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OsSCE1 Encoding SUMO E2-Conjugating Enzyme Involves in Drought Stress Response of *Oryza sativa*

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Abstract: Small ubiquitin-like modifier (SUMO)-conjugating enzymes are involved in post-translational regulatory processes in eukaryotes, including the conjugation of SUMO peptides to protein substrate (SUMOylation). SUMOylation plays an important role in improving plant tolerance to abiotic stress such as salt, drought, heat and cold. Herein, we reported the isolation of *OsSCE1* (LOC_Os10g39120) gene encoding a SUMO-conjugating enzyme from rice (*Oryza sativa* cv. Nipponbare) and its functional validation in response to drought stress. The E2 enzyme, OsSCE1, is one of three key enzymes involved in the conjugation of SUMO to its target proteins. Activated SUMO is transferred to the cysteine of an E2 enzyme and then to the target lysine residue of the substrate, with or without the help of an E3 SUMO ligase. Expression of *OsSCE1* was strongly induced by polyethylene glycol 6000 (PEG6000) treatment, which suggested *OsSCE1* may be involved in the drought stress response. Overexpression of *OsSCE1* (OsSCE1-OX) in Nipponbare reduced the tolerance to drought stress. Conversely, the drought tolerance was slightly improved by the knockdown of *OsSCE1* (OsSCE1-KD). These results were further supported by measurement of proline content in OsSCE1-OX and OsSCE1-KD transgenic lines under induced drought stress, which showed OsSCE1-KD transgenic lines accumulated higher proline content than the wild type, whereas OsSCE1-OX line had lower proline content than the wild type. These findings suggested *OsSCE1* may play a role as a negative regulator in response to drought stress in rice.

Key words: *Oryza sativa*; drought stress; small ubiquitin-like modifier; SUMO-conjugating enzyme; proline content; gene expression

Drought is a major environmental stress factor that significantly reduces crop growth and productivity. Through evolution, plants have developed various mechanisms in response to environmental stress, such as changes in their internal molecules, cellular processes and physiological processes (Xiong et al, 2014). Protein activity is modulated by adding small molecules to the target protein, i.e., a process known as post-translational modification (PTM). PTM process is a quick, reversible physiological response needed by living organisms to respond to environmental changes. Several important protein modifiers exist and are used in PTM, including phosphate, methyl, acetyl, lipid, sugar and small peptide (Castro et al, 2012). Ubiquitin is the most important small peptide associated with protein degradation, and the small ubiquitin-like modifier (SUMO) is one of many ubiquitin-like modifiers with a conformational structure and conjugation machinery similar to the ubiquitin system (Chaikam and Karlson, 2010; Castro et al, 2012).

An essential post-translational regulatory process in all eukaryotes, SUMO conjugation (SUMOylation) involves three key enzymes: the SUMO activating enzyme (SAE/E1-type), SUMO conjugating enzyme (SCE1/E2-type) and SUMO ligase (E3-type) (Karan and Subudhi, 2012). In *Arabidopsis*, however, E4-type

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enzymes (PIAL1 and PIAL2) have been reported (Tomanov et al, 2014). Activation of the SUMO carboxyl terminus, catalyzed by SAE/E1, leads to formation of a thioester and transfer of SUMO to a SUMO-conjugating enzyme (SCE1). Moreover, SCE/E2 links the SUMO carboxyl terminus to lysine ε-amino groups in the substrate, with or without the help of E3 ligases (Castro et al, 2012; Tomanov et al, 2014). E4-type enzymes, PIAL1 and PIAL2, were previously reported to function as SUMO ligases, which are capable of SUMO chain formation but require the SUMO-modified SUMO conjugating enzyme SCE1 for optimal activity (Tomanov et al, 2014). SUMOylation plays a role in different cellular processes and has been demonstrated a range of effects depending on its target (Verger et al, 2003; Miura et al, 2007a). Some SUMO modifications regulate transcription factor activities, which can correspond to gene expression for plant development, and responses to hormones and environmental cues (Kurepa et al, 2003; Lois et al, 2003; Miura et al, 2005, 2007b).

In plants, SUMOylation is essential for development and stress responses (Raorane et al, 2013). *In vivo*, the role of SUMO conjugation in the stress response in *Arabidopsis* has been described for various stresses, including drought (Catala et al, 2007; Miura et al, 2013; Zhang et al, 2013), low temperature (Miura et al, 2007b) and pathogens (Lee et al, 2006). The role of SUMOylation in plants was previously reviewed by Castro et al (2012). In rice (*Oryza sativa*), the transcripts of SUMOylation-related genes are identified under cold, salt and abscisic acid (ABA) stresses. Furthermore, SUMOylation-related genes show differential accumulation in various tissues during development, suggesting important roles in both rice development and stress responses (Chaikam and Karlson, 2010).

The relatively less well-studied *SCE1* encodes the E2 type SUMO-conjugating enzyme. In plants, the SCE family members play roles in abiotic stress responses. An E2 enzyme, SaSce9, from *Spartina alterniflora* plays roles in salinity and drought stress responses (Karan and Subudhi, 2012). Furthermore, SCE can respond to heat stress in rice (Nigam et al, 2008). However, the role of *SCE1* in drought stress response in rice has not yet been reported. We previously observed upregulation of an *OsSCE1* gene transcript in rice during drought stress, simulated by polyethylene glycol 6000 (PEG6000) treatment. Therefore, in this study, we performed the isolation and functional validation of the *OsSCE1* gene to better understand its role in the drought stress response in rice.

MATERIALS AND METHODS

Plant materials

Rice cultivar Nipponbare was used in this study.

Isolation of full-length cDNA and sequence analysis of OsSCE1 gene

Total RNA was extracted from leaves of mature rice plants using TRIzol reagent (Invitrogen, Carlsbad, USA) and used to isolate full length cDNA of *OsSCE1* by reverse transcriptase (RT)-PCR. For RT-PCR, specific primers (SCE1-F/SCE1-R) (Table 1) were designed based on the *OsSCE1* (LOC_Os10g39120) gene sequence, reported in the NCBI database. First strand cDNA was amplified using the Cloned AMV First-Strand cDNA Synthesis Kit (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Full-length cDNA was then used as the template for PCR amplification with KAPA2G robust DNA polymerase (KAPA Biosystems, Massachusetts, USA) under the following conditions: 95 ºC for 3 min, then 35 cycles of 95 ºC for 30 s, 55 ºC for 15 s and 72 ºC for 30 s, followed by a final step at 72 ºC for 5 min. Amplified cDNA fragments were cloned using pGEMT-Easy vector system (Promega, Madison, USA) and sequenced (1st BASE DNA Sequencing Services, Singapore). The full-length *OsSCE1* gene sequence was analyzed by the DnaMan1 software (Lynnon Biosoft, Leiden, Netherlands) and BLAST sequence analysis tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple sequence alignment of SCE1 proteins was performed by CLUSTALW (Feng and Doolittle, 1987; Thompson et al, 1994) and subsequently visualized as phylogenetic trees with resampling using 1000 bootstrap replications. Both the multiple sequence alignment and phylogenetic analysis were carried out using DnaMan1.

Establishment of OsSCE1-OX and OsSCE1-KD transgenic rice

Transgenic rice plants overexpressing the *OsSCE1* gene (OsSCE1-OX) were generated using the pCAMBIA1305 expression vector (Roberts et al, 1997) by introducing isolated *OsSCE1* full-length genes into *Bgl*II/*Bst*EII sites (Table 1), driven by CaMV35S promoter. Generation of knockdown rice plants