BMB
reports

Response and transcriptional regulation of rice SUMOylation system during development and stress conditions

Vijay Chaikam[1,2] *& Dale T. Karlson*[1,3,*]

[1]Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506-6108, USA, [2]Department of Agronomy, Purdue University, West Lafayette IN 47907, [3]Monsanto Company, 110 TW Alexander Drive, RTP, NC 27709

Modification of proteins by the reversible covalent addition of the small ubiquitin like modifier (SUMO) protein has important consequences affecting target protein stability, sub-cellular localization, and protein-protein interactions. SUMOylation involves a cascade of enzymatic reactions, which resembles the process of ubiquitination. In this study, we characterized the SUMOylation system from an important crop plant, rice, and show that it responds to cold, salt and ABA stress conditions on a protein level via the accumulation of SUMOylated proteins. We also characterized the transcriptional regulation of individual SUMOylation cascade components during stress and development. During stress conditions, majority of the SUMO cascade components are transcriptionally down regulated. SUMO conjugate proteins and SUMO cascade component transcripts accumulated differentially in various tissues during plant development with highest levels in reproductive tissues. Taken together, these data suggest a role for SUMOylation in rice development and stress responses. [BMB reports 2010; 43(2): 103-109]

INTRODUCTION

Post-translational modifications of proteins through the reversible covalent attachment of small proteins like ubiquitin and ubiquitin-like modifiers have critical effects on protein stability and biological activities. Small ubiquitin-like modifier (SUMO) proteins possess a similar 3-dimensional structure as that of ubiquitin, but only show ~18% identity at the amino acid level and have an additional ~15 N-terminal amino acids. Ubiquitination and SUMOylation of target proteins occurs via a cascade of similar enzymatic reactions including the sequential action of E1 enzymes for SUMO activation, E2 enzymes for conjugation and E3 enzymes for ligation. In spite of the structural and mechanistic similarities, ubiquitination and SUMOylation have very different biological consequences. Ubiquitination mostly associates with the proteasomal degradation of target proteins, whereas SUMOylation has various effects on target protein localization, stability, transcription factor activity, interactions with other proteins, other post-translational modifications and effects on chromatin structure (reviewed in (1, 2)).

All of the SUMOylation system components are present in plant genomes and some have been characterized on a biochemical and genetic level in Arabidopsis where they are essential for plant development (3-6). During abiotic stress conditions, the SUMOylation system affects plant survival via the accumulation of SUMO conjugates (5-11). SUMOylation also modulates ABA signaling (12), mediates bacterial resistance (13) and is involved in viral pathogenesis (14, 15). Even though the effects of SUMOylation are well understood at a molecular and whole organism level, the regulation of the SUMOylation system in plants remains poorly understood. SUMOylation can be regulated at four levels: (a) by altering the gene expression of SUMO cascade components (b) by regulating the enzyme activity of proteins involved in SUMOylation (c) by regulating the recruitment of E3 ligases and (d) by employing cross talk with other post-translational modifications (16, 17). In this study, we report the first comprehensive sequence analysis and transcriptional regulation of all SUMO component genes in the economically important monocot rice model system. We also show that during plant development SUMO conjugates accumulate at higher levels in actively growing tissues and that the SUMOylation system responds to stress conditions via the accumulation of SUMO conjugated proteins.

RESULTS AND DISCUSSION

Sequence analysis

All of the SUMO cascade component gene sequences were analyzed by aligning the rice sequences with Arabidopsis, human and yeast sequences. We documented important domains and critical residues in each set of proteins (Supplemental Fig. 1). Among the three SUMO genes in rice, OsSUMO1 and OsSUMO2 are highly homologous with 89% amino acid identity. OsSUMO3 showed less than 40% identity with other

*Corresponding author. Tel: 9194065704; Fax: 9194065757;
E-mail: dale.karlson@monsanto.com

Received 17 September 2009, Accepted 6 November 2009

Keywords: Abiotic stress, Development, SUMO, SUMOylation, Sequence analysis

plant, yeast and human SUMO proteins. The SUMOylation pathway contains two E1 enzymes, named SAE1 and SAE2, which mutually stabilize each other (18) and together form a functional heterodimer. Rice contains a single *SAE1* gene (*OsSAE1a*) which shows 60% identity with both of the Arabidopsis SAE1 proteins. Similar to other studied organisms, the rice genome encodes a single *SAE2* gene (Os*SAE2*) which exhibits 79% identity with At*SAE2*. Three E2 genes named *OsSCE1a, OsSCE1b*, and *OsSCE1c* are encoded within the rice genome. OsSCE1a and OsSCE1b proteins are very closely related to AtSCE1a with 89% and 86% identity respectively. On the other hand, OsSCE1c shows less than 60% identity with other rice and Arabidopsis E2 proteins. In contrast to Arabidopsis, which contains a single PIAS-type E3 ligase, two PIAS-

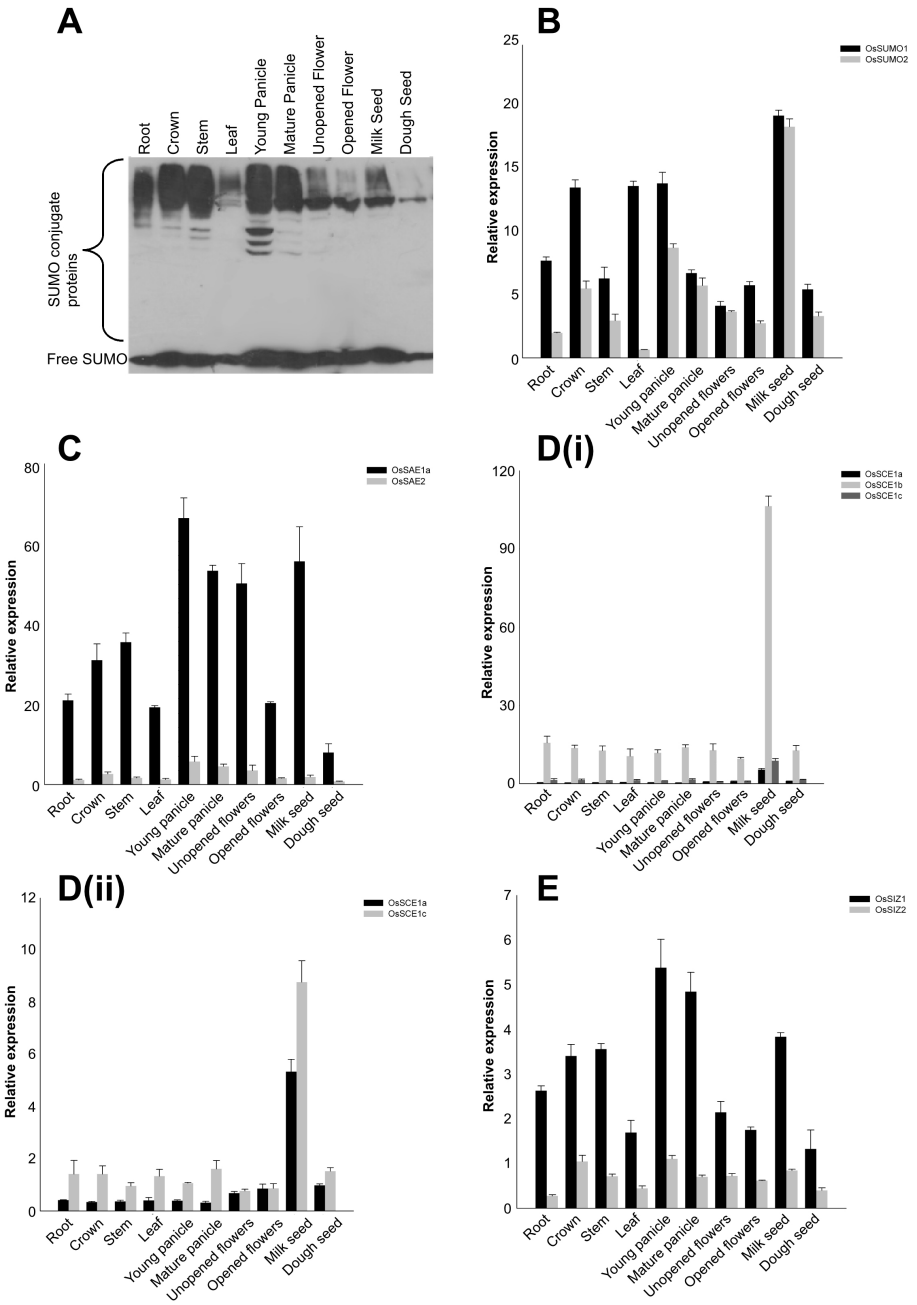

Fig. 1. SUMOylation profiles and SUMO cascade component RNA accumulation in different tissues. (A) SUMOylation profiles in tissues from vegetative and reproductive tissues were determined by western blot analysis of 25 μg of total proteins. Relative mRNA accumulation levels of rice (B) SUMO genes (C) E1 genes D(i) and D(ii) E2 genes and (E) E3 genes were determined by quantitative real-time PCR by obtaining absolute copy number for each gene and normalizing with the absolute copy number of the *18srRNA* gene. Note the differences in the scale of the Y-axis in the different graphs. Note that all of the SUMO components are expressed in all tissues and the majority of them are highly abundant in reproductive tissues of young panicles and milk seeds.

type E3 ligase genes were identified in rice (*OsSIZ1*; Os05g 0125000 and *OsSIZ2*; Os03g0719100) (2).

Developmental changes in SUMOylation levels and expression of SUMOylation cascade components

To study the changes in SUMOylation profiles during development, we isolated total proteins from various tissues during different rice development stages and subjected them to western blot analysis using a polyclonal antibody developed against the AtSUMO1 protein (Fig. 1A). OsSUMO1 and OsSUMO2 proteins show high homology to AtSUMO1 with 89% and 84% identity at the amino acid level, respectively. Since the AtSUMO1 antibody was suggested to cross react with the AtSUMO2 protein (10), which has 87% amino acid identity with that of AtSUMO1, we hypothesized that the anti-AtSUMO1 antibody could recognize rice SUMO proteins and their conjugates. Using Arabidopsis crude extracts for western blot analysis, the anti-AtSUMO1 antibody recognized an abundant 14 kDa species in Arabidopsis which likely represents AtSUMO1/2 species (10). Similarly, the AtSUMO1 antibody recognized an abundant 14 kDa species in rice which likely represents OsSUMO1/OsSUMO2 species (Fig. 1A). In addition, high levels of high molecular weight SUMOylated proteins accumulated in large amounts in tissues such as crown, stem and panicle which correlate with active growth and development. The lowest amount of SUMO conjugates, along with a low amount of free SUMO protein, was observed in leaf and dough seed tissues. In roots, stems and milk seed tissues, moderate amounts of SUMOylated proteins were found. These data indicate that SUMOylation of proteins may have a specialized function in actively growing tissues. These observations are further supported by the proven role of SUMOylation and its components in plant growth and development at a functional level in Arabidopsis (3-6).

To understand the role of individual SUMO cascade components and their regulation during development, we studied their individual expression patterns within different tissues by quantitative real-time PCR analysis. In our expression analysis, we were only able to detect *OsSUMO1* and *OsSUMO2* transcripts among the three SUMO genes tested (Fig. 1B). After evaluating multiple primer pairs and using cDNA derived from different tissues, we were still unable to amplify *OsSUMO3* (data not shown). In Arabidopsis, even though the genome encodes nine SUMO genes, only four of them are expressed (6, 10). Expression of *OsSUMO1* is at least two-folds higher than that of *OsSUMO2* in vegetative tissues, however, they are expressed at comparable levels in reproductive tissues like mature panicles and developing seeds. High levels of expression for both of these genes can be found in milk seeds and young panicle tissues. These correlative data suggest that both genes may be functionally important during plant reproductive development. Furthermore, these data also indicate that OsSUMO1 has a more generalized function, whereas OsSUMO2 may perform specialized functions during plant reproductive development. *OsSUMO2* is expressed at very low levels in leaf tissue, which may contribute to the low levels of free SUMO and SUMOylated proteins detected in leaf tissue (Fig. 1A).

Among the E1 genes, *OsSAE1a* is expressed at very high levels compared to Os*SAE2* in all of the examined tissues (Fig 1C). A similar observation was noted in Arabidopsis where *AtSAE1a* and *AtSAE1b* are highly expressed relative to *AtSAE2* (6). However, it is interesting to note that the SUMO activation enzymes SAE1 and SAE2 form a functional heterodimer suggesting that these proteins should be present in equal amounts. *AtSAE2* transcript was proposed to be more efficiently translated and/or the protein may be more stable which would result in a stoichiometrically equal level for both of the E1 proteins (6). Similar mechanisms may also apply for rice E1 proteins. In rice, both *SAE1a* and *SAE2* transcripts are highly expressed in reproductive tissues like panicles, developing flowers and seeds, indicating a potential role for these genes during reproductive development.

Among the three SUMO conjugation enzyme genes, *OsSCE1b* is expressed at very high levels (at least 15 folds more) compared to *OsSCE1a* and *OsSCE1c* in all of the tissues studied (Fig. 1D(i)). All three of the E2 transcripts are specifically enriched in milk seed tissue (Fig. 1D(i) and D(ii)). These results indicate that *OsSCE1b* is the most transcriptionally active E2 gene and may function as the major SUMO conjugating enzyme in rice. *OsSCE1a* and *OsSCE1c* are expressed in all of the tissues at relatively low levels (Fig. 1D(ii)).

Among the two PIAS-type E3 ligases, *OsSIZ1* is expressed at least two-fold higher than that of *OsSIZ2* in all of the tested tissues. These correlative data indicate that *OsSIZ1* may function as the major SUMO ligase in rice (Fig. 1E). Both *OsSIZ1* and *OsSIZ2* are highly expressed in panicle and milk seed tissues and *OsSIZ2* was specifically enriched in crown tissue. Low expression of *OsSIZ1* and *OsSIZ2* was noticed in leaf tissue which may contribute to the lowest levels of SUMOylated proteins which were identified in leaves (Fig. 1A).

Response of SUMOylation system to abiotic stress conditions

To understand the role of SUMOylation in rice stress responses, we examined the changes in SUMOylation profiles after cold, salt and ABA stress treatments in rice seedlings over a period of 24 hrs. Changes in SUMOylation profiles were subsequently monitored with western blot analysis. To understand the role and regulation of individual components of SUMOylation cascade during stress conditions, we studied the changes in transcript levels with real-time PCR analysis.

(A) Cold stress

During cold stress at 4^{o}C, roots accumulated high molecular weight SUMO conjugates after only 1 hr of treatment. These conjugates were maintained at relatively similar levels during the remaining 24 hrs of treatment (Fig. 2A). This accumulation likely resulted from the appearance of new SUMO conjugates or from an increased amount in the levels of SUMOylated pro-

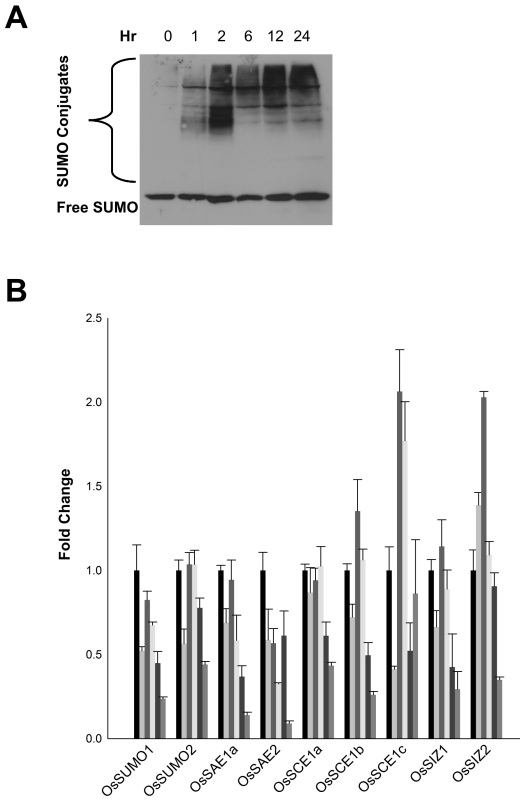

Fig. 2. Cold stress induces the accumulation of SUMO conjugates and affects SUMO component gene expression. (A) *In planta* SUMOylation profiles in root tissue of 1 week old seedlings grown in a growth chamber maintained at 30°C (0 hr time point) and then transferred into cold water maintained in a growth chamber at 4°C. Fifteen μg of total proteins were isolated and subjected to western blot analysis. Note the accumulation of high molecular weight SUMO conjugates after cold stress treatment. (B) Changes in mRNA levels of rice SUMOylation components with cold treatment were determined by quantitative real-time PCR. Changes in transcript abundance were represented as fold change by calibrating the relative mRNA levels of each time point with the relative mRNA level of the 0 hr time point. Note the down regulation of transcript levels for SUMO and E1 genes. Among E2 and E3 genes, *OsSCE1b, OsSCE1c* and *OsSIZ2* are transiently up regulated.

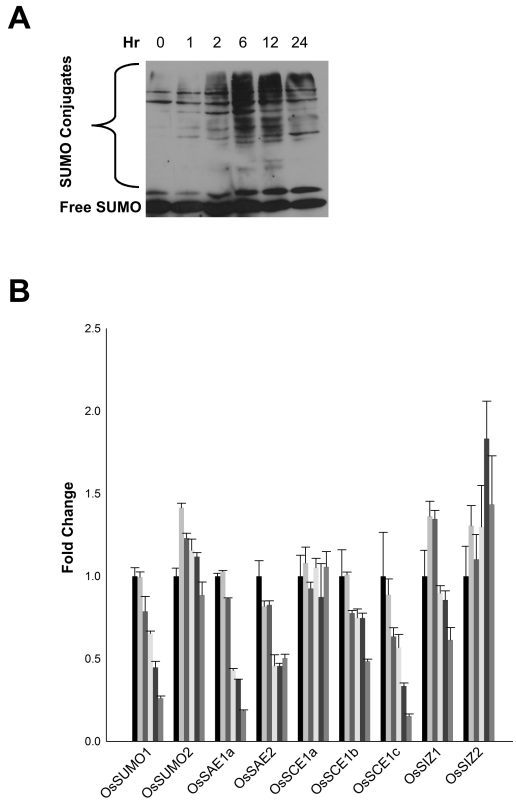

Fig. 3. Salt stress induces the accumulation of SUMO conjugates and effects SUMO component gene expression. (A) Western blot analysis of 15 μg of total proteins from 1 week old seedling root tissue grown in tap water (0 hr) and after transfer to 250 mM salt solution. Note the accumulation of high molecular weight SUMO conjugates after transferring to salt solution. (B) Changes in mRNA levels of rice SUMOylation components with salt stress as determined by quantitative real-time PCR. Fold change was calculated as described for Fig. 2B. Note the down regulation of SUMO and E1 and E2 (except *OsSCE1a*) encoding genes. Among the E3 genes, *OsSIZ1* and *OsSIZ2* were up-regulated.

teins that were present prior to initiation of the stress treatments. A similar accumulation of SUMOylated proteins was noted in Arabidopsis upon cold stress, which was mediated to a large extent by the E3 enzyme AtSIZ1 (8). In addition, a master regulator of cold responses in Arabidopsis (ICE1) was identified as a target for SUMOylation. SUMOylation of ICE1 enhances its stability and affects its ability to mediate transcriptional regulation (8). AtSIZ1 T-DNA insertion lines are freezing sensitive and impaired in their capacity to cold acclimate. The accumulation of SUMO conjugates in rice indicates that SUMOylation likely plays an important and similar role in the response to cold stress in this monocot model system.

In response to cold stress, the majority of the SUMO cascade component transcript levels were decreased (Fig. 2B). Most prominently, SUMO transcripts and SUMO activation enzyme transcripts were reduced by more than half of their original level after 24 hrs of cold treatment. However, transcripts for the SUMO conjugation enzymes *OsSCE1b*, *OsSCE1c* and the PIAS-type SUMO ligation enzyme *OsSIZ2* showed a transient increase during early periods of cold treatment. These increases in gene expression may contribute to the accumulation of SUMOylated proteins after exposure to cold stress. Given the importance of SUMO ligases in increasing the efficiency of SUMOylation and specificity (19-21), the increase in their transcription may result in the accumulation of ligase proteins and consequently an increase of SUMO conjugated proteins.

(B) Salt stress

Compared to the rapid changes observed during cold stress, SUMO conjugates gradually accumulated during 12 hrs of salt treatment and slightly decreased after 24 hrs (Fig. 3A). The observed increase in SUMOylated proteins may affect plant survival during salt stress. In Arabidopsis, the SUMOylation system was recently shown to respond to salt stress. Double mutants for two SUMO proteases (OTS1 and OTS2) showed extreme sensitivity to salt stress and overexpression of *OTS1* increased salt tolerance. These data clearly indicate an important role for the SUMOylation system in response to salt stress (11). Our data are in good accordance and thus corroborate the hypothesis that increased accumulation of SUMO conjugates during salt stress may have functional implications in rice.

OsSUMO1 transcripts decreased to a large extent after exposure to salt stress, whereas, *OsSUMO2* transcripts transiently increased and were maintained nearly at initial levels (Fig. 3B). Both SUMO E1 enzyme transcripts are decreased in response to salt stress. Among the E2 enzymes, *OsSCE1a* transcript levels were maintained at similar levels, whereas, *OsSCE1b* and *OsSCE1c* showed a significant reduction relative to initial transcript levels. Among the E3 genes, *OsSIZ1* showed a transient increase, whereas, *OsSIZ2* began to increase after 6 hrs of treatment. Comparative analysis of SUMO conjugate profiles and real-time PCR data enabled us to conclude that OsSIZ2 may play an important role in mediating SUMO conjugate accumulation during salt stress in rice.

(C) ABA treatment

Abscisic acid mediates plant responses to stress conditions such as cold, salinity and drought (22, 23). In Arabidopsis, using transgenic plants overexpressing *AtSUMO1* and *AtSUMO2*, along with co-suppression lines of *AtSCE1a*, ABA was shown to play a dual role in ABA signaling by positively effecting the induction of ABA responsive genes and by attenuating the ABA signaling pathway that leads to growth inhibition (12). However, it is not known if ABA mediates these physiological and molecular responses by affecting the SUMO conjugate levels of proteins in plants. Here, we show that ABA treatment effects SUMO conjugate accumulation in rice (Fig. 4A). Changes in profiles were reflected after 1 hr of ABA treatment by the appearance of new SUMOylated proteins. These data further support the hypothesis that the SUMOylation of proteins in rice is a functionally important component in the response of rice to abiotic stress.

After treating with ABA, the majority of SUMO cascade component transcripts exhibited a transient decrease during the first 2 hrs of treatment and subsequently returned to basal levels (Fig. 4B). The *OsSUMO2* gene showed a prominent positive response 6 hrs subsequent to ABA treatment, where its transcript expression was induced six-fold. Conversely, *OsSUMO1* was not induced, thereby indicating a specific role for OsSUMO2 in ABA stress response. Among the E1 genes, *OsSAE1a* transcript levels increased after 6 hrs and E2 genes show a transient decrease followed by restoration to basal levels. Among the E3 ligases, *OsSIZ2* transcript levels were induced 2-folds in 24 hrs. These transcript expression analyses indicate that OsSIZ2 may be responsible for increased SUMOylation levels during ABA treatment and that the majority of rice proteins may be conjugated by OsSUMO2.

In summary, we demonstrated that rice responds to different abiotic stress treatments by accumulating SUMO conjugated proteins. This study also indicated that individual components of the SUMOylation system are regulated at the transcriptional level during stress conditions. Studies on the expression of individual SUMO components and the accumulation of SUMO conjugates in different tissues indicated that SUMOylation may play an important role in plant growth and reproductive

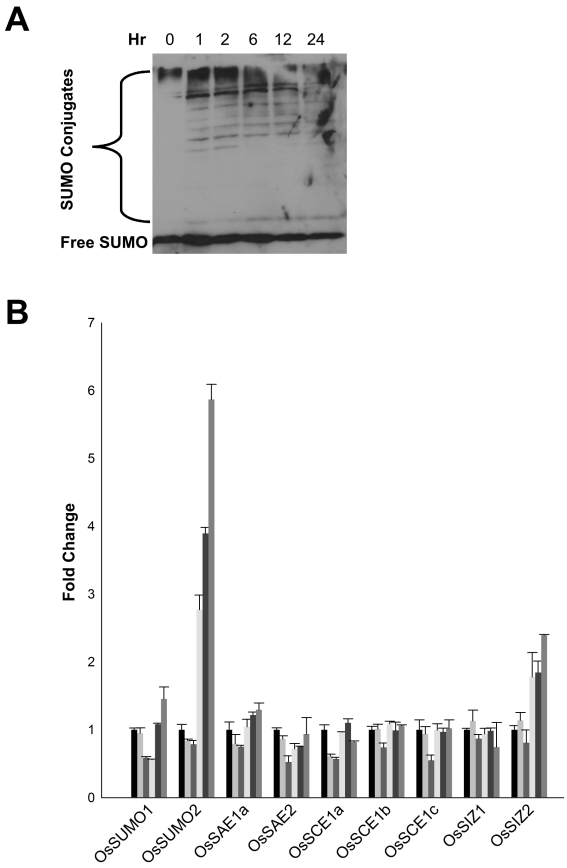

Fig. 4. ABA induces accumulation of SUMO conjugates and affects SUMO component gene expression. (A) SUMO conjugate levels from 1 week old seedling root tissue grown in tap water (0 hr) and after transferring to 50 mM ABA solution were determined by western blotting with 15 μg of total protein extracts. Note the accumulation of high molecular weight SUMO conjugates after transferring to ABA solution (B) Changes in mRNA levels of rice SUMOylation components were determined by quantitative real-time PCR analysis. Fold change was calculated as described for Fig. 2B. *OsSUMO2* and *OsSIZ2* exhibited five and two fold up-regulation in response to ABA.

development in rice.

MATERIALS AND METHODS

Sequence analysis

Rice SUMOylation system components have been previously identified (2). In this study, we obtained sequences for individual components from the Rice Annotation Project Database (RAP-DB) (http://rapdb.dna.affrc.go.jp/). Known protein/DNA sequences were used as search queries to BLAST against rice sequence databases to determine if the rice genome encodes additional SUMO, E1, E2 and E3 proteins. We were unable to identify any additional sequences through our database searching (data not shown). Arabidopsis sequences were obtained from TAIR (http://www.arabidopsis.org) and human and yeast sequences were obtained from the NCBI database. Sequences were aligned using ClustalW (24) and were searched for conserved domains using the Pfam database (25). Percentage identity among sequences was determined by using the Vector NTI® Software (Invitrogen, Carlsbad, CA, USA).

Plant materials and cDNA preparation

Rice seed sterilization, growth conditions in either growth chambers or glasshouses, stress treatments, RNA isolation and cDNA preparation were performed as previously described (26).

Real-time PCR analysis

Primers were designed using 3'UTR sequences for SUMO, E1, E2 and E3 genes to amplify 210 bp regions (Supplemental Table 1). 18SrRNA was employed as a control gene for normalization and gene specific primers were designed as previously described (26). All of the genes were initially amplified using young panicle cDNA as the template with GoTaq® Flexi DNA Polymerase (Promega, Madison, WI, USA) and cloned into the pGEMT- easy vector to generate standard curves. Plasmids were quantified using a NanoDropND-1000 (NanoDrop, Wilmington, DE, USA) and copy number was calculated based on molecular mass. Absolute standard curves were prepared using SYBR® Green (Applied Biosystems, Foster City, CA, USA) based upon the quantification of plasmid serial dilutions. Absolute quantities were calculated in each sample for 18SrRNA and all SUMO cascade genes. For the developmental series expression analysis, normalized values ($*10^6$) were shown as relative expression. For each stress treatment, fold change was calculated by calibrating the relative expression for each time point with the relative expression of the time zero sample.

Western blots

Proteins were isolated and quantified as previously described (26). The only exception was the inclusion of 2 mM N-ethylmaleimide into the SDS loading buffer (12) to prevent deSUMOylation during protein isolation and further storage. For stress treatments, 15 μg of total proteins were subjected to SDS-PAGE and transferred to nitrocellulose membranes according to standard protocols. For developmental SUMOylation profiles, 25 μg of total protein was used for western blot analysis. Membranes were probed with AtSUMO1 primary antibody (10) (Abcam, Cambridge, MA, USA) followed by incubation with an HRP conjugated secondary anti-rabbit antibody. Western blots were developed on CL-XPosure X-ray film (Pierce, Rockford, IL, USA) by employing the SuperSignal West Pico chemiluminescent substrate (Pierce, Rockford, IL, USA).

Acknowledgements

The authors thank Drs. Kenji Miura and Jed Doelling for helpful discussions and Dr. Jianbo Yao and his lab for assistance in Western blot analysis. West Virginia Agriculture and Forestry Experiment Station Scientific Article No. 3055.

REFERENCES

1. Zhao, J. (2007) Sumoylation regulates diverse biological processes. *Cell. Mol. Life Sci.* **64**, 3017-3033.
2. Miura, K., Jin, J. B. and Hasegawa, P. M. (2007) Sumoylation, a post-translational regulatory process in plants. *Curr. Opin. Plant. Biol.* **10**, 495-502.
3. Jin, J. B., Jin, Y. H., Lee, J., Miura, K., Yoo, C. Y., Kim, W. Y., Van Oosten, M., Hyun, Y., Somers, D. E., Lee, I., Yun, D. J., Bressan, R. A. and Hasegawa, P. M. (2008) The SUMO E3 ligase, AtSIZ1, regulates flowering by controlling a salicylic acid-mediated floral promotion pathway and through affects on FLC chromatin structure. *Plant. J.* **53**, 530-540.
4. Murtas, G., Reeves, P. H., Fu, Y. F., Bancroft, I., Dean, C. and Coupland, G. (2003) A nuclear protease required for flowering-time regulation in Arabidopsis reduces the abundance of SMALL UBIQUITIN-RELATED MODIFIER conjugates. *Plant. Cell.* **15**, 2308-2319.
5. Catala, R., Ouyang, J., Abreu, I. A., Hu, Y., Seo, H., Zhang, X. and Chua, N. H. (2007) The Arabidopsis E3 SUMO ligase SIZ1 regulates plant growth and drought responses. *Plant. Cell.* **19**, 2952-2966.
6. Saracco, S. A., Miller, M. J., Kurepa, J. and Vierstra, R. D. (2007) Genetic analysis of SUMOylation in Arabidopsis: conjugation of SUMO1 and SUMO2 to nuclear proteins is essential. *Plant. Physiol.* **145**, 119-134.
7. Yoo, C. Y., Miura, K., Jin, J. B., Lee, J., Park, H. C., Salt, D. E., Yun, D. J., Bressan, R. A. and Hasegawa, P. M. (2006) SIZ1 small ubiquitin-like modifier E3 ligase facilitates basal thermotolerance in Arabidopsis independent of salicylic acid. *Plant. Physiol.* **142**, 1548-1558.
8. Miura, K., Jin, J. B., Lee, J., Yoo, C. Y., Stirm, V., Miura, T., Ashworth, E. N., Bressan, R. A., Yun, D. J. and Hasegawa, P. M. (2007) SIZ1-mediated sumoylation of ICE1 controls CBF3/DREB1A expression and freezing tolerance in Arabidopsis. *Plant. Cell.* **19**, 1403-1414.
9. Downes, B. and Vierstra, R. D. (2005) Post-translational

regulation in plants employing a diverse set of polypeptide tags. *Biochem. Soc. Trans.* **33**, 393-399.
10. Kurepa, J., Walker, J. M., Smalle, J., Gosink, M. M., Davis, S. J., Durham, T. L., Sung, D. Y. and Vierstra, R. D. (2003) The small ubiquitin-like modifier (SUMO) protein modification system in Arabidopsis. Accumulation of SUMO1 and -2 conjugates is increased by stress. *J. Biol. Chem.* **278**, 6862-6872.
11. Conti, L., Price, G., O'Donnell, E., Schwessinger, B., Dominy, P. and Sadanandom, A. (2008) Small Ubiquitin-Like Modifier Proteases OVERLY TOLERANT TO SALT1 and -2 Regulate Salt Stress Responses in Arabidopsis. *Plant. Cell.* **20**, 2894-2908.
12. Lois, L. M., Lima, C. D. and Chua, N. H. (2003) Small ubiquitin-like modifier modulates abscisic acid signaling in Arabidopsis. *Plant. Cell.* **15**, 1347-1359.
13. Lee, J., Nam, J., Park, H. C., Na, G., Miura, K., Jin, J. B., Yoo, C. Y., Baek, D., Kim, D. H., Jeong, J. C., Kim, D., Lee, S. Y., Salt, D. E., Mengiste, T., Gong, Q., Ma, S., Bohnert, H. J., Kwak, S. S., Bressan, R. A., Hasegawa, P. M. and Yun, D. J. (2007) Salicylic acid-mediated innate immunity in Arabidopsis is regulated by SIZ1 SUMO E3 ligase. *Plant. J.* **49**, 79-90.
14. Roden, J., Eardley, L., Hotson, A., Cao, Y. and Mudgett, M. B. (2004) Characterization of the Xanthomonas AvrXv4 effector, a SUMO protease translocated into plant cells. *Mol. Plant. Microbe. Interact.* **17**, 633-643.
15. Castillo, A. G., Kong, L. J., Hanley-Bowdoin, L. and Bejarano, E. R. (2004) Interaction between a geminivirus replication protein and the plant sumoylation system. *J. Virol.* **78**, 2758-2769.
16. Liu, B. and Shuai, K. (2008) Regulation of the sumoylation system in gene expression. *Curr. Opin. Cell. Biol.* **20**, 288-293.
17. Bossis, G. and Melchior, F. (2006) SUMO: regulating the regulator. *Cell. Div.* **1**, 13.
18. Boggio, R., Passafaro, A. and Chiocca, S. (2007) Targeting SUMO E1 to ubiquitin ligases: a viral strategy to counteract sumoylation. *J. Biol. Chem.* **282**, 15376-15382.
19. Melchior, F., Schergaut, M. and Pichler, A. (2003) SUMO: ligases, isopeptidases and nuclear pores. *Trends. Biochem. Sci.* **28**, 612-618.
20. Palvimo, J. J. (2007) PIAS proteins as regulators of small ubiquitin-related modifier (SUMO) modifications and transcription. *Biochem. Soc. Trans.* **35**, 1405-1408.
21. Weissman, A. M. (2001) Themes and variations on ubiquitylation. *Nat. Rev. Mol. Cell. Biol.* **2**, 169-178.
22. Mahajan, S. and Tuteja, N. (2005) Cold, salinity and drought stresses: an overview. *Arch. Biochem. Biophys.* **444**, 139-158.
23. Xiong, L., Schumaker, K. S. and Zhu, J. K. (2002) Cell signaling during cold, drought, and salt stress. *Plant. Cell.* **14** *Suppl,* S165-183.
24. Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics.* **23**, 2947-2948.
25. Finn, R. D., Tate, J., Mistry, J., Coggill, P. C., Sammut, S. J., Hotz, H. R., Ceric, G., Forslund, K., Eddy, S. R., Sonnhammer, E. L. and Bateman, A. (2008) The Pfam protein families database. *Nucleic. Acids. Res.* **36**, D281-288.
26. Chaikam, V. and Karlson, D. (2008) Functional characterization of two cold shock domain proteins from Oryza sativa. *Plant. Cell. Environ.* **31**, 995-1006.