °C), salt (300 mM at 25 °C), and ABA (100 μM at 25 °C) were applied, T-DNA-tagging lines harboring pGA2144 or pGA2707 (Jeon et al., 2000) were screened by GUS assays, as previously reported (Lee et al., 2004). Histochemical GUS-staining was performed as described by Dai et al. (1996) except for the addition of 20% methanol in the staining solution. Samples were incubated in an X-gluc solution according to the method of Jeon et al. (2000). Chlorophyll was removed with 70% and 95% ethanol. The GUS-stained samples were examined under a Leica MZ dissecting microscope (Leica, Germany).

2.3. T-DNA flanking sequence analysis

Genomic DNA was extracted from the mature leaves of selected lines according to the method of Chen and Ronald (1999) after the samples were ground with an MM300 Mixer Mill (Retsch, Germany). To analyze the T-DNA flanking sequences, we performed inverse PCR as described previously (Jeong et al., 2002). H3IPCR1 and GUS2, as the first-PCR primers, and H3IPCR2 and GUS1, as the second-PCR primers, were used for amplifications after cutting with *Hin*dIII. The primer sequences were as follows: H3IPCR1, 5′-CGAGACAACGCAGAGAAAG-3′; GUS2, 5′-CTGCATATAACCTGCACATTAGC-3′; H3IPCR2, 5′-TTCGTACTCGCCTCTCTCC-3′; and GUS1, 5′-GGATACAAGTCTGTACCTTG-3′. Template DNAs were amplified by PCR runs that comprised 35 cycles at 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 5 min. The PCR products were directly sequenced. Genomic sequences containing the tagging sequence were retrieved from the Chinese Rice Genome Database (http://www.btn.genomics.org.cn/rice/) (Yu et al., 2002) and annotated using a Rice Genome Automated Annotation System (RiceGAAS; http://www.ricegaas.dna.affrc.go.jp), the FGENESH program (http://linux1.softberry.com/berry.phtml), and BLASTP programs (http://www.ncbi.nlm.nih.gov/BLAST/). In addition, the flanking gene of the tagging sequence was confirmed by searches of the Rice Genome database (http://signal.salk.edu/cgi-bin/RiceGE; http://rice.plantbiology.msu.edu/).

2.4. Genotyping of OsGASR1 mutant lines

To distinguish the genotypes for progeny of the tagging lines, we conducted PCR analysis with 250 ng of genomic DNA as template, performing 35 cycles of 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min. Primers included P1 (5′-GATAAATAGTGGCCAGCAACC-3′) and P2 (5′-TACGATCGATCATGGCAAGC-3′) for the non-T-DNA insertion, or P1 and P3 (5′-CTGCATATAACCTGCACATTAGC-3′) for T-DNA insertion. The primer for the P3 sequence was the same as for the GUS2 primer.

2.5. Southern blot analyses

Genomic DNA was extracted from the mature leaves of mutant lines by the cetyltrimethylammonium bromide method (Roger and Bendich, 1988). Southern blot analyses were performed as described by Lee et al. (2005), using *GUS* as a probe.

2.6. RT-PCR and quantitative real-time PCR analysis

For RT-PCR and real-time PCR, total RNAs were isolated with an RNeasy Plant Mini Kit and DNaseI treatment (Qiagen, Germany). The cDNAs were then synthesized with a Sprint RT Complete-Oligo $(dT)_{18}$ Kit (Clontech, USA), according to the manufacturer's instructions. Gene-specific primers are presented in Supplementary Table S1. As controls, we used primers specific to the rice actin gene *RAc1*, the elongation factor gene *OseEF1-a*, and a ubiquitin gene, *OsUBQ5* (McElroy et al., 1990; Jain et al., 2006). Template DNAs were amplified by PCR that consisted of 22–40 cycles at 95 °C for 1 min, 52–59 °C for 1 min, and 72 °C for 1 min. Quantitative real-time PCR (qRT-PCR) was performed using a SYBR FAST Universal qPCR Kit (KAPA Bioscience, USA) and ABI 7500 (Applied Biosystems, USA) according to the manufacturers' instructions. The PCR conditions included pre-denaturation at 95 °C for 30 s, then 40 cycles of 95 °C for 5 s and 60 °C for 34 s. Relative expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001) with *RAc1* as an internal control.

2.7. Scanning electron microscopy

The second leaf blades were treated with a primary fixation solution containing 2% paraformaldehyde and 2% glutaraldehyde in a 50 mM sodium cacodylate buffer (pH 7.2), and then with a post-fixation solution containing 1% osmium tetroxide in the same buffer. After dehydration through an ethanol series, samples were dried using liquid carbon dioxide and then coated with gold. The leaf surfaces were observed with a field-emission scanning electron microscope (Carl Zeiss, Germany).

3. Results

3.1. Identification of T-DNA tagging lines of an abiotic stress-inducible gene, OsGASR1

We screened rice T-DNA tagging lines for genes induced by various stress treatments (Lee et al., 2004). Because the T-DNA carried the promoterless *GUS*, the insertion generated a fusion between an endogenous gene and the GUS reporter. Line 0-175-70 showed *GUS* induction after ABA or salt treatment (Fig. 1A). The T-DNA was inserted 46 bp upstream from the translational start codon of *OsGASR1* (LOC_Os03g55290), where *GUS* was inserted in the same orientation as the tagged gene (Fig. 1B). *OsGASR1* encodes a protein of 93 amino acid residues (AB192574; Furukawa et al., 2006) that shares approximately 40% homology with *OsGASR2* (AB192575; Furukawa et al., 2006) and *OsGSR1* (AY604180; Wang et al., 2009).

Homozygous progenies of the T-DNA insertion in *OsGASR1*, named *osgasr1-1*, were identified by genotyping Line 0-175-70 (Fig. 2A). Whereas all of the homozygous plants displayed salt- and ABA-responsive *GUS* expression (Fig. 1A), the segregating WT progenies were *GUS*-negative. This confirmed that *GUS* expression was due to the T-DNA insertion near *OsGASR1*.