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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*

Santiago A. Trupkin,[1,2,*] Gabriela A. Auge,[1,3,*] Jian-Kang Zhu,† Rodolfo A. Sánchez,* and Javier F. Botto[4,*]

*Universidad de Buenos Aires, Facultad de Agronomía, Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina; and †Shanghai Center for Plant Stress Biology, Chinese Academy of Sciences, Shanghai, People's Republic of China; and Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, Indiana 47907, USA

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q1 *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 q2 SOS3, its protein-protein interacting partner, have been described as central players in salt-stress responses, while q3 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. 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 q4 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 q5 et al. 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- q6

[1] These authors contributed equally to this work.

[2] Current address: Instituto de Floricultura, Centro de Investigación de Recursos Naturales, Instituto Nacional de Tecnología Agropecuaria, Nicolás Repetto y de los Reseros, Hurlingham, Buenos Aires, Argentina.

[3] Current address: Fundación Instituto Leloir, Instituto de Investigeciones Bioquimicas de Buenos Aires (IIBBA), CONICET, Buenos Aires, Argentina.

[4] Author for correspondence; e-mail: botto@agro.uba.ar.

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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 q7 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 q8 partners to the integration of the information and the response to cues of diverse origin.

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*, q9 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 catab- q10 olism 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- q11 INTERACTING FACTOR3–LIKE5 (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 $\mu mol\ m^{-2}\ s^{-1}$ 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 gibberellin (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
q12 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

q13 Long-wavelength far-red light established a calculated Pfr/P of 3% (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
q14 of 87% (30 μmol $m^{-2}s^{-1}$), provided by a light-emitting diode with a light-emitting maximum set at 660 nm (Cavadevices, Buenos Aires, Argentina). Red light was measured with a Li-COR LI-188B sensor (Lincoln, NE). Far-red light was 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,
q15 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). 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 pro-

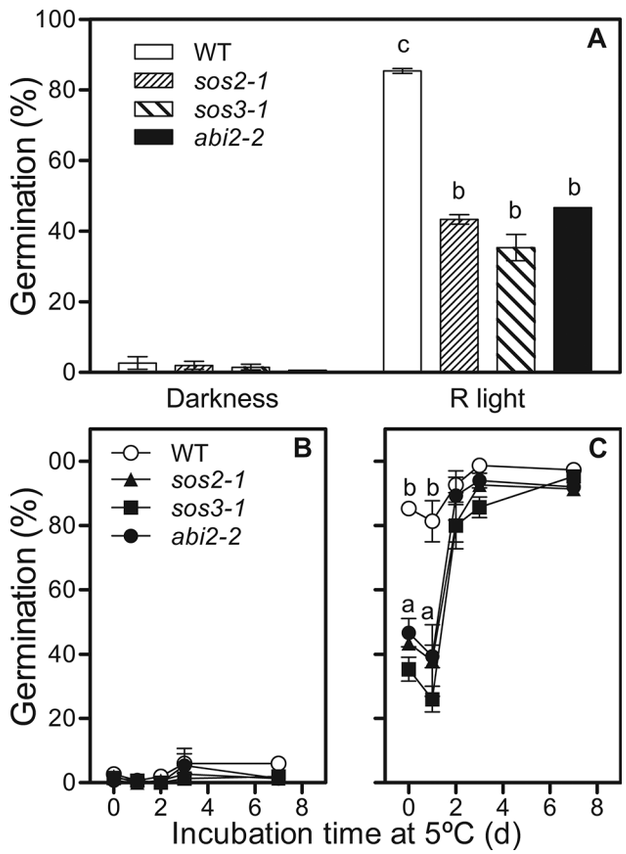

Fig. 1 Germination responses of *sos2*, *sos3*, and *abi2* mutant seeds. *A*, Nonstratified seeds kept in darkness or irradiated with a red light pulse. *B*, *C*, Cold-stratified seeds kept in darkness (*B*) or after a red light pulse (*C*). Different letters indicate significant differences among means,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

moted 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, q16 we explored the effect on dormancy breaking by cold stratification and light. 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*). 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 treat- q17 ment [$F = 50.54$, $P < 0.0001$] 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 sensi- q18 tivity to GA. Seeds of *sos2*, *sos3*, and *abi2* germinated less than the 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.

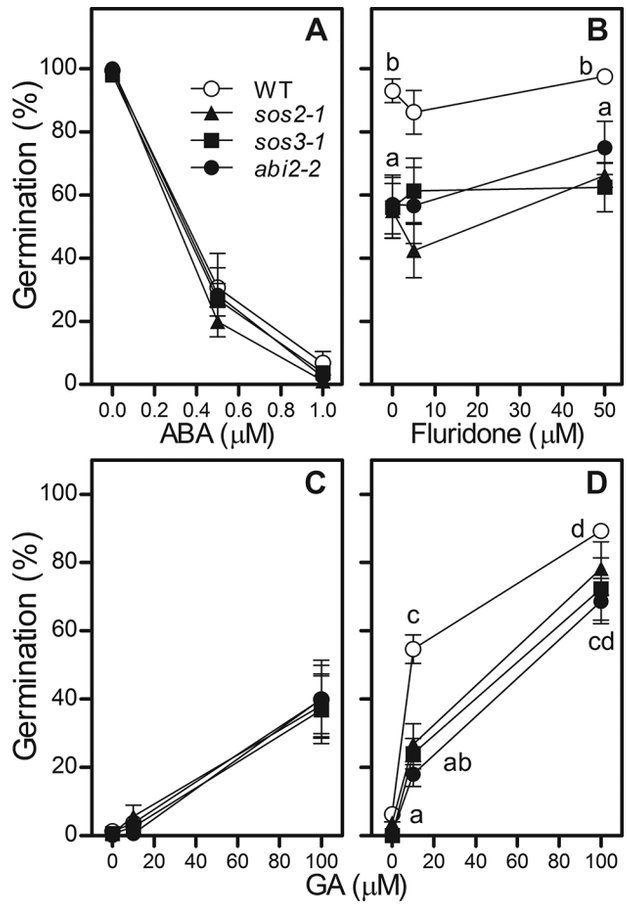

Fig. 2 *sos2*, *sos3*, and *abi2* seeds have normal germination sensitivity to exogenous abscisic acid (ABA) but are hyposensitive to exogenous gibberellic acid (GA) under red light. *A*, ABA dose-response curve of cold-stratified seeds exposed to a red light pulse. *B*, Fluridone dose response of seeds 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 q19 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*). q20 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 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

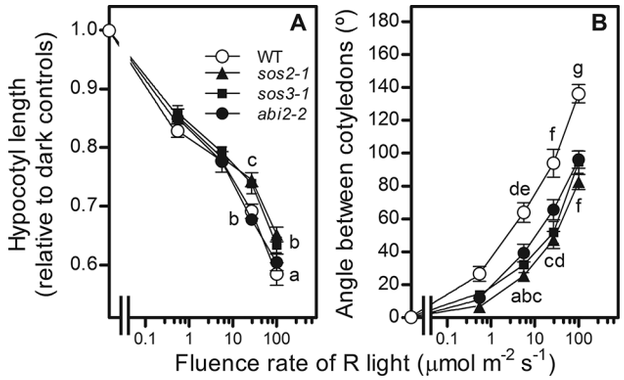

Fig. 3 Seedling deetiolation is reduced in *sos2*, *sos3*, and *abi2* mutants. Shown are the fluence rate response of hypocotyl length (*A*) and cotyledon opening (*B*) under continuous red light. Different letters denote significant differences among means, determined using the Bonferroni posttest ($P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

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 q21 *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, q22 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*, q23 and *ABI2* are expressed in a broad range of plant tissues and organs, including senescent leaves, floral organs, the root elongation zone, and guard cells, as well as during the late stages of seed development 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 was upregulated in seedlings exposed q24 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 GAs, 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 sensitivity to ABA (Gualberti et al. 2002). In addition, null *spl8* mutants showed reduced germination inhibition after exposure q25

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.

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 q26 (Reed et al. 1993; Quail et al. 1995). Mutants in *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 q27 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. q28 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 FT messenger RNA abun- q29 dance (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 genetic relationship between these genes q30 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. The SOS2 has been q31 previously shown to be involved in salt, reactive oxygen species, and flowering signaling (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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Literature Cited

Arana MV, M Sánchez-Lamas, B Strasser, SE Ibarra, PD Cerdán, JF Botto, RA Sánchez 2014 Functional diversity of phytochrome family in the control of light and gibberellin-mediated germination in *Arabidopsis*. Plant Cell Environ 37:2014–2023.

Auge GA, S Perelman, CD Crocco, RA Sánchez, JF Botto 2009 Gene expression analysis of light-modulated germination in tomato seeds. New Phytol 183:301–314.

Botto JF, RA Sánchez, JJ Casal 1995 Role of phytochrome B in the induction of seed germination by light in *Arabidopsis thaliana*. J Plant Physiol 146:307–312.

Botto JF, RA Sánchez, GC Whitelam, JJ Casal 1996 Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade light in *Arabidopsis*. Plant Physiol 110:439–444.

Capella M, PA Ribone, AL Arce, RL Chan 2015 *Arabidopsis thaliana* HomeoBox 1 (AtHB1), a homedomain-leucine zipper I (HD-Zip I) transcription factor, is regulated by PHYTOCHROME-INTERACTING FACTOR 1 to promote hypocotyl elongation. New Phytol 207:669–682.

Chen X, Q Yao, X Gao, C Jiang, NP Harberd, X Fu 2016 Shoot-to-root mobile transcription factor HY5 coordinates plant carbon and nitrogen acquisition. Curr Biol 26:640–646.

Chinnusamy V, K Schumaker, J-K Zhu 2004 Molecular genetic perspectives on cross-talk and specificity in abiotic stress signaling in plants. J Exp Bot 55:225–236.

Choi G, H Yi, J Lee, YK Kwon, MS Soh, B Shin, Z Luka, TR Hahn, PS Song 1999 Phytochrome signalling is mediated through nucleoside diphosphate kinase 2. Nature 401:610–613.

q32 de Montaigu A, A Giakountis, M Rubin, R Tóth, F Cremer, V Sokolova, A Porri, M Reymond, C Weinig, G Coupland 2015 Natural diversity in daily rhythms of gene expression contributes to phenotypic variation. Proc Nat Acad Sci USA 112:905–910.

Derkx MPM, E Vermeer, CM Karssen 1994 Gibberellins in seeds of *Arabidopsis thaliana*: biological activities, identification and effects of light and chilling on endogenous levels. Plant Growth Regul 15: 223–234.

Finch-Savage WE, G Leubner-Metzger 2006 Seed dormancy and the control of germination. New Phytol 171:501–523.

Flowers TJ, TD Colmer 2008 Salinity tolerance in halophytes. New Phytol 179:945–963.

Fowler S, K Lee, H Onouchi, A Samach, K Richardson, B Morris, G Coupland, J Putterill 1999 GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. EMBO J 18:4679–4688.

Gangappa SN, JF Botto 2014 The BBX family of plant transcription factors. Trends Plant Sci 19:460–470.

Gong D, Y Guo, KS Schumaker, JK Zhu 2004 The SOS3 family of calcium sensors and SOS2 family of protein kinases in *Arabidopsis*. Plant Physiol 134:919–926.

Gualberti G, M Papi, L Belluci, I Ricci, D Bouchez, C Camilleri, P Costantino, P Vittorioso 2002 Mutations in the Dof zinc finger genes *DAG2* and *DAG1* influence with opposite effects the germination of *Arabidopsis* seeds. Plant Cell 14:1253–1263.

Halfter U, M Ishitani, JK Zhu 2000 The *Arabidopsis* SOS2 protein kinase physically interacts with and is activated by the calcium-binding protein SOS3. Proc Nat Acad Sci USA 97:3735–3740.

Ishitani M, J Liu, U Halfter, CS Kim, M Wei, JK Zhu 2000 SOS3 function in plant salt tolerance requires N-myristoylation and calcium binding. Plant Cell 12:1667–1677.

Ji H, JM Pardo, G Batelli, MJ Van Oosten, RA Bressan, X Li 2013 The salt overly sensitive (SOS) pathway: established and emerging roles. Mol Plant 6:275–286.

Kaplan-Levy RN, PB Brewer, T Quon, DR Smyth 2012 The trihelix family of transcription factors—light, stress and development. Trends Plant Sci 17:163–171.

Kim W-Y, Z Ali, H-J Park, SJ Park, J-Y Cha, J Perez-Hormaeche, FJ Quintero, et al 2013 Release of SOS2 kinase from sequestration with GIGANTEA determines salt tolerance in *Arabidopsis*. Nature Comm 4:1352–1364.

Le Gourrierec J, V Delaporte, M Ayadi, YF Li, DX Zhou 2002 Functional analysis of *Arabidopsis* transcription factor GT-1 in the expression of light-regulated genes. Genome Lett 1:77–82.

Leung J, S Merlot, J Giraudat 1997 The *Arabidopsis* ABSCISIC ACID-INSENSITIVE2 (ABI2) and ABI1 genes encode homologous protein phosphatases 2C involved in abscisic acid signal transduction. Plant Cell 9:759–771.

Liu J, M Ishitani, U Halfter, CS Kim, JK Zhu 2000 The *Arabidopsis thaliana SOS2* gene encodes a protein kinase that is required for salt tolerance. Proc Nati Acad Sci USA 97:3703–3734.

Liu J, JK Zhu 1998 A calcium sensor homolog required for plant salt tolerance. Science 280:1943–1945.

Matsushita A, T Furumoto, S Ishida, Y Takahashi 2007 AGF1, an AT-Hook protein, is necessary for the negative feedback of AtGA3ox1 encoding GA 3-oxidase. Plant Physiol 143:1152–1162.

Merlot S, F Gosti, D Guerrier, A Vavasseur, J Giraudat 2001 The ABI1 and ABI2 protein phosphatases 2C act in a negative feedback regulatory loop of the abscisic acid signalling pathway. Plant J 25:295–303.

Mishra P, KC Panigrahi 2015 GIGANTEA—an emerging story. Front Plant Sci 6:8.

Mizoguchi T, L Wright, S Fujiwara, F Cremer, K Lee, H Onouchi, A Mouradov, et al 2005 Distinct roles of GIGANTEA in promoting flowering and regulating circadian rhythms in *Arabidopsis*. Plant Cell 17:2255–2270.

Nakabayashi K, M Okamoto, T Koshiba, Y Kamiya, E Nambara 2005 Genome-wide profiling of stored mRNA in *Arabidopsis thaliana* seed germination: epigenetic and genetic regulation of transcription in seed. Plant J 41:697–709.

Ogawa M, A Hanada, Y Yamauchi, A Kuwahara, Y Kamiya, S Yamaguchi 2003 Gibberellin biosynthesis and response during *Arabidopsis* seed germination. Plant Cell 15:1591–1604.

Oh E, H Kang, S Yamaguchi, J Park, D Lee, Y Kamiya, G Choi 2009 Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in *Arabidopsis*. Plant Cell 21:403–419.

Oh E, S Yamaguchi, J Hu, J Yusuke, B Jung, I Paik, H-S Lee, et al 2007 PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the *GAI* and *RGA* promoters in *Arabidopsis* seeds. Plant Cell 19:1192–1208.

Ohta M, Y Guo, U Halfter, JK Zhu 2003 A novel domain in the protein kinase SOS2 mediates interaction with the protein phosphatase 2C ABI2. Proc Nati Acad Sci USA 100:11771–11776.

Oliverio KA, M Crepy, EL Martin-Tryon, R Milich, SL Harmer, J Putterill, MJ Yanovsky, JJ Casal 2007 GIGANTEA regulates phytochrome A–mediated photomorphogenesis independently of its role in the circadian clock. Plant Physiol 144:495–502.

Park HJ, WY Kim, DJ Yun 2013 A role for GIGANTEA: keeping the balance between flowering and salinity stress tolerance. Plant Signal Behav 8:e24820.

Penfield S, EM Josse, R Kannangara, AD Gilday, KJ Halliday, IA Graham 2005 Cold and light control seed germination through the bHLH transcription factor SPATULA. Curr Biol 15:1998–2006.

Quail PH, MT Boylan, BM Parks, TW Short, Y Xu, D Wagner 1995 Phytochromes: photosensory perception and signal transduction. Science 268:675–680.

Quintero FJ, M Ohta, H Shi, JK Zhu, JM Pardo 2002 Reconstitution in yeast of the *Arabidopsis* SOS signaling pathway for Na+ homeostasis. Proc Natl Acad Sci USA 99:9061–9066.

Quintero FJ, J Martinez-Atienza, I Villalta, X Jiang, WY Kim, Z Ali, H Fujii, et al 2011 Activation of the plasma membrane Na/H antiporter salt-overly-sensitive 1 (SOS1) by phosphorylation of an autoinhibitory C-terminal domain. Proc Natl Acad Sci USA 108:2611–2616.

Reed JW, P Nagpal, DS Poole, M Furuya, J Chory 1993 Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. Plant Cell 5:147–157.

Richter R, C Behringer, IK Müller, C Schwechheimer 2010 The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. Genes Dev 24:2093–2104.

Rubio S, A Rodrigues, A Saez, MB Dizon, A Galle, TH Kim, J Santiago, et al 2009 Triple loss of function of protein phosphatases type 2C leads to partial constitutive response to endogenous abscisic acid. Plant Physiol 150:1345–1355.

Seo M, A Hanada, A Kuwahara, A Endo, M Okamoto, Y Yamauchi, H North, et al 2006 Regulation of hormone metabolism in *Arabidopsis* seeds: phytochrome regulation of abscisic acid metabolism and abscisic acid regulation of gibberellin metabolism. Plant J 48: 354–366.

Shi H, M Ishitani, C Kim, JK Zhu 2000 The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na^+/H^+ antiporter. Proc Nati Acad Sci USA 97:6896–6901.

Shinomura T, A Nagatani, J Chory, M Furuya 1994 The induction of seed germination in *Arabidopsis thaliana* is regulated principally by phytochrome B and secondarily by phytochrome A. Plant Physiol 104:363–371.

Steffens NO, C Galuschka, M Schindler, L Bulow, R Hehl 2004 AthaMap: an online resource for in silico transcription factor binding sites in the *Arabidopsis thaliana* genome. Nucleic Acids Res 32: 368–372.

Unte US, AM Sorensen, P Pesaresi, M Gandikota, D Leister, H Saedler, P Huijser 2003 *SPL8*, an SBP-box gene that affects pollen sac development in *Arabidopsis*. Plant Cell 15:1009–1019.

Verslues PE, G Batelli, S Grillo, F Agius, YS Kim, J Zhu, M Agarwal, et al 2007 Interaction of SOS2 with nucleoside diphosphate kinase 2 and catalases reveals a point of connection between salt stress and H_2O_2 signaling in *Arabidopsis thaliana*. Mol Cell Biol 27: 7771–7780.

Winter D, B Vinegar, H Nahal, R Ammar, GV Wilson, NJ Provart 2007 An "electronic fluorescent pictograph" browser for exploring and analyzing large-scale biological data sets. PLoS ONE 2:e718.

Xing S, M Salinas, A Garcia-Molina, S Höhmann, R Berndtgen, P Huijser 2013 *SPL8* and miR156-targeted *SPL* genes redundantly regulate *Arabidopsis gynoecium* differential patterning. Plant J 75: 566–577.

Xu D, J Li, SN Gangappa, C Hettiarachchi, F Lin, MX Andersson, Y Jiang, et al 2014 Convergence of light and ABA signaling on the *ABI5* promoter. PLoS Genet 10:e1004197.

Yamaguchi S, MW Smith, RGS Brown, Y Kamiya, T Sun 1998 Phytochrome regulation and differential expression of gibberellin 3β-hydroxylase genes in germinating *Arabidopsis* seed. Plant Cell 10: 2115–2126.

Yamauchi Y, M Ogawa, A Kuwahara, A Hanada, Y Kamiya, S Yamaguchi 2004 Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. Plant Cell 16:367–378.

Yamauchi Y, N Takeda-Kamiya, A Hanada, M Ogawa, A Kuwahara, M Seo, Y Kamiya, S Yamaguchi 2007 Contribution of gibberellin deactivation by AtGA2ox2 to the suppression of germination of dark-imbibed *Arabidopsis thaliana* seeds. Plant Cell Physiol 48: 61555–61561.

Zhang Y, S Schwarz, H Saedler, P Huijser 2007 SPL8, a local regulator in a subset of gibberellin mediated developmental processes in *Arabidopsis*. Plant Mol Biol 63:429–439.

Zhao J, DS Favero, H Peng, MM Neff 2013 *Arabidopsis thaliana* AHL family modulates hypocotyl growth redundantly by interacting with each other via the PPC/DUF296 domain. Proc Natl Acad Sci USA 110:E4688–E4697.

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Appendix from Trupkin et al., "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*"

(Int. J. Plant Sci., vol. 178, no. 6, p. 000)

Table A1. Summary of Putative *cis* Elements and Transcription Factors (TFs) Found in *SOS2*, *SOS3*, and *ABI2* Promoters

TF	TF family	Ratio	Present in	Response	Reference(s)
AtLEC2	ABI3/VP1	1.25	*SOS2*, *SOS3*	Seed maturation; embryogenesis; auxin response; gibberellin response; dormancy induction	Stone et al. 2001; Curaba et al. 2004; Braybrook et al. 2006
REM1(1)	ABI3/VP1	1.66	*SOS2*, *SOS3*	Flower development	Mantegazza et al. 2014
ABI4(2)	AP2/EREBP	1.62	*SOS3*	Seed development; ABA response	Niu et al. 2002; Hu et al. 2012
DEAR3(1)	AP2/EREBP	1.02	*SOS2*, *SOS3*, *ABI2*	Drought and cold responses	Shinozaki and Yamaguchi-Shinozaki 2000
DREB2C(2)	AP2/EREBP	1.8	*SOS2*	Heat, salt, and oxidative response; seed germination; ABA response	Lim et al. 2007; Hwang et al. 2012; Je et al. 2014; Song et al. 2014
NtERF2	AP2/EREBP	1.12	*SOS2*, *SOS3*	Defense response; ethylene response	Ohme-Takagi et al. 2000
RAP2.6(2)	AP2/EREBP	1.1	*SOS2*, *SOS3*	Defense response; salt and osmotic stress; ABA response	Zhu et al. 2010; Ali et al. 2013
TEIL	AP2/EREBP	1.32	*SOS2*, *SOS3*, *ABI2*	Ethylene response; sulphur deficiency response	Kosugi and Ohashi 2000; Wawrzyńska et al. 2009
TOE1(2)	AP2/EREBP	1.34	*SOS2*, *ABI2*	Flowering time regulation (GIGANTEA)	Jung et al. 2007
TOE2(3)	AP2/EREBP	1.7	*SOS2*, *SOS3*, *ABI2*	Flowering time regulation	Zhang et al. 2015
WRI1	AP2/EREBP	1.46	*SOS2*, *SOS3*, *ABI2*	Lipid storage during seed maturation	Maeo et al. 2009
ETT(2)	ARF	2.52	*SOS2*, *SOS3*, *ABI2*	Flower Development; auxin response	Okushima et al. 2005
AHL12(1)	AT-Hook	1.49	*SOS2*, *SOS3*, *ABI2*	Light-regulated hypocotyl growth	Zhao et al. 2013
AHL12(2)	AT-Hook	2.02	*SOS2*, *SOS3*, *ABI2*	Light-regulated hypocotyl growth	Zhao et al. 2013
AHL12(3)	AT-Hook	1.73	*SOS2*, *SOS3*, *ABI2*	Light-regulated hypocotyl growth	Zhao et al. 2013
AHL20(1)	AT-Hook	1.7	*SOS2*, *SOS3*, *ABI2*	Defense response; light-regulated hypocotyl growth	Lu et al. 2010; Zhao et al. 2013
AHL20(2)	AT-Hook	1.84	*SOS2*, *SOS3*, *ABI2*	Defense response; light-regulated hypocotyl growth	Lu et al. 2010; Zhao et al. 2013
AHL20(3)	AT-Hook	2.03	*SOS2*, *SOS3*, *ABI2*	Defense response; light-regulated hypocotyl growth	Lu et al. 2010; Zhao et al. 2013
AHL25(1)	AT-Hook	1.68	*SOS2*, *SOS3*, *ABI2*	Negative regulation of gibberellic acid signaling pathway; light-regulated hypocotyl growth	Matsushita et al. 2007; Zhao et al. 2013
AHL25(2)	AT-Hook	1.83	*SOS2*, *SOS3*, *ABI2*	Negative regulation of gibberellic acid signaling pathway; light-regulated hypocotyl growth	Matsushita et al. 2007; Zhao et al. 2013
AHL25(3)	AT-Hook	1.85	*SOS2*, *SOS3*, *ABI2*	Negative regulation of gibberellic acid signaling pathway; light-regulated hypocotyl growth	Matsushita et al. 2007; Zhao et al. 2013
bZIP60(2)	bZIP	1.36	*SOS3*, *ABI2*	Endoplasmic reticulum stress response	Iwata et al. 2008
O2	bZIP	1.34	*SOS3*, *ABI2*	Seed maturation; ABA response	Lara et al. 2003
ID1	C2H2(Zn)	1.11	*SOS2*, *SOS3*, *ABI2*	Flowering time regulation	Kozaki et al. 2004
ZAT14	C2H2(Zn)	1.03	*SOS2*, *SOS3*, *ABI2*	Osmotic and salt response?	Mittler et al. 2006
DAG2	C2C2(Zn)Dof	1.2	*SOS2*, *SOS3*, *ABI2*	Seed germination; gibberellin response; light response; cold response	Gualberti et al. 2002
GATA-1	C2C2(Zn)GATA	1.96	*SOS3*	Chloroplast development; cell growth and cell cycle regulation; cytokinin response; deetiolation	Chiang et al. 2012

Table A1 (*Continued*)

TF	TF family	Ratio	Present in	Response	Reference(s)
GATA12	C2C2(Zn)GATA	1.14	*SOS2*, *SOS3*, *ABI2*	Seed germination; gibberellin response; light response; flowering time regulation	Teakle et al. 2002; Richter et al. 2010
YAB1	C2C2(Zn)YABBY	1.27	*SOS2*, *SOS3*, *ABI2*	Gibberellin response; leaf development; flower development; abaxial cell fate	Siegfried et al. 1999; Meister et al. 2005; Dai et al. 2007; Ha et al. 2010
YAB5	C2C2(Zn)YABBY	1.04	*SOS2*, *SOS3*, *ABI2*	Leaf development; flower development; abaxial cell fate	Siegfried et al. 1999; Meister et al. 2005; Ha et al. 2010
CBP60g	CAMTA-like	7.1	*SOS2*	Defense response	Wang et al. 2009, 2011
SARD1	CAMTA-like	7.1	*SOS2*	Defense response	Wang et al. 2009, 2011
E2Fa	E2F/DP	84.19	*SOS2*	Cell cycle regulation	De Veylder et al. 2002
E2Fc	E2F/DP	77.41	*SOS2*	Cell cycle regulation; photomorphogenic response	de Jager et al. 2009; Berckmans et al. 2011
E2Fd	E2F/DP	77.41	*SOS2*	Cell cycle regulation; auxin response	Sozzani et al. 2010
ARR2	GARP/ARR-B	1.41	*ABI2*	Pollen development; cytokinin response; defense response; root and hypocotyl growth; leaf senescence	Lohrmann et al. 2001; Choi et al. 2010; Kim et al. 2012
ARR11(3)	GARP/ARR-B	1.11	*SOS2*, *SOS3*, *ABI2*	Cytokinin response	Imamura et al. 2003
GLK1(1)	GARP	1.54	*SOS2*, *ABI2*	Chloroplast development; leaf senescence; defense response	Kobayashi et al. 2013; Rauf et al. 2013; Murmu et al. 2014
GLK1(2)	GARP	1.04	*SOS2*, *ABI2*	Chloroplast development; leaf senescence; defense response	Kobayashi et al. 2013; Rauf et al. 2013; Murmu et al. 2014
AGP1	GATA	1.25	*SOS2*, *SOS3*, *ABI2*	Defense response	Sugimoto et al. 2003
ZML2	GATA	4.59	*SOS3*, *ABI2*	Excess light response	Shaikhali et al. 2012
WOX13(2)	HD-HOX	1.54	*SOS2*, *SOS3*, *ABI2*	Fruit development	Romera-Branchat et al. 2012
ATHB1	HD-ZIP	2.04	*SOS2*, *SOS3*, *ABI2*	Light-regulated hypocotyl growth; leaf development	Aoyama et al. 1995; Capella et al. 2015
ATHB5	HD-ZIP	2.72	*ABI2*	Seed germination; seedling growth; ABA response; auxin response	Johannesson et al. 2003; De Smet et al. 2013
ATHB12	HD-ZIP	1.19	*SOS2*, *SOS3*	Drought and osmotic response; ABA response; defense response	Lee and Chun 1998; J. Park et al. 2011; M. Y. Park et al. 2011
ATHB51	HD-ZIP	2.09	*SOS2*, *SOS3*, *ABI2*	Leaf development; organ identity	Saddic et al. 2006
HAHB4	HD-ZIP	2.35	*SOS2*, *SOS3*, *ABI2*	Drought and salt response; ABA, ethylene, and jasmonic acid response; defense response	Dezar et al. 2005; Manavella et al. 2006, 2008
ICU4	HD-ZIP	1.27	*SOS2*, *SOS3*, *ABI2*	Vascular development; shoot organogenesis; embryo development	Smith and Long 2010; Duclercq et al. 2011; Du et al. 2015
HSFB2a(2)	HSF	1.19	*SOS3*, *ABI2*	Flower development (gametophytic)	Wunderlich et al. 2014
TBF1	HSF	2.34	*SOS3*	Defense response	Pajerowska-Mukhtar et al. 2012
LBD16	LOB/AS2	1.53	*SOS2*, *SOS3*, *ABI2*	Lateral root formation; auxin response	Lee et al. 2009
AG	MADS	1.89	*SOS2*, *ABI2*	Flower development; ovule development	Mizukami and Ma 1992; Flanagan et al. 1996; Pinyopich et al. 2003

Table A1 (*Continued*)

TF	TF family	Ratio	Present in	Response	Reference(s)
AGL1	MADS	1.08	*SOS2*, *ABI2*	Flower development; ovule development	Mizukami and Ma 1992; Flanagan et al. 1996; Pinyopich et al. 2003
AGL2	MADS	1.75	*SOS2*, *SOS3*, *ABI2*	Flower organ identity	Pelaz et al. 2000
AGL15	MADS	1.25	*SOS2*, *SOS3*, *ABI2*	Seed maturation; gibberellin response; flowering time regulation; flower senescence	Fang and Fernandez 2002; Wang et al. 2004; Adamczyk et al. 2007
AtMYB61	MYB	1.07	*SOS2*, *SOS3*	Seed germination; photomorphogenesis; lignin deposition	Penfield et al. 2001; Newman et al. 2004
AtMYB77	MYB	1.09	*SOS3*	Auxin response; lateral root growth	Shin et al. 2007; Zhao et al. 2014
CCA1(1)	MYB	4.47	*SOS3*, *ABI2*	Circadian regulation	Green and Tobin 1999
MYB.PH3(1)	MYB	2.27	*SOS3*	na	na
MYB46(1)	MYB	1.23	*SOS2*, *SOS3*, *ABI2*	Defense response; cell wall biogenesis	Ramírez et al. 2011; Zhong and Ye 2012
MYB52(1)	MYB	1.17	*SOS2*, *SOS3*, *ABI2*	Drought response; ABA response; cell wall biogenesis	Ko et al. 2009; J. Park et al. 2011; M. Y. Park et al. 2011
MYB52(2)	MYB	1.67	*SOS2*, *SOS3*, *ABI2*	Drought response; ABA response; cell wall biogenesis	Ko et al. 2009; J Park et al. 2011; M. Y. Park et al. 2011
MYB83	MYB	1.23	*SOS2*, *SOS3*, *ABI2*	Cell wall biogenesis	Zhong and Ye 2012
RVE1(1)	MYB	2.3	*SOS2*, *SOS3*, *ABI2*	Cold response; auxin and circadian growth regulation	Rawat et al. 2009; Meissner et al. 2013
RVE1(2)	MYB	1.2	*SOS2*, *SOS3*, *ABI2*	Cold response; auxin and circadian growth regulation	Rawat et al. 2009; Meissner et al. 2013
ANAC46	NAC	1.2	*SOS2*, *SOS3*, *ABI2*	na	na
ANAC55(1)	NAC	3.31	*SOS2*, *SOS3*, *ABI2*	Defense response; jasmonic acid and salicylic acid response; leaf senescence	Bu et al. 2008; Zheng et al. 2012; Hickman et al. 2013
ANAC55(2)	NAC	3.27	*SOS2*, *SOS3*, *ABI2*	Defense response; jasmonic acid and salicylic acid response; leaf senescence	Bu et al. 2008; Zheng et al. 2012; Hickman et al. 2013
ANAC58	NAC	2.34	*SOS2*, *SOS3*, *ABI2*	na	na
TaNAC69(2)	NAC	3.77	*SOS3*, *ABI2*	Drought and salt response; ABA response	Huang et al. 2015
AtSPL8	SBP	1.98	*SOS2*, *SOS3*, *ABI2*	Pollen and gynoecium development; auxin response; seed germination and root elongation modulated by gibberellin	Unte et al. 2003; Zhang et al. 2007; Xing et al. 2013
SPL14	SBP	3.78	*ABI2*	Defense response; development	Stone et al. 2005
STY1(1)	SRS	3.41	*SOS2*	Leaf and flower development; auxin response	Eklund et al. 2010
PCF5	TCP	1.38	*SOS3*	na	na
TCP16(1)	TCP	5.07	*SOS3*	Pollen development	Takeda et al. 2006
TCP16(2)	TCP	9.77	*SOS3*	Pollen development	Takeda et al. 2006
TCP15(1)	TCP	8.84	*ABI2*	Cell cycle regulation; leaf and flower development; auxin response	Kieffer et al. 2011; Uberti-Manassero et al. 2012
GT1	Trihelix	1.72	*SOS2*, *SOS3*, *ABI2*	Light, salt, and defense regulation; flower development; seed/embryo maturation	Le Gourrierec et al. 2002; Kaplan-Levy et al. 2012

Table A1 (*Continued*)

TF	TF family	Ratio	Present in	Response	Reference(s)
GT-3b	Trihelix	3.95	*ABI2*	Salt response; defense response	Park et al. 2004
WRKY6	WRKY(Zn)	6.51	*SOS2*	Leaf senescence; defense response; phosphate and boron deficiency response	Robatzek and Somssich 2001; Chen et al. 2009; Kasajima et al. 2010
WRKY11	WRKY(Zn)	10.45	*SOS2*	Defense response	Journot-Catalino et al. 2006
WRKY12	WRKY(Zn)	2.3	*SOS2*, *SOS3*	Cell wall biogenesis	Wang et al. 2010
WRKY18(1)	WRKY(Zn)	2.6	*SOS2*, *SOS3*	Seed germination; ABA response; salt and osmotic response; defense response	Xu et al. 2006; Chen et al. 2010
WRKY38(1)	WRKY(Zn)	2.75	*SOS2*, *SOS3*	Defense response	Kim et al. 2008
WRKY45	WRKY(Zn)	5.47	*SOS2*, *SOS3*	Phosphate starvation response; ion homeostasis	Wang et al. 2014
ZAP1	WRKY(Zn)	4.51	*SOS2*	na	na
TSS	other	3.52	*SOS2*, *ABI2*	na	na

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 shown. ABA = abscisic acid; na = not applicable.

Literature Cited Only in the Appendix

Adamczyk BJ, MD Lehti-Shiu, DE Fernandez 2007 The MADS domain factors AGL15 and AGL18 act redundantly as repressors of the floral transition in *Arabidopsis*. Plant J 50:1007–1019.

Ali MA, A Abbas, DP Kreil, H Bohlmann 2013 Overexpression of the transcription factor RAP2.6 leads to enhanced callose deposition in syncytia and enhanced resistance against the beet cyst nematode *Heterodera schachtii* in *Arabidopsis* roots. BMC Plant Biol 13:47.

Aoyama T, C-H Dong, Y Wu, M Carabelli, G Sessa, I Ruberti, G Morelli, N-H Chua 1995 Ectopic expression of the *Arabidopsis* transcriptional activator Athb-1 alters leaf cell fate in tobacco. Plant Cell 7:1773–1785.

Berckmans B, T Lammens, H Van Den Daele, Z Magyar, L Bögre, L De Veylder 2011 Light-dependent regulation of *DEL1* is determined by the antagonistic action of E2Fb and E2Fc. Plant Physiol 157:1440–1451.

Braybrook SA, SL Stone, S Park, AQ Bui, BH Le, RL Fischer, RB Goldberg, JJ Harada 2006 Genes directly regulated by LEAFY COTYLEDON2 provide insight into the control of embryo maturation and somatic embryogenesis. Proc Natl Acad Sci USA 103:3468–3473.

Bu Q, H Jiang, CB Li, Q Zhai, J Zhang, X Wu, J Sun, Q Xie, C Li 2008 Role of the *Arabidopsis thaliana* NAC transcription factors ANAC019 and ANAC055 in regulating jasmonic acid-signaled defense responses. Cell Res 18:756–767.

Chen H, Z Lai, J Shi, Y Xiao, Z Chen, X Xu 2010 Roles of *Arabidopsis* WRKY18, WRKY40 and WRKY60 transcription factors in plant responses to abscisic acid and abiotic stress. BMC Plant Biol 10:281.

Chen Y-F, L-Q Li, Q Xu, YH Kong, H Wang, W-H Wu 2009 The WRKY6 transcription factor modulates *PHOSPHATE1* expression in response to low Pi stress in *Arabidopsis*. Plant Cell 21:3554–3566.

Chiang YH, YO Zubo, W Tapken, HJ Kim, AM Lavanway, L Howard, M Pilon, et al 2012 Functional characterization of the GATA transcription factors GNC and CGA1 reveals their key role in chloroplast development, growth, and division in *Arabidopsis*. Plant Physiol 160:332–348.

Choi J, SU Huh, M Kojima, H Sakakibara, KH Paek, I Hwang 2010 The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in *Arabidopsis*. Dev Cell 19:284–295.

Curaba J, T Moritz, R Blervaque, F Parcy, V Raz, M Herzog, G Vachon 2004 *AtGA3ox2*, a key gene responsible for bioactive gibberellin biosynthesis, is regulated during embryogenesis by *LEAFY COTYLEDON2* and *FUSCA3* in *Arabidopsis*. Plant Physiol 136:3660–3669.

Dai M, Y Zhao, Q Ma, Y Hu, P Hedden, Q Zhang, DX Zhou 2007 The rice *YABBY1* gene is involved in the feedback regulation of gibberellin metabolism. Plant Physiol 144:121–133.

de Jager SM, S Scofield, RP Huntley, AS Robinson, BG den Boer, JA Murray 2009 Dissecting regulatory pathways of G1/S control in *Arabidopsis*: common and distinct targets of CYCD3;1, E2Fa and E2Fc. Plant Mol Biol 71:345–365.

De Smet I, S Lau, JS Ehrismann, I Axiotis, M Kolb, M Kientz, D Wijers, G Jürgens 2013 Transcriptional repression of *BODENLOS* by HD-ZIP transcription factor HB5 in *Arabidopsis thaliana*. J Exp Bot 64:3009–3019.

De Veylder L, T Beeckman, G Beemster, J de Almeida Engler, S Ormenese, S Maes, M Naudts, et al 2002 Control of proliferation endoreduplication and differentiation by the *Arabidopsis* E2Fa-Dpa transcription factor. EMBO J 21:1360–1368.

Dezar CA, GM Gago, DH Gonzalez, RL Chan 2005 *Hahb-4*, a sunflower homeobox-leucine zipper gene, is a developmental regulator and confers drought tolerance to *Arabidopsis thaliana* plants. Transgenic Research 14:429–440.

Du Q, H Wang 2015 The role of HD-ZIP III transcription factors and *miR165/166* in vascular development and secondary cell wall formation. Plant Signal Behav 10:e1078955. doi:101080/1559232420151078955. **q33**

Duclercq J, YPA Ndong, F Guerineau, RS Sangwan, M Catterou 2011 *Arabidopsis* shoot organogenesis is enhanced by an amino acid change in the ATHB15 transcription factor. Plant Biol 13:317–324. **q34**

Eklund DM, V Staldal, I Valsecchi, I Cierlik, C Eriksson, K Hiratsu, M Ohme-Takagi, et al 2010 The *Arabidopsis thaliana* STYLISH1 protein acts as a transcriptional activator regulating auxin biosynthesis. Plant Cell 22:349–363.

Fang S, DE Fernandez 2002 Effect of regulated overexpression of the MADS domain factor AGL15 on flower senescence and fruit maturation. Plant Physiol 130:78–89.

Flanagan CA, Y Hu, H Ma 1996 Specific expression of the *AGL1* MADS-box gene suggests regulatory functions in *Arabidopsis* gynoecium and ovule development. Plant J 10:343–353.

Green RM, EM Tobin 1999 Loss of the circadian clock–associated protein 1 in *Arabidopsis* results in altered clock-regulated gene expression. Proc Natl Acad Sci USA 96:4176–4179.

Ha CM, JH Jun, JC Fletcher 2010 *BLADE-ON-PETIOLE1* and *2* control *Arabidopsis* leaf morphogenesis by regulating *YABBY* and *KNOX* gene activity. Genetics 186:197–206. doi:101534/genetics110118703. **q35**

Hickman R, C Hill, CA Penfold, E Breeze, L Bowden, JD Moore 2013 A local regulatory network around three NAC transcription factors in stress responses and senescence in *Arabidopsis* leaves. Plant J 75:26–39.

Hu YF, YP Li, J Zhang, H Liu, M Tian, Y Huang 2012 Binding of ABI4 to a CACCG motif mediates the ABA-induced expression of the *ZmSSI* gene in maize (*Zea mays* L) endosperm. J Exp Bot 63:5979–5989.

Huang Q, Y Wang, B Li, J Chang, M Chen, K Li, G Yang, G He 2015 TaNAC29, a NAC transcription factor from wheat, enhances salt and drought tolerance in transgenic *Arabidopsis*. BMC Plant Biol 15:268.

Hwang JE, CJ Lim, H Chen, J Je, C Song, CO Lim 2012 Overexpression of *Arabidopsis* dehydration-responsive element-binding protein 2C confers tolerance to oxidative stress. Mol Cell 33:135–140.

Imamura A, T Kiba, Y Tajima, T Yamashino, T Mizuno 2003 In vivo and in vitro characterization of the ARR11 response regulator implicated in the His-to-Asp phosphorelay signal transduction in *Arabidopsis thaliana*. Plant Cell Physiol 44:122–131.

Iwata Y, NV Fedoroff, N Koizumi 2008 *Arabidopsis* bZIP60 is a proteolysis-activated transcription factor involved in the endoplasmic reticulum stress response. Plant Cell 20:3107–3121.

Je J, H Chen, C Song, CO Lim 2014 *Arabidopsis* DREB2C modulates ABA biosynthesis during germination. Biochem Biophys Res Commun 452:91–98.

Johannesson H, Y Wang, J Hanson, P Engstrom 2003 The *Arabidopsis thaliana* homeobox gene *ATHB5* is a potential regulator of abscisic acid responsiveness in developing seedlings. Plant Mol Biol 51:719–729.

Journot-Catalino N, IE Somssich, D Roby, T Kroj 2006 The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in *Arabidopsis thaliana*. Plant Cell 18:3289–3302.

Jung JH, YH Seo, PJ Seo, JL Reyes, J Yun, NH Chua, CM Park 2007 The *GIGANTEA*-regulated MicroRNA172 mediates photoperiodic flowering independent of *CONSTANS* in *Arabidopsis*. Plant Cell 19:2736–2748.

Kasajima I, Y Ide, M Yokota Hirai, T Fujiwara 2010 WRKY6 is involved in the response to boron deficiency in *Arabidopsis thaliana*. Physiol Plant 139:80–92.

Kieffer M, V Master, R Waites, B Davies 2011 TCP14 and TCP15 affect internode length and leaf shape in *Arabidopsis*. Plant J 68:147–158.

Kim K, H Ryu, YH Cho, E Scacchi, S Sabatini, I Hwang 2012 Cytokinin-facilitated proteolysis of ARABIDOPSIS RESPONSE REGULATOR2 attenuates signaling output in two-component circuitry. Plant J 69:934–945.

Kim KC, Z Lai, B Fan, Z Chen 2008 *Arabidopsis* WRKY38 and WRKY62 transcription factors interact with histone deacetylase 19 in basal defense. Plant Cell 20:2357–2371.

Ko JH, WC Kim, KH Han 2009 Ectopic expression of MYB46 identifies transcriptional regulatory genes involved in secondary wall biosynthesis in *Arabidopsis*. Plant J 60:649–665.

Kobayashi K, D Sasaki, K Noguchi, D Fujinuma, H Komatsu, M Kobayashi, M Sato, et al 2013 Photosynthesis of root chloroplasts developed in *Arabidopsis* lines overexpressing *GOLDEN2-LIKE* transcription factors. Plant Cell Physiol 54:1365–1377.

Kosugi S, Y Ohashi 2000 Cloning and DNA-binding properties of a tobacco Ethylene-Insensitive3 (EIN3) homolog. Nucleic Acids Res 28:960–967.

Kozaki A, S Hake, J Colasanti 2004 The maize ID1 flowering time regulator is a zinc finger protein with novel DNA binding properties. Nucleic Acids Res 32:1710–1720.

Lara P, L Oñate-Sánchez, Z Abraham, C Ferrándiz, I Díaz, P Carbonero, J Vicente-Carbajosa 2003 Synergistic activation of seed storage protein gene expression in *Arabidopsis* by ABI3 and two bZIPs related to OPAQUE2. J Biol Chem 278:21003–21011.

Lee HW, NY Kim, DJ Lee, J Kim 2009 *LBD18/ASL20* regulates lateral root formation in combination with *LBD16/ASL18* downstream of *ARF7* and *ARF19* in *Arabidopsis*. Plant Physiol 151:1377–1389.

Lee Y-H, J-Y Chun 1998 A new homeodomain-leucine zipper gene from *Arabidopsis thaliana* induced by water stress and abscisic acid treatment. Plant Mol Biol 37:377–384.

Lim CJ, JE Hwang, H Chen, JK Hong, KA Yang, MS Choi, KO Lee 2007 Over-expression of the *Arabidopsis* DRE/CRT-binding transcription factor *DREB2C* enhances thermotolerance. Biochem Biophys Res Commun 362:431–436.

Lohrmann J, U Sweere, E Zabaleta, I Baurle, C Keitel, L Kozma-Bognar, A Brennicke, et al 2001 The response regulator ARR2: a pollen-specific transcription factor involved in the expression of nuclear genes for components of mitochondrial complex I in *Arabidopsis*. Mol Genet Genomics 265:2–13.

Lu H, Y Zou, N Feng 2010 Overexpression of *AHL20* negatively regulates defenses in *Arabidopsis*. J Integr Plant Biol 52:801–808.

Maeo K, T Tokuda, A Ayame, N Mitsui, T Kawai, H Tsukagoshi, S Ishiguro, K Nakamura 2009 An AP2-type transcription factor WRINKLED1 of *Arabidopsis thaliana* binds to AW-box sequence conserved among proximal upstream regions of genes involved in fatty acid synthesis. Plant J 60:476–487.

Manavella PA, AL Arce, CA Dezar, F Bitton, FP Renou, M Crespi, RL Chan 2006 Cross-talk between ethylene and drought signalling pathways is mediated by the sunflower Hahb-4 transcription factor. Plant J 48:125–137.

Manavella PA, CA Dezar, G Bonaventure, IT Baldwin, RL Chan 2008 HAHB4, a sunflower HD-Zip protein, integrates signals from the jasmonic acid and ethylene pathways during wounding and biotic stress responses. Plant J 56:376–388.

Mantegazza O, V Gregis, MA Mendes, P Morandini, M Alves-Ferreira, CM Patreze, SM Nardeli, MM Kater, L Colombo 2014 Analysis of the *Arabidopsis REM* gene family predicts functions during flower development. Ann Bot 114:1507–1515.

Meissner M, E Orsini, M Ruschhaupt, AE Melchinger, DK Hincha, AG Heyer 2013 Mapping quantitative trait loci for freezing tolerance in a recombinant inbred line population of *Arabidopsis thaliana* accessions Tenela and C24 reveals REVEILLE1 as negative regulator of cold acclimation. Plant Cell Environ 36:1256–1267.

Meister RJ, H Oldenhof, JL Bowman, CS Gasser 2005 Multiple protein regions contribute to differential activities of YABBY proteins in reproductive development. Plant Physiol 137:651–662.

Mittler R, Y Kim, L Song, J Coutu, A Coutu, S Ciftci-Yilmaz, H Lee, B Stevenson, JK Zhu 2006 Gain- and loss-of-function mutations in *Zat10* enhance the tolerance of plants to abiotic stress. FEBS Lett 580:6537–6542.

Mizukami Y, H Ma 1992 Ectopic expression of the floral homeotic gene *AGAMOUS* in transgenic *Arabidopsis* plants alters floral organ identity. Cell 71:119–131.

Murmu J, M Wilton, G Allard, R Pandeya, D Desveaux, J Singh, R Subramaniam 2014 *Arabidopsis* GOLDEN2-LIKE (GLK) transcription factors activate jasmonic acid (JA)–dependent disease susceptibility to the biotrophic pathogen *Hyaloperonospora arabidopsidis*, as well as JA-independent plant immunity against the necrotrophic pathogen *Botrytis cinerea*. Mol Plant Pathol 15:174–184.

Newman LJ, DE Perazza, L Juda, MM Campbell 2004 Involvement of the R2R3-MYB, *At*MYB61, in the ectopic lignification and dark-photomorphogenic components of the *det3* mutant phenotype. Plant J 37:239–250.

Niu X, T Helentjaris, NJ Bate 2002 Maize ABI4 binds coupling element1 in abscisic acid and sugar response genes. Plant Cell 14:2565–2575.

Ohme-Tagaki M, K Suzuki, H Shinshi 2000 Regulation of ethylene-induced transcription of defense genes. Plant Cell Physiol 41:1187–1192.

Okushima Y, PJ Overvoordea, K Arimaa, JM Alonso, A Chana, JR Ecker, C Changa, et al 2005 Functional genomic analysis of the *AUXIN RESPONSE FACTOR* gene family members in *Arabidopsis thaliana*: unique and overlapping functions of *ARF7* and *ARF19*. Plant Cell 17:444–463.

Pajerowska-Mukhtar KM, W Wang, Y Tada, N Oka, CL Tucker, JP Fonseca, X Dong 2012 The HSF-like transcription factor TBF1 is a major molecular switch for plant growth-to-defense transition. Curr Biol 22:103–112.

Park HC, ML Kim, YH Kang, JM Jeon, JH Yoo, MC Kim, CY Park, et al 2004 Pathogen- and NaCl-induced expression of the SCaM-4 promoter is mediated in part by a GT-1 box that interacts with a GT-1-like transcription factor. Plant Physiol 135:2150–2161.

Park J, HJ Lee, CI Cheon, SH Kim, YS Hur, C-K Auh, K-H Im, et al 2011 The *Arabidopsis thaliana* homeobox gene *ATHB12* is involved in symptom development caused by geminivirus infection. PLoS ONE 6:e20054.

Park MY, JY Kang, SY Kim 2011 Overexpression of AtMYB52 confers ABA hypersensitivity and drought tolerance. Mol Cells 31:447–454.

Pelaz S, GS Ditta, E Baumann, E Wisman, MF Yanofsky 2000 B and C floral organ identity functions require *SEPALLATA* MADS-box genes. Nature 405:200–203.

Penfield S, RC Meissner, AA Shoue, NC Carpita, MW Bevan 2001 *MYB61* is required for mucilage deposition and extrusion in the *Arabidopsis* seed coat. Plant Cell 13:2777–2791.

Pinyopich A, GS Ditta, B Savidge, SJ Liljegren, E Baumann, E Wisman, MF Yanofsky 2003 Assessing the redundancy of MADS-box genes during carpel and ovule development. Nature 424:85–88.

Ramírez V, A Agorio, A Coego, J García-Andrade, MJ Hernández, B Balaguer, PBF Ouwerkerk, I Zarra, P Vera 2011 MYB46 modulates disease susceptibility to *Botrytis cinerea* in *Arabidopsis*. Plant Physiol 155:1920–1935.

Rauf M, M Arif, H Dortay, LP Matallana-Ramírez, MT Waters, H Gil Nam, PO Lim, et al 2013 ORE1 balances leaf senescence against maintenance by antagonizing G2-like-mediated transcription. EMBO Rep 14:382–388.

Rawat R, J Schwartz, MA Jones, I Sairanen, Y Cheng, CR Andersson, Y Zhao, K Ljung, SL Harmer 2009 REVEILLE1, a Myb-like transcription factor, integrates the circadian clock and auxin pathways. Proc Natl Acad Sci USA 106:16883–16888.

Robatzek S, IE Somssich 2001 A new member of the *Arabidopsis* WRKY transcription factor family, *At*WRKY6, is associated with both senescence- and defence-related processes. Plant J 28:123–133.

Romera-Branchat M, JJ Ripoll, MF Yanofsky, S Pelaz 2012 The *WOX13* homeobox gene promotes replum formation in the *Arabidopsis thaliana* fruit. Plant J 73:37–49.

Saddic LA, BR Huvermann, S Bezhani, YH Su, CM Winter, CS Kwon, RP Collum, D Wagner 2006 The LEAFY target LMI1 is a meristem identity regulator and acts together with LEAFY to regulate expression of CAULIFLOWER. Development 133:1673–1682.

Shaikhali J, J de Dios Barajas-Lopéz, K Ötvös, D Kremnev, AS Garcia, V Srivastava, G Wingsle, et al 2012 The CRYPTOCHROME1-dependent response to excess light is mediated through the transcriptional activators ZINC FINGER PROTEIN EXPRESSED IN INFLORESCENCE MERISTEM LIKE1 and ZML2 in *Arabidopsis*. Plant Cell 24:3009–3025.

Shin R, AY Burch, KA Huppert, SB Tiwari, AS Murphy, TJ Guilfoyle, DP Schachtman 2007 The *Arabidopsis* transcription factor MYB77 modulates auxin signal transduction. Plant Cell 19:2440–2453.

Shinozaki K, K Yamaguchi-Shinozaki 2000 Molecular response to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223.

Siegfried KR, Y Eshed, SF Baum, D Otsuga, GN Drews, JL Bowman 1999 Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis*. Development 126:4117–4128.

Smith ZR, JA Long 2010 Control of *Arabidopsis* apical-basal embryo polarity by antagonistic transcription factors. Nature 464:423–426.

Song C, J Je, JK Hong, CO Lim 2014 Ectopic expression of an *Arabidopsis* dehydration-responsive element-binding factor *DREB2C* improves salt stress tolerance in crucifers. Plant Cell Rep 33:1239–1254.

Sozzani R, C Maggio, R Giordo, E Umana, JT Ascencio-Ibañez, L Hanley-Bowdoin, C Bergounioux et al 2010 The E2FD/DEL2 factor is a component of a regulatory network controlling cell proliferation and development in *Arabidopsis*. Plant Mol Biol 72:381–395.

Stone JM, X Liang, ER Nekl, JJ Stiers 2005 *Arabidopsis AtSPL14*, a plant-specific SBP-domain transcription factor, participates in plant development and sensitivity to fumonisin B1. Plant J 41:744–754.

Stone SL, LW Kwong, KM Yee, J Pelletier, L Lepiniec, RL Fischer, RB Goldberg, JJA Harada 2001 *LEAFY COTYLEDON2* encodes a B3 domain transcription factor that induces embryo development. Proc Natl Acad Sci USA 98:11806–11811.

Sugimoto K, S Takeda, H Hirochika 2003 Transcriptional activation mediated by binding of a plant GATA-type zinc finger protein AGP1 to the AG-motif (AGATCCAA) of the wound-inducible *Myb* gene *NtMyb2*. Plant J 36:550–564.

Takeda T, K Amano, MA Ohto, K Nakamura, S Sato, T Kato, S Tabata, C Ueguchi 2006 RNA interference of the *Arabidopsis* putative transcription factor *TCP16* gene results in abortion of early pollen development. Plant Mol Biol 61:165–177.

Teakle GR, IW Manfield, JF Graham, PM Gilmartin 2002 *Arabidopsis thaliana* GATA factors: organisation, expression and DNA-binding characteristics. Plant Mol Biol 50:43–57.

Uberti-Manassero NG, LE Lucero, IL Viola, AC Vegetti, DH Gonzalez 2012 The class I protein AtTCP15 modulates plant development through a pathway that overlaps with the one affected by CIN-like TCP proteins. J Exp Bot 63:809–823.

Wang H, U Avci, J Nakashima, MG Hahn, F Chen, RA Dixon 2010 Mutation of WRKY transcription factors initiates pith secondary wall formation and increases stem biomass in dicotyledonous plants. Proc Natl Acad Sci USA 107:22338–22343.

Wang H, LV Caruso, AB Downie, SE Perry 2004 The embryo MADS domain protein AGAMOUS-Like 15 directly regulates expression of a gene encoding an enzyme involved in gibberellin metabolism. Plant Cell 16:1206–1219.

Wang H, Q Xu, H Kong, Y Chen, JY Duan, WH Wu, YF Chen 2014 *Arabidopsis* WRKY45 transcription factor activates *PHOSPHATE TRANSPORTER1;1* expression in response to phosphate starvation. Plant Physiol 164:2020–2029.

Wang L, K Tsuda, M Sato, JD Cohen, F Katagiri, J Glazebrook 2009 *Arabidopsis* CaM binding protein CBP60g contributes to MAMP-induced SA accumulation and is involved in disease resistance against *Pseudomonas syringae*. PLoS Pathog 5:e1000301.

Wang L, K Tsuda, W Truman, M Sato, V Nguyen, F Katagiri, J Glazebrook 2011 CBP60g and SARD1 play partially redundant critical roles in salicylic acid signaling. Plant J 67:1029–1041.

Wawrzyńska A, M Lewandowska, A Sirko 2010 *Nicotiana tabacum* EIL2 directly regulates expression of at least one tobacco gene induced by sulphur starvation. J Exp Bot 61:889–900.

Wunderlich M, R Gross-Hardt, F Schoffl 2014 Heat shock factor HSFB2a involved in gametophyte development of *Arabidopsis thaliana* and its expression is controlled by a heat-inducible long non-coding antisense RNA. Plant Mol Biol 85:541–550.

Xu X, C Chen, B Fan, Z Chen 2006 Physical and functional interactions between pathogen-induced *Arabidopsis* WRKY18, WRKY40, and WRKY60 transcription factors. Plant Cell 18:1310–1326.

Zhang B, L Wang, L Zeng, C Zhang, H Ma 2015 *Arabidopsis* TOE proteins convey a photoperiodic signal to antagonize CONSTANS and regulate flowering time. Genes Dev 29:975–987.

Zhao Y, L Xing, X Wang, YJ Hou, J Gao, P Wang, CG Duan, X Zhu, et al 2014 The ABA receptor PYL8 promotes lateral root growth by enhancing MYB77-dependent transcription of auxin-responsive genes. Sci Signal 7:ra53.

Zheng X-Y, NW Spivey, W Zeng, P-P Liu, ZQ Fu, DF Klessig, SY He, X Dong 2012 Coronatine promotes *Pseudomonas syringae* virulence in plants by activating a signaling cascade that inhibits salicylic acid accumulation. Cell Host Microbe 11:587–596.

Zhong R, ZH Ye 2012 MYB46 and MYB83 bind to the SMRE sites and directly activate a suite of transcription factors and secondary wall biosynthetic genes. Plant Cell Physiol 53:368–380.

Zhu J-K 2000 Genetic analysis of plant salt tolerance using *Arabidopsis*. Plant Physiol 124:941–948.

——— 2003 Regulation of ion homeostasis under salt stress. Curr Opin Plant Biol 6:441–445.

Zhu J-K, J Liu, L Xiong 1998 Genetic analysis of salt tolerance in *Arabidopsis*: evidence for a critical role of potassium nutrition. Plant Cell 10:1181–1191.

Zhu Q, J Zhang, X Gao, J Tong, L Xiao, W Li, H Zhang 2010 The *Arabidopsis* AP2/ERF transcription factor *RAP26* participates in ABA, salt and osmotic stress responses. Gene 457:1–12.

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Q9. AU: Has phyB been expanded correctly? Also, for consistency, R and FR have been expanded as red light and far-red light throughout; okay?
Q10. AU: Is "and promote ABA catabolism in the seeds" okay as edited?
Q11. AU: Could "PHYTOCHROME-INTERACTING FACTOR3–LIKE5" read "PHYTOCHROME-INTERACTING FACTOR 3–LIKE 5" (i.e., with spaces before the numbers)?
Q12. AU: Is "seedlings were transferred" okay as edited? If not, please otherwise clarify what was transferred.
Q13. AU: Please ensure that the first two sentences of the "Light Sources" section are okay as edited.
Q14. AU: Has LED been expanded correctly?
Q15. AU: Correct to edit to read "2-kb," or did you not mean "kilobase"? Also, is "−2000-bp" okay as edited? By any chance did you mean "~2000-bp"? Perhaps the minus sign should be omitted, to read "(2000-bp)"? If so, could "(2000-bp)" be omitted, as it is redundant?
Q16. AU: Should "the effect on dormancy breaking" read "the effect of dormancy breaking"?
Q17. AU: Should "Red light promoted germination when seeds were incubated in 10 and 100 μM GA compared with the dark control" read "Red light promoted more germination when seeds were incubated in 10 and 100 μM GA compared with the dark control" or something similar? Perhaps "Red light promoted germination when seeds were incubated in 10 and 100 μM GA but not when they were incubated with the dark control"? I don't quite understand the comparison.
Q18. AU: Should "Seeds of *sos2*, *sos3*, and *abi2* germinated less than the WT" read "Seeds of *sos2*, *sos3*, and *abi2* germinated less frequently than those of the WT" or something similar? Again, the comparison is not quite clear to me.
Q19. AU: Okay to edit to read "Continuous Red Light" in the head here?
Q20. AU: In the figure 3 legend, is "Shown are the fluence rate response of hypocotyl length (*A*) and cotyledon opening (*B*) under continuous red light" okay as edited? Or should it read "Shown are the fluence rate response of hypocotyl length (*A*) and cotyledon opening under continuous red light (*B*)"?
Q21. AU: In table A1: (*a*) Correct to change the year of publication from Wawrzyńska et al. 2009 to 2010? (*b*) Correct to change Veylder et al. 2002 to De Veylder et al. 2002? (*c*) Correct to change Murmu et al. 2013 to Murmu et al. 2014? (*d*) Correct to change Newman et al. 2003 to Newman et al. 2004? (*e*) Correct to change Uberti-Manassero et al. 2011 to Uberti-Manassero et al. 2012? (*f*) Because two different Park et al. 2011 references were included, on three occasions in table A1 "Park et al. 2011" was changed to "J. Park et al. 2011; M. Y. Park et al. 2011"; okay? If not, please specify which Park et al. 2001 reference was meant in each of the three instances.
Q22. AU: Okay to edit "vascular" to read "vasculature," so that all of the elements in the list are nouns? If not, please clarify what was meant.
Q23. AU: Is "are expressed in a broad range of plant tissues and organs, including senescent leaves, floral organs, the root elongation zone, and guard cells, as well as during the late stages of seed development and pollen development" okay as edited, to avoid the implication that the late stages of seed development and pollen development are plant tissues or organs?
Q24. AU: Should "*SOS2* expression was upregulated" read "*SOS2* expression is upregulated"?
Q25. AU: Is "after exposure to PAC" okay as edited?
Q26. AU: Should "Mutants in *SOS2*, *SOS3*, and *ABI2*" read "Mutants in *SOS2*, *SOS3*, of *ABI2*"?
Q27. AU: Is "light can exert its function by interfering with the PIF1-ATHB1 signaling pathway, promoting photomorphogenesis" okay as edited? Perhaps ""light can exert its function by interfering with the PIF1-ATHB1 signaling pathway, which promotes photomorphogenesis" would be better?
Q28. AU: Is "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" okay as edited?
Q29. AU: Please expand FT, if possible.
Q30. AU: Should "genetic" be removed here, to read "The relationship between these genes and the molecular mechanism of action of these proteins"?
Q31. AU: Is "The SOS2 has been previously shown to be involved in salt, reactive oxygen species, and flowering signaling" okay as edited?

Q32. AU: Correct to change the year of publication from de Montaigu et al. 2014 to 2015?
Q33. AU: Is the publication information added to Du and Wang 2015 accurate as edited?
Q34. AU: Correct to change the year of publication from Duclercq et al. 2010 to Duclercq et al. 2011?
Q35. AU: Is the publication information added to Ha et al. 2010 accurate?