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SHORT COMMUNICATION

SALT OVERLY SENSITIVE 2 (SOS2) AND INTERACTING PARTNERS SOS3 AND ABSCISIC ACID–INSENSITIVE 2 (ABI2) PROMOTE RED-LIGHT-DEPENDENT GERMINATION AND SEEDLING DEETIOLATION IN *ARABIDOPSIS*

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Premise of research. Plants cope with complex environments throughout their life cycles. The perception and integration of information acquired from different environmental parameters are key to responding appropriately and securing plant survival. In *Arabidopsis thaliana*, SALT OVERLY SENSITIVE 2 (SOS2) and SOS3, its protein-interacting partner, have been described as central players in salt-stress responses, while another SOS2-interacting partner, ABSCISIC ACID (ABA)–INSENSITIVE 2 (ABI2), is involved in ABA signaling. SOS2 was also suggested to be involved in the photocontrol of seed germination, although its function in photomorphogenesis is not fully understood. Here we studied the role played by SOS2, SOS3, and ABI2 in light-dependent responses in *Arabidopsis* mutants. We assessed whether SOS2, SOS3, and ABI2 modulate light- and hormone-regulated seed germination and seedling deetiolation.

Methodology. We examined physiological responses in wild-type and SOS2, SOS3, and ABI2 knockout mutants of *A. thaliana* exposed to red light and explored whether these proteins modulate germination (by changing hormone sensitivity) and seedling deetiolation. In addition, we analyzed the presence of *cis*-regulatory elements (CREs) in their promoters and expression profiles from public microarrays to confirm their function in photomorphogenesis.

Pivotal results. Germination experiments demonstrate that SOS2, SOS3, and ABI2 are positive regulators of germination induced by red light. Differences in germination between the wild type and mutants are explained by changes in gibberellin sensitivity. Analysis of hypocotyl growth inhibition and cotyledon opening suggest that these components are also required for full seedling deetiolation under red light. Furthermore, identification of CREs in gene promoters and expression analysis matched the proposed role for these genes in both processes.

Conclusions. SOS2, SOS3, and ABI2 are known components of salt signaling, and here we demonstrate that they are also positive elements of light signaling by regulating seedling deetiolation and altering gibberellic acid sensitivity during germination. These results suggest that they act as integrators of different signaling pathways.

Keywords: Arabidopsis thaliana, light signaling, salt signaling, salt overly sensitive (SOS) pathway, cross talk.

Online enhancements: appendix table.

Introduction

Soil salinity is one of the environmental factors that affect crop productivity. Most crop species are salt sensitive, and their tolerance levels vary greatly (Flowers and Colmer 2008). Under salt stress, roots respond to maintain ion homeostasis through the salt overly sensitive (SOS) signaling pathway (Liu and Zhu 1998; Ishitani et al. 2000; Zhu 2000, 2003). Loss of SOS2, SOS3, and SOS1 proteins, elements in the salt-signaling pathway, results in different levels of NaCl hypersensitivity in *Arabidopsis*. SOS3 is a calcium-binding protein that functions as a cytosolic calcium sensor detecting Ca^{2+} oscillations triggered by excess Na^{+} (Ishitani et al. 2000; Gong et al. 2004). To a lesser extent, SOS3 can also be partially associated with cell membranes independently of *N*-myristoylation, a post-translational modification essential for salt tolerance (Ishitani et al. 2000). After Ca^{2+} binding, SOS3 interacts and activates the serine/threonine protein kinase SOS2 through the FISL mo-

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tif (Liu and Zhu 1998; Halfter et al. 2000; Liu et al. 2000; Ohta et al. 2003), recruiting SOS2 to the plasma membrane and leading to activation of SOS1, a Na^+/H^+ antiporter that causes extrusion of excessive Na^+ from the cytosol to the apoplast (Shi et al. 2000; Quintero et al. 2002, 2011).

Interestingly, SOS2 function relates to different signaling pathways. SOS2 has been shown to interact with components of the abscisic acid (ABA) signaling pathway, such as ABA-INSENSITIVE 2 (ABI2), a protein phosphatase 2C (Ohta et al. 2003). ABA may play a role in salt tolerance through the interaction between SOS2 and ABI2, since the dominant negative mutant *abi2-1* is more tolerant to salt shock and survives in up to 150 mM NaCl (Ohta et al. 2003). However, the role played by ABA in salt tolerance is not clear, as loss-of-function mutant alleles, such as *abi2-2*, or revertant alleles of *abi2-1* have not been tested under the same conditions (Merlot et al. 2001; Rubio et al. 2009). By yeast two-hybrid assays, it was demonstrated that ABI2 binds to SOS2 through a protein phosphatase interaction (PPI) motif, suggesting a tight regulation between the phosphatase and kinase activities of these proteins (Ohta et al. 2003). Furthermore, both enzimatic activities may be simultaneously regulated by the proximity of SOS3. Mutations in conserved amino acid residues in the PPI motif abolish the interaction between SOS2 and ABI2. In contrast, a mutation in the FISL motif disrupts the interaction between SOS2 and SOS3 but has no effect on the interaction between SOS2 and ABI2 (Ohta et al. 2003), suggesting that ABI2 can bind to SOS2 independently of SOS3. The increased salt tolerance and ABA insensitivity in the dominant *abi2-1* is explained because the mutation disrupted the interaction between SOS2 and ABI2 (Leung et al. 1997; Quintero et al. 2002; Ohta et al. 2003). Interestingly, *sos2* and *sos3* mutants are not defective in ABA responses (Liu and Zhu 1998; Zhu et al. 1998; Chinnusamy et al. 2004), highlighting the importance of the interactions between SOS2 and certain partners for the integration of endogenous and environmental signals.

In addition, there is some evidence demonstrating the connection between salt stress and light signaling pathways. The SOS2-interacting protein, NUCLEOSIDE DIPHOSPHATE KINASE 2 (NDPK2), physically interacts with phytochrome A, promoting seedling photomorphogenesis (Choi et al. 1999), and the interaction between NDPK2 and SOS2 contributes positively, inducing germination under salt stress (Verslues et al. 2007). Interestingly, in a microarray study of tomato seeds the expression of *SOS2* was downregulated when germination was inhibited by continuous far-red light, and a subsequent pulse of red light upregulated the expression of this gene (Auge et al. 2009). Another point of connection between salt stress and light-signaling pathways is the physical interaction between SOS2 and GIGANTEA, a nuclear protein involved in the promotion of flowering, that prevents SOS2-based activation of SOS1 under normal growth conditions but is degraded in response to salt stress (Kim et al. 2013; Park et al. 2013; Mishra and Panigrahi 2015).

Germination is a process highly regulated by environmental cues. Seeds integrate information from various ambient factors that translate into signals conveyed by plant hormones, such as gibberellic acid (GA) and ABA, to promote or inhibit germination (Finch-Savage and Leubner-Metzger 2006). In *Arabidopsis*, phytochrome B (phyB) is the main photoreceptor sensing the red light (600–700 nm) to far-red light (700–800 nm) ratio of the light spectrum that promotes seed germination and seedling deetiolation under red light. In laboratory conditions, germination is maximal when a saturating red light pulse establishes high levels of the active form of the phytochrome (Pfr) and is reversed by a subsequent far-red light pulse (Shinomura et al. 1994; Botto et al. 1995; Botto et al. 1996). To promote germination, light perceived by the phytochromes stimulates GA biosynthesis and signaling and represses GA catabolism. Phytochromes can also reduce ABA biosynthesis and signaling and promote ABA catabolism in the seeds (Yamaguchi et al. 1998; Nakabayashi et al. 2005; Seo et al. 2006; Oh et al. 2007; Yamauchi et al. 2007; Xu et al. 2014). All of these changes are mainly produced by the degradation of the key negative regulator PHYTOCHROME-INTERACTING FACTOR 3–LIKE 5 (PIL5) in a phyB-dependent manner (Oh et al. 2007, 2009). Although some ABA and salt-signaling proteins have been explored in photomorphogenesis, the functional roles played by ABI2 and SOS3 in light responses have not been adequately characterized.

Here we studied the role played by SOS2, SOS3, and ABI2 in seed germination and seedling photomorphogenic development and demonstrated that SOS2 and interacting proteins are positive regulators of photomorphogenesis under red light. Interestingly, they exert this promotion by partially increasing GA sensitivity in the seed tissues.

Material and Methods

Plants and Growth Conditions

Arabidopsis thaliana Columbia wild-type (WT) and knockout mutant lines *sos2-1* (Liu et al. 2000), *sos3-1* (Halfter et al. 2000), and *abi2-2* (SALK_015166; Rubio et al. 2009) were grown in a temperate greenhouse with a natural photoperiod and 500 μmol m^{-2} s^{-1} of maximal irradiation at an average temperature of 20°C. Plants were grown together, and mature seeds were harvested on the same day. Seeds of three different plants of each genotype were collected, bulked, and stored in dry conditions in darkness at room temperature for 2 wk, followed by storage at 5°C until they were used for experiments.

Germination Experiments

Twenty-five seeds of each genotype were sown in triplicate clear plastic boxes containing 4 mL of 0.8% (w/v) agar. To set minimum and identical Pfr levels, soaked seeds were incubated for 1 h in darkness and then irradiated with a 30-min saturating long-wavelength far-red light pulse provided by an RG9 filter at room temperature (22° ± 2°C). After the far-red light pulse, the germination treatments were as follows.

Red light promoting pulse. To test the response to a germination-promoting red light pulse, seeds were either immediately exposed to a 30-min red light pulse or kept in darkness.

Cold stratification. To assess the effect of dormancy breakage by cold stratification, seeds were incubated for 0, 1, 2, 3, or 7 d at 5°C in the dark and then kept in darkness or irradiated with a red light pulse.

ABA sensitivity. To test the sensitivity to exogenously applied ABA, seeds were incubated in 0.5 or 1 μM ABA solution

(Sigma-Aldrich, St. Louis, MO) for 7 d at 5°C in the dark and then irradiated with a red light pulse. To test the effect of the ABA synthesis inhibitor fluridone (Sigma-Aldrich), seeds were incubated in 5 or 50 μM fluridone solution for 24 h at 25°C in the dark until germination was induced by a red light pulse.

GA sensitivity. To test the sensitivity to exogenously applied GA, seeds were incubated with paclobutrazol (PAC; 10 μM; Syngenta, Basel, Switzerland) to reduce endogenous GA synthesis and 10 or 100 μM GA_3 (Sigma-Aldrich) for 24 h at 4°C in darkness followed by a red light treatment.

For all of the experiments described above, seeds were transferred to darkness for 3 d at 25°C after the final light treatments until germination counting (total number of seeds showing radicle protrusion). Results are mean values from at least three independent experiments.

Deetiolation Experiments

Twenty seeds of each genotype were incubated for 1 h in darkness in clear plastic boxes containing 4 mL of 0.8% (w/v) agar, exposed to a saturating 30-min far-red light pulse to reduce Pfr levels, and then incubated in darkness for 3 d at 4°C to homogenize germination. Germination was induced by 1 h of red light followed by 23 h in the dark, and then seedlings were transferred to different light treatments for 3 d at 25°C with the exception of the dark controls, which were kept in darkness. Hypocotyl length and the angle between cotyledons were measured in the 12 tallest seedlings per box with a ruler and a protractor, respectively. The hypocotyl length of seedlings exposed to light was expressed relative to the hypocotyl length of the dark controls. Results are mean values from four independent experiments of at least three replicates each.

Light Sources

Long-wavelength far-red light established a calculated Pfr/P of 0.03 (30 μmol m^{-2} s^{-1}), provided by an incandescent lamp in combination with a water filter and an RG9 filter (Schott, Maintz, Germany). Red light established a calculated Pfr/P of 0.87 (30 μmol m^{-1} s^{-1}), provided by light-emitting diodes (LEDs) with a light-emitting maximum set at 660 nm (Cavadevices, Buenos Aires, Argentina). Red and far-red light were measured with a Skye SKR 110 sensor (Skye Instruments, Llandrindod Wells, United Kingdom), and values were corrected by a factor that accounts for the sensor-limited sensitivity in that wavelength range.

Identification of cis*-Regulatory Elements (CREs) and Expression Meta-analysis*

To identify *cis* elements in *SOS2*, *SOS3*, and *ABI2* promoters, the AthaMap database was used to analyze the 2-kb (−2000-bp) promoter sequence of each gene (Steffens et al. 2004; http://www.athamap.de). The total strand distribution of putative CREs was the sum of the individual CRE numbers in each promoter. Statistically overrepresented elements (overrepresentation ratio = occurrence/theoretical) were calculated from an expectation value for each element. The tool enabled identification and selection according to a set threshold score of common transcription factor binding sites that provide information about the *cis* elements. *Arabidopsis* genome identifier codes of the putative transcription factors were used to investigate their functions in the literature. Expression of *SOS2*, *SOS3*, and *ABI2* genes throughout the *Arabidopsis* plant was based on data from the eFP Browser microarray database (Winter et al. 2007). Expression of the three genes was relativized to the reference gene At4g34270 (TIP41-like protein) and visualized from the online repositories.

Results

SOS2, SOS3, *and* ABI2 *Promote Germination by Red Light Pulse*

Previous observations suggest a possible point of convergence between light and salt-signaling pathways through *SOS2* (Auge et al. 2009). To study further this signaling connection, we explored the germination response to red light of an SOS2 mutant, *sos2-1*, and known mutant alleles of SOS2-interacting proteins, *sos3-1* and *abi2-2* (hereafter, *sos2*, *sos3*, and *abi2*, respectively).

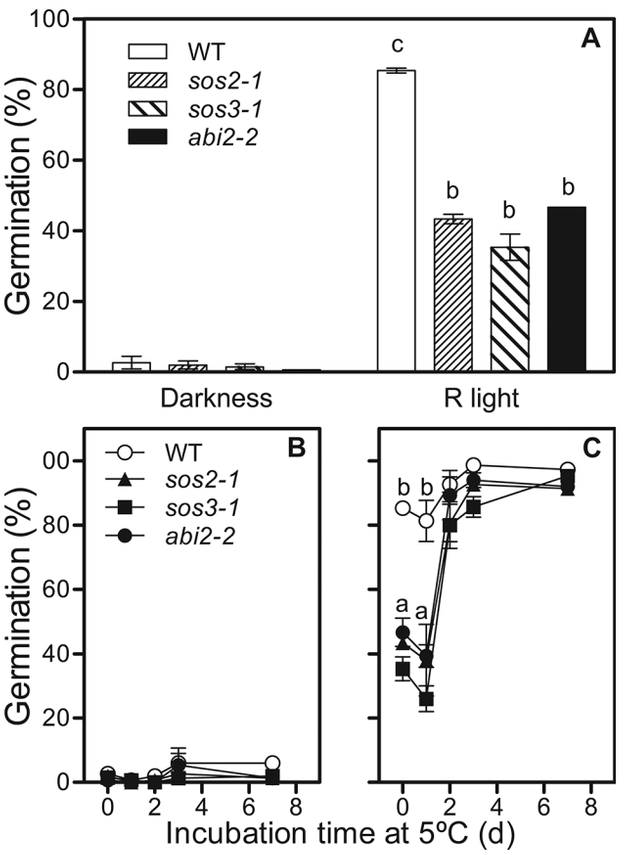

Fig. 1 Germination responses of *sos2*, *sos3*, and *abi2* mutant seeds. *A*, Nonstratified seeds kept in darkness or irradiated with a red light pulse. *B*, *C*, Cold-stratified seeds kept in darkness (*B*) or after a red light pulse (*C*). Different letters indicate significant differences among means, determined using the Bonferroni posttest ($P < 0.005$). WT = wild type.

Without stratification, all of the genotypes showed negligible germination in darkness, indicating a strong light requirement for germination (fig. 1*A*). A red light pulse strongly promoted germination in the WT, while the *sos2*, *sos3*, and *abi2* mutants showed a consistently lower germination response (genotype × red light treatment interaction [$F = 46.52$, $P < 0.0001$] is significant; fig. 1*A*). These results suggest that SOS2, SOS3, and ABI2 are positive regulators of light-induced seed germination.

Since the differential response could be caused by differences in dormancy levels between the WT and the mutants, we explored the effect of cold stratification and light on dormancy breaking. Darkness incubation did not promote germination, and the response was independent of the incubation time at 5°C, showing again a strong light requirement independently of the cold period (fig. 1*B*, 1*C*). Two or more days of cold treatment before the perception of a red light pulse were enough to reduce the dormancy levels of mutant seeds and dissipate phenotypic differences (genotype × cold treatment interaction [$F = 5.91$, $P < 0.0001$] is significant; fig. 1*C*).

SOS2, SOS3, *and* ABI2 *Increase Gibberellin Sensitivity in Seeds*

The reduced germination observed in the mutants (fig. 1*A*) could be caused by changes in sensitivity and/or endogenous levels of ABA and/or GA. Since the sensitivity to red light signals is influenced in mutant seeds by their degree of dormancy (fig. 1*C*), we first tested the response to exogenous application of ABA and fluridone (an inhibitor of ABA biosynthesis) after a germination-inducing red light pulse. Increasing ABA concentrations reduced germination in cold-stratified seeds in all of the genotypes (genotype [$F = 0.62$, $P = 0.61$] and genotype × ABA interaction [$F = 0.28$, $P = 0.94$] are not significant, while ABA [$F = 422.38$, $P < 0.0001$] is significant; fig. 2*A*). Furthermore, mutant seeds that were not cold stratified maintained their differences compared with the WT when increasing doses of fluridone were applied (fluridone [$F = 2.86$, $P = 0.07$] and genotype × fluridone interaction [$F = 0.48$, $P = 0.82$] are not significant, while genotype [$F = 13.27$, $P < 0.0001$] is significant; fig. 2*B*). These results suggest that these genes do not regulate the sensitivity to this hormone.

To investigate whether the reduced germination response of mutant genotypes under red light is due to a lower sensitivity to GA, we blocked endogenous biosynthesis with PAC and incubated the seeds in different concentrations of exogenous GA. Germination in darkness was low without or with a low concentration of GA and was induced with 100 μM GA, although no differences between genotypes were observed (genotype [$F = 0.06$, $P = 0.98$] and genotype × GA treatment interaction [$F = 0.07$, $P = 0.99$] are not significant, while GA treatment [$F = 50.54$, $P < 0.0001$] is significant; fig. 2*C*). Red light promoted germination when seeds were incubated in 10 and 100 μM GA compared with the dark control (fig. 2*D*), showing that red light promotes germination by changing the sensitivity to GA. Seeds of *sos2*, *sos3*, and *abi2* showed smaller germination values than WT in 10 μM GA, but germination differences between genotypes disappeared when seeds were incubated in 100 μM GA (genotype × GA treatment interaction [$F = 3.10$, $P = 0.008$] is significant; fig. 2*D*). Altogether, the results show that phenotypic differences in germination observed in mutant seeds correlate with reduced sensitivity to GA.

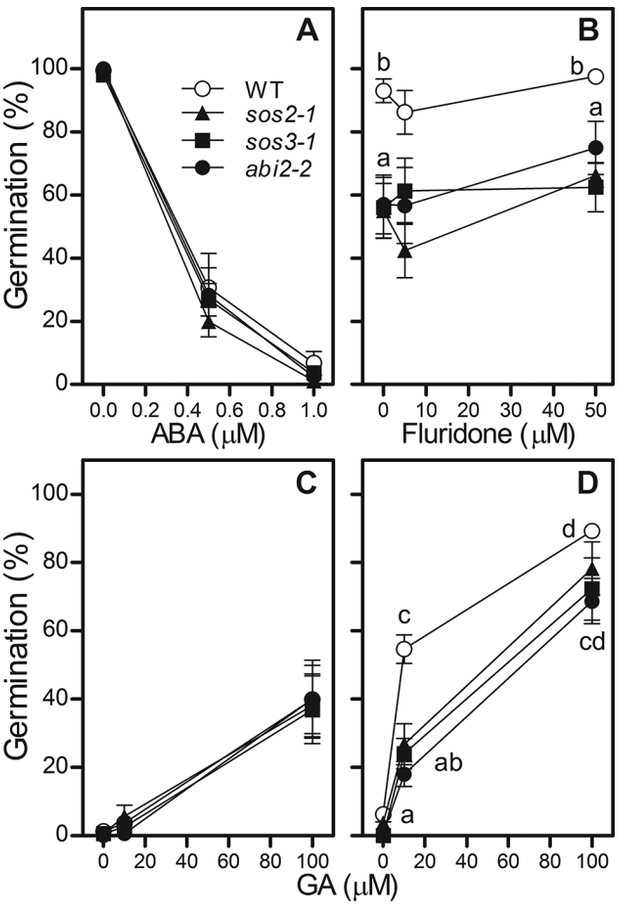

Fig. 2 *sos2*, *sos3*, and *abi2* seeds have normal germination sensitivity to exogenous abscisic acid (ABA) but are hyposensitive to exogenous gibberellic acid (GA) under red light. *A*, ABA dose-response curve of cold-stratified seeds exposed to a red light pulse. *B*, Fluridone dose response of seeds exposed to a red light pulse. *C*, *D*, Dose-response GA curves (plus paclobutrazol) for seeds kept in darkness (*C*) or exposed to a red light pulse (*D*). Different letters denote significant differences among means, determined using the Bonferroni posttest ($P < 0.005$). WT = wild type.

SOS2, SOS3, *and* ABI2 *Are Required for Seedling Deetiolation Responses in Continuous Red Light*

To investigate possible roles played by these genes in deetiolation responses, we cultivated seedlings under different irradiance levels of continuous red light. Increasing fluence rates of continuous red light reduced the relative hypocotyl elongation compared with that of the darkness control, reaching a maximum hypocotyl length inhibition in seedlings grown at 100 μmol m^{-2} s^{-1} (WT = 40% of hypocotyl inhibition; fig. 3*A*). The *sos2* and *sos3* mutants were hyposensitive to continuous red light and displayed significantly longer hypocotyls than the WT at higher irradiances; however, *abi2* seedlings showed a phenotype similar to that of the WT (genotype × R fluence rate

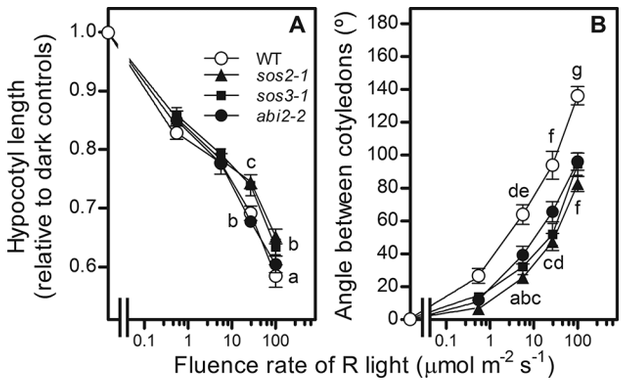

Fig. 3 Seedling deetiolation is reduced in *sos2*, *sos3*, and *abi2* mutants. *A*, Fluence rate response of hypocotyl length. *B*, Cotyledon opening under continuous red light. Different letters denote significant differences among means, determined using the Bonferroni posttest ($P < 0.005$). WT = wild type.

interaction [$F = 1.44$, $P = 0.18$] is not significant, while genotype [$F = 8.78$, $P < 0.0001$] and R fluence rate [$F = 220.63$, $P < 0.0001$] separately are significant; fig. 3*A*). Moreover, seedlings of the three mutant genotypes showed a clear reduction in cotyledon opening under intermediate and high irradiances (genotype × R fluence rate interaction [$F = 1.83$, $P = 0.07$] is not significant, while genotype [$F = 45.19$, $P < 0.0001$] and R fluence rate [$F = 191.53$, $P < 0.0001$] separately are significant; fig. 3*B*). These results suggest that some components of the SOS signaling pathway have overlapping but also different functions in the promotion of seedling photomorphogenesis.

SOS2, SOS3, and ABI2 Are Transcriptionally Regulated in Germination and Seedling Deetiolation

To gain insight into the transcriptional regulation of *SOS2*, *SOS3*, and *ABI2* genes, we analyzed the presence of CREs in their promoters and gene expression from a public microarray database. The analysis of CREs in *SOS2*, *SOS3*, and *ABI2* promoters revealed that the three genes share many *cis* elements, suggesting similar features of transcriptional regulation. Thirty-three different CREs related to 17 different transcription factor families were found in all three promoters. Twenty-one CREs were found within different combinations of two gene promoters, and ten, eight, and five CREs were specific to *SOS2*, *SOS3*, and *ABI2* promoters, respectively (see table A1, available online). The CREs identified in the three gene promoters are associated with many developmental processes, such as leaf senescence, organ plant development (leaf, flower, seed, vasculature, pollen, and gynoecium), lateral root formation, and cell wall biogenesis. The CREs are also associated with salt responses and other stresses (table 1). We also found binding sites on the *SOS2*, *SOS3*, and *ABI2* promoters associated with ABA and GA regulation. In addition, many CREs were associated with transcription factors involved in seed development, seed germination, seedling deetiolation, and light response (table 1).

Arabidopsis eFP Browser data showed that *SOS2*, *SOS3*, and *ABI2* are expressed in a broad range of plant tissues and organs, including senescent leaves, floral organs, late stages of seed development, root elongation zone, guard cells, and pollen development. In addition, *SOS2*, *SOS3*, and *ABI2* expression is upregulated in biosynthetic *ga1-3* mutant seeds imbibed in water and exogenous GA, and *SOS2* and *ABI2* expression is also upregulated by light in leaves and seedlings grown under light/dark cycles. Furthermore, *SOS2* expression is upregulated in seedlings exposed to different light qualities.

Discussion

In this article, we demonstrated that *Arabidopsis thaliana* SOS2 and two of its known interacting partners, SOS3 and ABI2, are positive regulators in the red light pathways that control germination and seedling deetiolation. These results uncover new functions for these genes in addition to the well-established function in salt signaling, suggesting that they play broader roles in plant development.

Under our conditions, *sos2*, *sos3*, and *abi2* mutants showed a reduced germination response after red light irradiation (fig. 1), indicating that SOS2, SOS3, and ABI2 are positive regulators of the red light–mediated germination pathway. Light promotes seed germination by modifying the GA/ABA balance and sensitivity. On red light exposure, phytochromes reduce ABA levels and increase GA synthesis through degradation of the negative regulator PIL5 (Oh et al. 2007, 2009). Light can also repress ABA signaling through downregulation of *ABI3* and *ABI5* (Nakabayashi et al. 2005; Xu et al. 2014). Under our conditions, we did not see changes in ABA sensitivity in the mutants, suggesting that SOS2, SOS3, and ABI2 do not regulate the germination response to red light by affecting the sensitivity to ABA levels or its signaling pathway. The normal ABA sensitivity is consistent with previous reports demonstrating that *sos2* and *sos3* loss-of-function mutants are specifically defective in salt tolerance but not ABA responses (Liu and Zhu 1998; Zhu et al. 1998; Chinnusamy et al. 2004), while loss-of-function *abi2* mutants displayed normal germination and normal ABA sensitivity for germination in stratified and nonstratified seeds incubated under white light (Leung et al. 1997; Merlot et al. 2001; Rubio et al. 2009).

In imbibed *Arabidopsis* seeds, light and cold treatments stimulate the synthesis and perception of GA, leading to germination (Derkx et al. 1994; Ogawa et al. 2003; Yamauchi et al. 2004; Penfield et al. 2005). phyB is the major phytochrome that regulates sensitivity to exogenous GA for red light–induced germination (Finch-Savage and Leubner-Metzger 2006; Oh et al. 2007; Arana et al. 2014). Our results show that mutants had lower sensitivity to GA after red light exposure; therefore, the lower germination observed in mutants could be explained by reduced sensitivity to GA, reduced red light–induced GA synthesis, or a combination of both (figs. 1, 2). In addition, differences in germination between the WT and mutants disappeared within two or more days of cold stratification and under a high concentration of GA. Interestingly, many CREs identified in the promoters of *SOS2*, *SOS3*, and *ABI2* are binding sites of GA signaling–associated transcription factors, including DOF-AFFECTING GERMINATION 2 (DAG2) and SQUAMOSA PROMOTER BINDING PROTEIN-LIKE 8 (AtSPL8; see table 1). In accordance with this, *dag2* mutant seeds have lower rates of light-induced germination under white light than the WT, probably due to a reduced sensitivity to GA and normal

Table 1

Putative *cis* Elements and Transcription Factors (TFs) Shared between *SOS2*, *SOS3*, and *ABI2* Promoters

TF	TF family	Overrepresentation ratio	Response
DEAR3(1)	AP2/EREBP	1.02	Drought and cold responses
TEIL	AP2/EREBP	1.32	Ethylene response; sulphur deficiency response
TOE2(3)	AP2/EREBP	1.7	Flowering time regulation
WRI1	AP2/EREBP	1.46	Lipid storage during seed maturation
ETT(2)	ARF	2.52	Flower development; auxin response
AHL12(1)	AT-Hook	1.49	Light-regulated hypocotyl growth
AHL12(2)	AT-Hook	2.02	Light-regulated hypocotyl growth
AHL12(3)	AT-Hook	1.73	Light-regulated hypocotyl growth
AHL20(1)	AT-Hook	1.7	Defense response; light-regulated hypocotyl growth
AHL20(2)	AT-Hook	1.84	Defense response; light-regulated hypocotyl growth
AHL20(3)	AT-Hook	2.03	Defense response; light-regulated hypocotyl growth
AHL25(1)	AT-Hook	1.68	Negative regulation of gibberellic acid signaling pathway; light-regulated hypocotyl growth
AHL25(2)	AT-Hook	1.83	Negative regulation of gibberellic acid signaling pathway; light-regulated hypocotyl growth
AHL25(3)	AT-Hook	1.85	Negative regulation of gibberellic acid signaling pathway; light-regulated hypocotyl growth
ID1	C2H2(Zn)	1.11	Flowering time regulation
ZAT14	C2H2(Zn)	1.03	Osmotic and salt response?
DAG2	C2C2(Zn)Dof	1.2	Seed germination; gibberellin response; light response; cold response
GATA12	C2C2(Zn)GATA	1.14	Seed germination; gibberellin response; light response; flowering time regulation
YAB1	C2C2(Zn)YABBY	1.27	Gibberellin response; leaf development; flower development; abaxial cell fate
YAB5	C2C2(Zn)YABBY	1.04	Leaf development; flower development; abaxial cell fate
ARR11(3)	GARP/ARR-B	1.11	Cytokinin response
AGP1	GATA	1.25	Defense response
WOX13(2)	HD-HOX	1.54	Fruit development
ATHB1	HD-ZIP	2.04	Light-regulated hypocotyl growth; leaf development
ATHB51	HD-ZIP	2.09	Leaf development; organ identity
HAHB4	HD-ZIP	2.35	Drought and salt response; ABA, ethylene, and jasmonic acid response; defense response
ICU4	HD-ZIP	1.27	Vascular development; shoot organogenesis; embryo development
LBD16	LOB/AS2	1.53	Lateral root formation; auxin response
AGL2	MADS	1.75	Flower organ identity
AGL15	MADS	1.25	Seed maturation; gibberellin response; flowering time regulation; flower senescence
MYB46(1)	MYB	1.23	Defense response; cell wall biogenesis
MYB52(1)	MYB	1.17	Drought response; ABA response; cell wall biogenesis
MYB52(2)	MYB	1.67	Drought response; ABA response; cell wall biogenesis
MYB83	MYB	1.23	Cell wall biogenesis
RVE1(1)	MYB	2.3	Cold response; auxin and circadian growth regulation
RVE1(2)	MYB	1.2	Cold response; auxin and circadian growth regulation
ANAC46	NAC	1.2	na
ANAC55(1)	NAC	3.31	Defense response: jasmonic acid and salicylic acid response; leaf senescence
ANAC55(2)	NAC	3.27	Defense response; jasmonic acid and salicylic acid response; leaf senescence
ANAC58	NAC	2.34	na
AtSPL8	SBP	1.98	Pollen and gynoecium development; auxin response; seed germination and root elongation modulated by gibberellin
GT1	Trihelix	1.72	Light, salt, and defense regulation; flower development; seed/embryo maturation

Note. Analysis of overrepresentation ratio (occurrence/theoretical) was made simultaneously with the three genes; *cis*-regulatory elements (CREs) with overall ratios higher than 1 are showed. ABA = abscisic acid; na, not applicable.

sensitivity to ABA (Gualberti et al. 2002). In addition, null *spl8* mutants showed reduced germination inhibition after exposure to PAC (Zhang et al. 2007). These observations, together with the reduced sensitivity to GA in *sos2*, *sos3*, and *abi2* (fig. 2) and the GA induction of *SOS2*, *SOS3*, and *ABI2* expression during imbibition (Ogawa et al. 2003), support a functional role for these regulators in the promotion of germination through GA signaling.

phyB is the main photoreceptor implicated in the inhibition of hypocotyl growth and cotyledon opening under red light (Reed et al. 1993; Quail et al. 1995). Mutants of *SOS2*, *SOS3*, and *ABI2* showed reduced deetiolation compared with the WT, and several CREs on *SOS2*, *SOS3*, and *ABI2* promoters are binding sites for transcription factors related to light and hormone responses, suggesting that these genes are involved as positive regulators of seedling photomorphogenesis. AT-HOOK MOTIF CONTAINING NUCLEAR LOCALIZED (AHL12, AHL20, AHL25), ARABIDOPSIS THALIANA HOMEOBOX 1 (ATHB1), and GT1, a trihelix transcription factor, are some of the proteins that can likely bind to the promoters of *SOS2*, *SOS3*, and *ABI2*, regulating their expression. Interestingly, AHL29 and other AHL transcription factors act redundantly, repressing hypocotyl growth under white light (Zhao et al. 2013). In addition, AHL25 is involved in gibberellin homeostasis through transcriptional activation of the biosynthetic *AtGA3ox1* gene (Matsushita et al. 2007), suggesting that AHL25 regulates plant development by affecting GA metabolism. Furthermore, GT1 transcription factor binds to GT elements on the promoters of light-induced genes (Le Gourrierec et al. 2002; Kaplan-Levy et al. 2012). On the other hand, light can exert its function by interfering with the PIF1-ATHB1 signaling pathway, promoting photomorphogenesis (Capella et al. 2015).

We have shown that SOS2, SOS3, and ABI2 promote photomorphogenesis, and this is in apparent contradiction with the more sensitive phenotype of *sos2* and *sos3* mutants (Zhu et al. 1998; Shi et al. 2000; Quintero et al. 2002) and the more tolerant phenotype of *abi2-1* when *Arabidopsis* seedlings grow in a saline medium (Ohta et al. 2003). These physiological results, together with yeast two-hybrid protein interaction assays, suggest that SOS3 acts as a positive regulator of SOS2 while ABI2 is a negative effector of SOS2 activity under stress conditions. We think that our results are in line with an increasing body of literature demonstrating that some proteins can be involved in different signaling pathways (Gangappa and Botto 2014; de Montaigu et al. 2015; Chen et al. 2016). In this context, the function of the protein in different signaling pathways depends on interactions with other proteins necessary for mediating a particular physiological process. One nice example is GIGANTEA, which is involved in photomorphogenesis and the circadian system in plants. GIGANTEA participates as a circadian component involved in the evening loop promoting flowering under long days and increasing CO and FLOWERING LOCUS T (FT) messenger RNA abundance (Fowler et al. 1999; Mizoguchi et al. 2005). GIGANTEA also has activity in photomorphogenesis (Oliverio et al. 2007; de Montaigu et al. 2015). Coupland and collaborators demonstrated that GIGANTEA allelic variants affect temporal patterns of gene expression in response to light independently of circadian control (de Montaigu et al. 2015). They suggest that this alternative mechanism to control gene expression provides an advantage by adjusting the activity of temporally regulated processes, avoiding the pleiotropic effects associated with severe disruptions of the circadian system (de Montaigu et al. 2015). Using the same rationale, we hypothesize that SOS2, SOS3, and ABI2 promote photomorphogenesis through an unknown molecular mechanism independently of the stress saline signaling pathway in which ABI2 acts, inhibiting SOS2 and SOS3 activity. The relationship between these genes and the molecular mechanism of action of these proteins in the promotion of photomophogenesis needs to be elucidated via the design of additional genetic and molecular experiments.

We have demonstrated here that SOS2, SOS3, and ABI2 are key players in seed germination and early plant development when seedlings are grow under red light. SOS2 has been previously shown to be involved in salt, reactive oxygen species, and flowering signaling pathways (Verslues et al. 2007; Ji et al. 2013; Kim et al. 2013; Park et al. 2013), suggesting that SOS2 and its interacting proteins, SOS3 and ABI2, can act as a point of cross talk between different environmental cues. The diversity of protein-protein interactions between them and other proteins may be a common molecular mechanism to properly decode into the cell the surrounding plant conditions. In accordance with this, we speculate that these molecular elements can form a dynamic complex with other proteins. The protein complex would change depending on the physiological context, sequestering or releasing interacting partners to dynamically decipher environmental signals and to finely adjust plant growth.

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