ABA receptor PYL9 promotes drought resistance and leaf senescence

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Drought stress is an important environmental factor limiting plant productivity. In this study, we screened drought-resistant transgenic plants from 65 *promoter-pyrabactin resistance 1-like* (*PYL*) abscisic acid (ABA) receptor gene combinations and discovered that *pRD29A::PYL9* transgenic lines showed dramatically increased drought resistance and drought-induced leaf senescence in both *Arabidopsis* and rice. Previous studies suggested that ABA promotes senescence by causing ethylene production. However, we found that ABA promotes leaf senescence in an ethylene-independent manner by activating sucrose nonfermenting 1-related protein kinase 2s (SnRK2s), which subsequently phosphorylate ABA-responsive element-binding factors (ABFs) and Related to ABA-Insensitive 3/VP1 (RAV1) transcription factors. The phosphorylated ABFs and RAV1 up-regulate the expression of *senescence-associated genes*, partly by up-regulating the expression of *Oresara 1*. The *pyl9* and *ABA-insensitive 1-1* single mutants, *pyl8-1pyl9* double mutant, and *snrk2.2/3/6* triple mutant showed reduced ABA-induced leaf senescence relative to the WT, whereas *pRD29A::PYL9* transgenic plants showed enhanced ABA-induced leaf senescence. We found that leaf senescence may benefit drought resistance by helping to generate an osmotic potential gradient, which is increased in *pRD29A::PYL9* transgenic plants and causes water to preferentially flow to developing tissues. Our results uncover the molecular mechanism of ABA-induced leaf senescence and suggest an important role of PYL9 and leaf senescence in promoting resistance to extreme drought stress.

drought stress | abscisic acid | PYL | dormancy | *Arabidopsis*

Cell and organ senescence causes programmed cell death to regulate the growth and development of organisms. In plants, leaf senescence increases the transfer of nutrients to developing and storage tissues. Recently, studies on transgenic tobacco showed that delayed leaf senescence increases plant resistance to drought stress (1). However, the senescence and abscission of older leaves and subsequent transfer of nutrients are known to increase plant survival under abiotic stresses, including drought, low or high temperatures, and darkness (2, 3). Senescence mainly develops in an age-dependent manner and is also triggered by environmental stresses and phytohormones, such as abscisic acid (ABA), ethylene, salicylic acid, and jasmonic acid, but delayed by cytokinin (4).

Senescence-associated genes (*SAG*s) are induced by leaf senescence. The expression of *SAG*s is tightly controlled by several senescence-promoting, plant-specific NAC (NAM, ATAF1, and CUC2) transcription factors, such as Oresara 1 (ORE1) (5), Oresara 1 sister 1 (ORS1) (6), and AtNAP (7). Environmental stimuli and phytohormones may regulate leaf senescence through NACs. Phytochrome-interacting factor 4 (PIF4) and PIF5 transcription factors promote dark-induced senescence by activating *ORE1* expression (8). The expression of *ORE1*, *AtNAP*, and *OsNAP* (ortholog of *AtNAP*) is up-regulated by ABA by an unknown molecular mechanism (7, 9).

ABA is an important hormone that regulates plant growth and development and responses to abiotic stresses, such as drought and high salinity (10). Although it is well-known that ABA promotes leaf senescence, the underlying molecular mechanism is obscure. Previous studies suggested that ABA promotes senescence by causing ethylene biosynthesis (11). ABA induces expression of several *SAG*s and yellowing of the leaves, which are typical phenomena associated with leaf senescence (9, 12). ABA is sensed by the pyrabactin resistance 1 and pyrabactin resistance 1-like (PYL)/regulatory component of abscisic acid receptor proteins (13, 14). The ABA-bound PYLs prevent clade A protein phosphatase type 2Cs (PP2Cs) from inhibiting the sucrose nonfermenting 1-related protein kinase 2s (SnRK2s). ABA-activated SnRK2s phosphorylate transcription factors, such as ABA-responsive element-binding factors (ABFs), and these phosphorylated ABFs regulate the expression of ABA-responsive genes (15). In *Arabidopsis*, 14 PYLs function diversely and redundantly in ABA and drought-stress signaling (16–19). Understanding how each

Significance

We identified transgenic plants that are extremely resistant to drought from a large-scale screening of transgenic plants overexpressing the pyrabactin resistance 1-like (PYL) family of abscisic acid (ABA) receptors. We explored how these plants resist drought by examining both short-term responses, such as stomatal closure, and long-term responses, such as senescence. The physiological roles of ABA-induced senescence under stress conditions and the underlying molecular mechanism are unclear. Here, we found that ABA induces senescence by activating ABA-responsive element-binding factors and Related to ABA-Insensitive 3/VP1 transcription factors through core ABA signaling. Our results suggest that PYL9 promotes drought resistance by not only limiting transpirational water loss but also, causing summer dormancy-like responses, such as senescence, in old leaves and growth inhibition in young tissues under severe drought conditions.

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PYL affects drought resistance would have both basic and applied importance. Because constitutive overexpression of stress tolerance genes might inhibit plant growth, the use of stress-inducible or organ-specific promoters should be advantageous.

In this study, we evaluated the drought resistance of 14 transgenic *Arabidopsis*-overexpressing *PYL*s driven by the constitutive *35S* cauliflower mosaic virus (CaMV) promoter, the stress-inducible *RD29A* promoter, the guard cell-specific *GC1* and *ROP11* promoters (20, 21), and the green tissue-specific *ribulose bisphosphate carboxylase small subunit* (*RBCS*) *RBCS-1A* promoter (22). We found that, relative to the WT and all other combinations, *pRD29A::PYL9* transgenic *Arabidopsis* plants had both greater drought resistance and accelerated drought- or ABA-induced leaf senescence. We discovered that ABA induces leaf senescence in an ethylene-independent manner and that PYL9 promotes ABA-induced leaf senescence by inhibiting PP2Cs and activating SnRK2s. ABA-activated SnRK2s then mediate leaf senescence by phosphorylation of Related to ABA-Insensitive 3/VP1 (RAV1) and ABF2 transcription factors, which then up-regulate the expression of *ORE1* and other *NAC* transcription factors, thereby activating expression of *SAG*s. Previous research has suggested that transgenic plants with delayed leaf senescence are more resistant to drought stress (1). However, we examined the importance of ABA-induced leaf senescence under drought stress and found that the increased leaf senescence in *pRD29A::PYL9* transgenic plants apparently helps generate a greater osmotic potential gradient, which causes water to preferentially flow to developing tissues. Therefore, hypersensitivity to ABA leads to increased senescence and death of old leaves but survival of young tissues during severely limited water conditions through promoting summer dormancy-like responses (23).

Results

Screening Transgenic *Arabidopsis* for Drought-Stress Survival. In *Arabidopsis*, the expression of many PYLs is down-regulated by osmotic stress, which may constitute a negative feedback loop that reduces drought responses (24). In principle, expression modifications that allow general, tissue-specific, or stress-inducible overexpression of *PYL*s should amplify ABA signaling and increase drought resistance in transgenic plants. Based on this assumption, the following five promoters were used for *PYL* overexpression: the *35S* CaMV promoter, the stress-inducible *RD29A* promoter, and the tissue-specific promoters *GC1*, *ROP11*, and *RBCS-1A*. Transgenic plants from a total of 65 different *promoter-PYL* combinations were generated and evaluated for drought-stress resistance (Fig. 1*A* and Fig. S1). The results indicated that drought resistance was increased by *PYL*s driven by *35S*, *pRD29A*, and *pGC1* promoters but not by the *pRBCS-1A* promoter. The *pGC1*-driven lines performed better than the lines driven by the other guard cell-specific promoter, *pROP11*. Among the combinations, survival was highest for *35S::PYL3/9/13*, *pRD29A::PYL7/9*, and *pGC1::PYL3/5/6/7/11* lines. These top drought-resistant lines preferentially cluster on the monomeric PYLs, especially PYL7 and PYL9, which have very high affinities to ABA (16).

We also evaluated the PYLs in a transient expression assay in *Arabidopsis* protoplasts (25). Highly ABA-induced 1 (HAI1), HAI2, and HAI3 PP2Cs interact with only a few of these PYLs, even in the presence of 10 μM ABA (24). Transfections of HAI1 inhibited *RD29B-LUC* expression. PYL3, PYL4, PYL6, or PYL13 did not inhibit HAI1, whereas cotransfections of HAI1 with PYL5, PYL7, or PYL9 strongly enabled the ABA-dependent induction of *RD29B-LUC* expression (Fig. S2*A*). Furthermore, cotransfections of PYL9 together with each of the PP2Cs strongly enabled the ABA-dependent induction of *RD29B-LUC* (Fig. S2*B*). Similar to the ABA-independent inhibition of ABA-insensitive 1 (ABI1) by PYL10 (16, 25), PYL9 can partially inhibit HAI3 activity in the absence of exogenous ABA. These results suggested that PYL9 strongly inhibits the phosphatase activities of clade A PP2Cs in plant cells, an inhibition that activates the core ABA signaling pathway and is presumably involved in the drought resistance of *pRD29A::PYL9* transgenic plants.

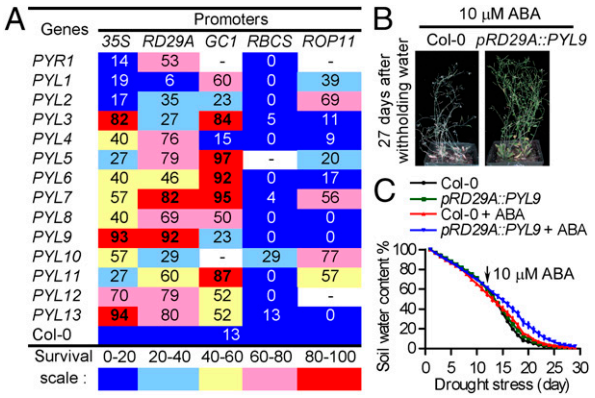

Fig. 1. Screening *PYL* transgenic lines for resistance to drought stress in *Arabidopsis*. (*A*) Drought-resistance screening of *PYL* transgenic *Arabidopsis*. Fourteen *Arabidopsis PYL*s and five promoters were used to generate 65 transgenic plants with different *promoter-PYL* combinations. Two-week-old plants were subjected to drought stress by withholding water for 20 d. Survival rates were calculated at 2 d after rehydration. –, No transgenic plants were obtained. (*B* and *C*) *pRD29A::PYL9* transgenic plants exhibit improved drought-stress resistance with ABA treatment. Plants were subjected to drought stress after flowering. After water was withheld for 12 d, plants were treated once with 10 μM ABA. (*B*) Images of representative seedlings. (*C*) Soil water content during the drought-stress period. Error bars indicate SEM ($n \geq 4$).

PYL9 transcripts were significantly more abundant in *pRD29A::PYL9* lines than in the WT under drought stress (Fig. S3*A*). Application of ABA after water was withheld for 12 d significantly increased the drought resistance of the *pRD29A::PYL9* transgenic plants after flowering (Fig. 1 *B* and *C*). The delayed wilting and drying in *pRD29A::PYL9* transgenic plants was correlated with a reduction in water loss from the soil, indicating a reduction in transpiration. This result revealed that *PYL9*, when driven by the *pRD29A* promoter, is useful for generating transgenic plants that are extremely resistant to drought when treated with ABA or ABA-mimicking compounds (26, 27). We chose the *pRD29A::PYL9* transgenic lines for additional study (Fig. S3*B*).

***pRD29A::PYL9* Confers Drought Resistance to Both *Arabidopsis* and Rice.** The *Arabidopsis pRD29A::PYL9* transgenic lines also exhibited increased drought resistance before flowering under short-day conditions (Fig. 2*A*). The greater drought-stress survival of *pRD29A::PYL9* lines was associated with reduced water loss (Fig. 2*B*), reduced cell membrane damage (Fig. 2*C*), reduced transpiration rate and stomatal conductance (Fig. S3 *C* and *D*), enhanced photosynthetic rate and water use efficiency (Fig. S3 *E* and *F*), reduced accumulation of toxic hydrogen peroxide, and enhanced activities of antioxidant enzymes (Fig. S3*G*). As a result, the total biomass was greater in *pRD29A::PYL9* lines than in the WT after drought treatment but did not differ statistically from the WT in the absence of drought treatment (Fig. S3*H*). These results showed that the *pRD29A::PYL9* transgene confers drought resistance to *Arabidopsis* in at least two ways (i.e., by reducing water loss and by reducing oxidative injury).

To determine whether *pRD29A::PYL9* may confer drought resistance in crop plants, we generated *pRD29A::PYL9* transgenic rice (*Oryza sativa* L.) in the *japonica* variety Zhonghua 11 (ZH11), in which *PYL9* expression was dramatically induced by drought stress (Fig. S3*I*). *pRD29A::PYL9* transgenic rice exhibited increased drought resistance (Fig. 2*D*). After a 2-wk drought treatment, nearly 50% of *pRD29A::PYL9* transgenic rice growing in soil survived, but only about 10% of the ZH11 WT plants survived (Fig. S3*J*). Although *pRD29A::PYL9* increased survival, total biomass, and cell

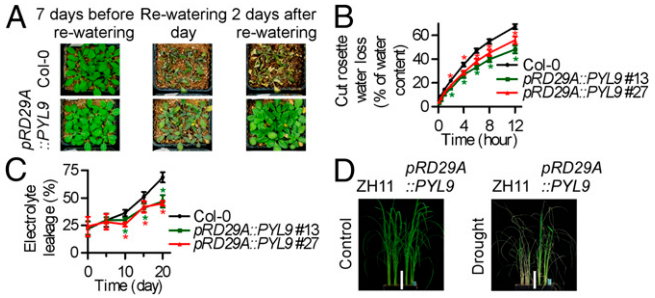

Fig. 2. *pRD29A::PYL9* transgenic plants exhibit improved drought-stress resistance in both *Arabidopsis* and rice. (*A*) *pRD29A::PYL9* confers drought resistance in *Arabidopsis*. Water was withheld from 3-wk-old *Arabidopsis* plants for 20 d under short-day conditions before watering was resumed. Representative images show plants 7 d before rewatering, on the day of rewatering, and 2 d after watering was resumed. (*B*) Cumulative transpirational water loss from rosettes of the WT (Col-0) and *pRD29A::PYL9* transgenic *Arabidopsis* at the indicated times after detachment. Error bars indicate SD ($n = 3$). (*C*) Electrolyte leakage of the WT (Col-0) and *pRD29A::PYL9* transgenic *Arabidopsis* at the indicated days after water was withheld. Error bars indicate SD ($n = 3$). (*D*) *pRD29A::PYL9* confers drought resistance in rice. Water was withheld from 4-wk-old rice plants for 14 d. Plants were photographed 14 d after watering was resumed. $^*P < 0.05$ (Student's *t* test).

membrane integrity of transgenic rice under drought conditions, the transgene did not adversely affect plant growth and development under well-watered conditions (Fig. S3 *H* and *J*). These results showed that *pRD29A::PYL9* increases drought resistance in rice without retarding growth under well-watered conditions.

PYL9 Promotes ABA-Induced Leaf Senescence in Both *Arabidopsis* and Rice. After the drought treatment in our experiments, it was evident that older leaves of *pRD29A::PYL9* lines became yellow, sooner than in the Columbia-0 (Col-0) WT (Fig. 2*A* and Fig. S4*A*). ABA-induced leaf yellowing was also accelerated in *pRD29A::PYL9* transgenic plants (Fig. 3*A*). Consistent with its visible phenotypes, *pRD29A::PYL9* lines had a lower chlorophyll level than the Col-0 WT after treatment with 20 μM ABA (Fig. 3*B*). *SAG*s are molecular markers of senescence and especially, ABA-induced senescence (12). Consistent with the elevated *PYL9* expression (Fig. S4*B*), both *SAG12* and *SAG13* were more strongly induced after ABA treatment in mature leaves of *pRD29A::PYL9* lines than in those of the WT (Fig. 3*C* and Fig. S4*C*). This result showed that *pRD29A::PYL9* accelerates ABA-induced leaf senescence of older leaves in *Arabidopsis*.

Enhanced drought survival and senescence are both associated with ABA signaling. To verify that PYL9 mediated these responses by making the plants hypersensitive to ABA, we analyzed the leaf yellowing of the *pyl9* transferred DNA (T-DNA) insertion mutant after ABA treatment of plate-grown seedlings. ABA-induced leaf yellowing was lower in the *pyl9* mutant than in the WT under low light (30–45 μmol m^{-2} s^{-1}) (Fig. 3 *D* and *E*) but not under normal light (80–100 μmol m^{-2} s^{-1}) (Fig. S4*D*) because of genetic redundancy. Moreover, the *pyl8-1pyl9* double mutant was less sensitive than the *pyl9* mutant to ABA-induced leaf yellowing, indicating that PYL9 and PYL8 function together in ABA-induced leaf senescence. Furthermore, *PYL9* is highly expressed in senescent leaves and stamens according to the *Arabidopsis* electronic fluorescent pictograph (eFP) browser, which is consistent with the expression of *SAG12* (Fig. S5). These results confirm that *PYL9* functions in both ABA-induced drought survival and senescence by hypersensitizing *Arabidopsis* to ABA.

ABA-induced leaf yellowing was also accelerated in *pRD29A::PYL9* transgenic rice (Fig. 3*F* and Fig. S4*E*). After ABA treatment, severe yellowing was evident in the third oldest leaves of the *pRD29A::PYL9* lines but not those of the ZH11 WT. Moreover, two *SAG*s, *Osh36* and *Osl85* (9), were more strongly induced after ABA treatment in the third-oldest leaves of *pRD29A::PYL9* rice lines than in those of the ZH11 WT (Fig. 3*G*). These results show that PYL9 also mediates ABA-induced leaf senescence in rice.

ABA Induces Leaf Senescence Through the Core ABA Signaling Pathway. To investigate whether ABA induction of senescence requires ethylene, we treated protoplasts with the ethylene biosynthesis inhibitor aminoethoxyvinylglycine (AVG). We found that AVG treatment decreased *SAG12-LUC* expression in the absence of ABA (Fig. S6*A*), consistent with the known role of ethylene in promoting senescence. However, AVG treatment did not inhibit either ABA-induced or PYL9-enhanced *SAG12-LUC* expression (Fig. S6*A*). We found that ABA-induced leaf yellowing was not reduced in the ethylene-resistant mutants *ein2-1* and *ein3-1* (Fig. S6*B*). Furthermore, ABA-induced and PYL9-enhanced *SAG12-LUC* expression was not blocked in *ein2-1* mutant protoplasts (Fig. S6*C*). These results suggest that the induction of senescence by ABA is not mediated through ethylene.

We generated transgenic *Arabidopsis* plants expressing HA- and YFP-tagged PYL9 under the native *PYL9* promoter (*ProPYL9:PYL9-HA-YFP*) (Fig. S7*A*) and isolated PYL9-associated proteins using tandem affinity purification (Fig. S7*B* and Dataset S1). The associated proteins mainly included several PP2Cs, such as HAB2, PP2CA, and ABI1, in an ABA- or osmotic stress-enhanced manner (Fig. S7*C*). PYL9 interacted with all PP2Cs tested in an ABA-independent manner in yeast two-hybrid (Y2H) assays (Fig. S7*D*). We fused the 788-bp fragment of the *SAG12* promoter (*SAG12-LUC*) (Fig. S7*E*) to the *LUC* reporter gene and used the construct as a senescence-responsive reporter. The 788-bp *SAG12* promoter contains the 9-mer sequence T(TAG)(GA)CGT(GA)(TCA)(TAG),

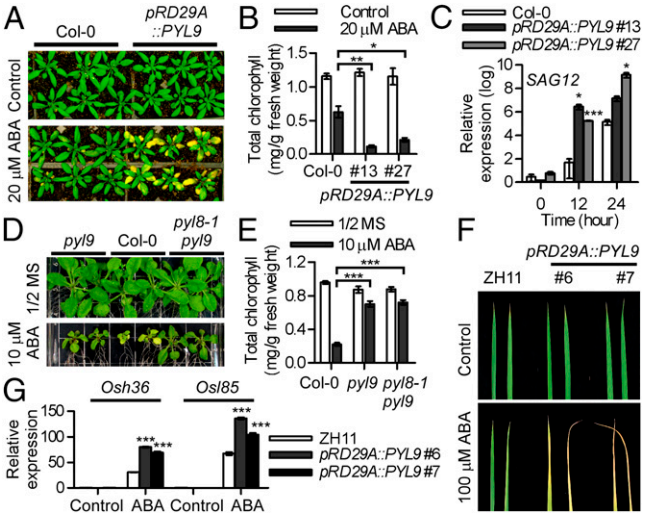

Fig. 3. PYL9 promotes ABA-induced leaf senescence in both *Arabidopsis* and rice. (*A* and *B*) *pRD29A::PYL9* accelerates ABA-induced leaf senescence in *Arabidopsis*. (*A*) Plants were photographed 2 d after they were sprayed with ABA. (*B*) Chlorophyll content in mature leaves of WT (Col-0) and *pRD29A::PYL9* lines. Error bars indicate SEM ($n = 3$). (*C*) Expression of *SAG12* in *pRD29A::PYL9* lines. The expression level of *SAG12* in Col-0 without ABA treatment was set at one. Error bars indicate SEM ($n = 3$). (*D* and *E*) Leaf growth and chlorophyll content of the WT (Col-0), the *pyl9* mutant, and the *pyl8-1pyl9* double mutant were documented at 17 d after the seedling were transferred to Murashige and Skoog (MS) medium with or without 10 μM ABA and grown under low light (30–45 μmol m^{-2} s^{-1}). Error bars indicate SEM ($n = 3$). (*F*) *pRD29A::PYL9* accelerates ABA-induced leaf senescence in rice. The third-oldest leaves of WT (ZH11) and *pRD29A::PYL9* rice lines were photographed. (*G*) Expression of *Osh36* and *Osl85* in *pRD29A::PYL9* rice lines. The expression level of *SAG*s in ZH11 without ABA treatment was set at one. $^*P < 0.05$ (Student's *t* test); $^{**}P < 0.01$ (Student's *t* test); $^{***}P < 0.001$ (Student's *t* test).

which is the preferred binding site of ORE1 (28). All PP2Cs decreased *SAG12-LUC* expression in the presence of ABA (Fig. S7 *F* and *G*). The inhibition of *SAG12-LUC* expression by ABI1 can be released by coexpression of *PYL9* (Fig. S7*F*). Moreover, ABA-induced leaf yellowing was weaker in the *abi1-1* mutant than in the Landsberg *erecta* (L*er*) WT (Fig. 4*A* and Fig. S7*H*). The *abi1-1* mutant is ABA-resistant and contains a G180D point mutation. PYLs do not interact with or inhibit ABI1^{G180D}, even in the presence of ABA (13). These results indicated that PP2Cs inhibit ABA-induced senescence.

We hypothesized that PYL9 may promote leaf senescence by activating SnRK2s. The *snrk2.2/3/6* triple mutant was insensitive to ABA-induced leaf yellowing (Fig. 4*B* and Fig. S7*I*). Moreover, *SAG12-LUC* expression was not enhanced by ABA treatment in *snrk2.2/3/6* triple-mutant protoplasts, but ABA induction of *SAG12-LUC* expression in such protoplasts could be recovered by transfection of *SnRK2.6* (Fig. 4*C*), suggesting that ABA-induced *SAG12* expression depends on the SnRK2s. The activation of *SAG12-LUC* expression by PYL9 was abolished in *snrk2.2/3/6* triple-mutant protoplasts, but such expression was also recovered with transfection of *SnRK2.6*. The activation of *SAG12-LUC* expression by SnRK2.6 was blocked by transfection of *ABI1*, which can be released by *PYL9*. These results suggest that PYL9 promotes ABA-induced leaf yellowing and *SAG12* expression through core ABA signaling.

Phosphorylation of ABFs by SnRK2s Facilitates ABA-Induced *SAG12* Promoter Activity in Leaf Protoplasts. ABA-activated SnRK2s phosphorylate ABF transcription factors, which activate these factors and enable them to regulate expression of ABA-responsive genes (15). To identify the transcription factors involved in ABA-induced leaf senescence, we cloned several ABFs, including ABF2, ABI5, enhanced EM level (EEL), and AREB3, and coexpressed them with *SAG12-LUC* in Col-0 leaf protoplasts (Fig. 4*D* and Fig. S8*A*). We found that *SAG12-LUC* expression in protoplasts was dramatically increased by ABF2, was less dramatically but significantly increased by ABI5 and EEL, and was not increased by AREB3 (Fig. 4*D*). The phosphorylation of ABF2 at amino acid residues S26, S86, S94, and T135 is important for stress-responsive gene expression in *Arabidopsis*, and these sites are putatively phosphorylated by SnRK2s (15, 25). Expression of *SnRK2.6* significantly enhanced the ability of ABF2 to increase *SAG12-LUC* expression in the presence of ABA. Furthermore, ABF2^{S26DS86DS94DT135D} constitutively increased *SAG12-LUC* expression in the *snrk2.2/3/6* triple-mutant protoplasts (Fig. 4*E*). These results suggested that phosphorylation of ABFs by SnRK2s promotes activity of the ABA-induced leaf senescence pathway.

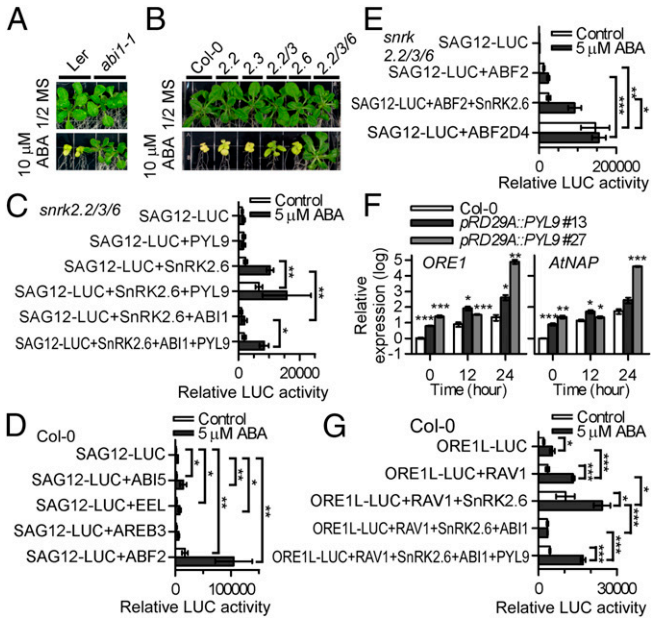

Fig. 4. Core ABA signaling promotes ABA-induced leaf senescence. (*A* and *B*) Leaf growth of the WT [Landsberg *erecta* (L*er*)]; the *abi1-1* mutant; the WT (Col-0); *snrk2.2*, *snrk2.3*, and *snrk2.6* single mutants; *snrk2.2/3* double mutant; and *snrk2.2/3/6* triple mutant at 13 d after seedlings were transferred to Murashige and Skoog (MS) medium with or without 10 μM ABA. (*C*) *SAG12-LUC* expression in *snrk2.2/3/6* triple-mutant protoplasts cotransformed with *SnRK2.6*, *ABI1*, and *PYL9*. Error bars indicate SEM ($n \geq 3$). (*D*) *SAG12-LUC* expression in Col-0 protoplasts cotransformed with *ABI5*, *EEL*, *AREB3*, and *ABF2*. Error bars indicate SEM ($n \geq 3$). (*E*) *SAG12-LUC* expression in *snrk2.2/3/6* triple-mutant protoplasts cotransformed with *SnRK2.6*, *ABF2*, and *ABF2*^{S26DS86DS94DT135D}. Error bars indicate SEM ($n = 4$). (*F*) Expression of *ORE1* and *AtNAP* in *pRD29A::PYL9* lines. The expression level of *ORE1* and *AtNAP* in Col-0 without ABA treatment was set at one. Error bars indicate SEM ($n = 3$). (*G*) *ORE1L-LUC* expression in Col-0 protoplasts cotransformed with *RAV1*, *SnRK2.6*, *ABI1*, and *PYL9*. Error bars indicate SEM ($n = 3$). *$P < 0.05$ (Student's *t* test); **$P < 0.01$ (Student's *t* test); ***$P < 0.001$ (Student's *t* test).

Leaf senescence is promoted by several NAC transcription factors, such as ORE1 (5), ORS1 (6), and AtNAP (7). ABA-induced leaf senescence was reported to be delayed in *ore1* mutant leaves (29). *SAG12-LUC* expression was clearly increased by ORE1 and AtNAP and slightly increased by ORS1 (Fig. S8*B*). The *ORE1* and *AtNAP* promoter regions contain several abscisic acid-responsive element (ABRE) motifs and RAV1 binding sites (Fig. S8*C*). ABRE motifs are the binding sites for the ABF transcription factors, which our results suggested to be positive regulators of senescence (Fig. 4 *D* and *E*). ABA-activated SnRK2s phosphorylate RAV1 (30), which positively regulates leaf senescence in *Arabidopsis* (31). According to the *Arabidopsis* eFP browser, expression of *ORE1*, *ORS1*, and *AtNAP* is enhanced by ABA (Fig. S8*D*). ABA treatment, indeed, induced the expression of *ORE1* and *AtNAP* in mature leaves, and the expression levels were higher in *pRD29A::PYL9* lines compared with those of the WT (Fig. 4*F*). We fused a 3,984-bp fragment of the *ORE1* promoter to the *LUC* reporter gene (*ORE1L-LUC*) (Fig. S8*E*) to use as a senescence-responsive reporter. According to the AthaMap, the 3,984-bp *ORE1* promoter contains multiple RAV1(1) [gCaACA(g/t)(a/t)] and RAV1(2) [caCCTG(a/g)] motifs, which are the preferred binding sites for RAV1. The *ORE1L-LUC* expression was enhanced by RAV1 and SnRK2.6 and repressed by ABI1 (Fig. 4*G*). Expression of *PYL9* released the inhibition of ABI1 on *ORE1-LUC* expression in an ABA-dependent manner. These results suggested that ABA core signaling up-regulates expression of *SAG*s through phosphorylation of both ABFs and RAV1 transcription factors.

Stressed *pRD29A::PYL9* Transgenic Plants Display Enhanced Osmotic Potential Gradients Between Senescing Leaves and Buds. *pRD29A::PYL9* transgenic plants are hypersensitive to ABA-induced leaf senescence (Fig. 3). In these plants, leaf yellowing spreads from older to younger leaves (Fig. 5*A*). Leaf wilting in *pRD29A::PYL9* transgenic plants was observed after 3 d of continuous ABA treatment, even in plants that were well-watered (Fig. 5*A*). This unusual event suggests that water transport to senescing leaves was reduced or blocked. Water moves from areas of high water potential to areas of low water potential, and plants control water potential, in part, by regulating osmotic potential ($\Psi\pi$). We found that ABA treatment reduced the osmotic potential in the developing bud tissue but not in the old leaves (Fig. 5*B*). The osmotic potential was lower in developing tissues of *pRD29A::PYL9* lines than in the WT but did not differ in old leaves of the transgenic plants vs. the WT. Thus, the osmotic potential gradient was greater in *pRD29A::PYL9* lines than in the WT. As noted above, this gradient would cause water to move preferentially to developing tissues but not to senescing leaves, especially in *pRD29A::PYL9* lines. Senescence, which is associated with the remobilization of carbohydrate and nitrogen from the senescing tissue to the developing or storage tissues, contributes

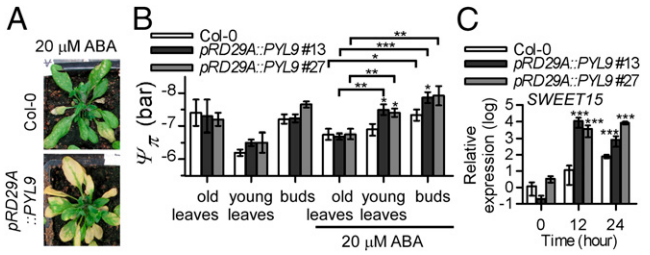

Fig. 5. *pRD29A::PYL9* transgenic plants exhibit an increased within-plant osmotic potential gradient. (*A* and *B*) *pRD29A::PYL9* accelerates ABA-induced drying in senescing leaves under well-watered conditions. Four-week-old *Arabidopsis* plants growing in soil were sprayed with 20 μM ABA plus 0.2% Tween-20. (*A*) Plants were photographed 4 d after they were sprayed with ABA. (*B*) *pRD29A::PYL9* transgenic plants exhibit improved osmoregulation in sink tissues. Samples were collected 2 d after ABA was sprayed. Error bars indicate SEM ($n = 6$). (*C*) Expression of *SWEET15* in *pRD29A::PYL9* lines. The expression level of *SWEET15* in Col-0 without ABA treatment was set at one. Error bars indicate SEM ($n = 3$). $^*P < 0.05$ (Student's *t* test); $^{**}P < 0.01$ (Student's *t* test); $^{***}P < 0.001$ (Student's *t* test).

to osmotic potential regulation. Carbohydrate is transported as sucrose to sink tissues through the phloem. The key step for phloem loading is sucrose efflux, which is mediated by SWEET proteins (32). We determined that *SWEET15*/*SAG29* is induced in senescing *Arabidopsis* leaves. Induction of *SWEET15* expression is greater in mature leaves of *pRD29A::PYL9* lines than in those of the WT after ABA treatment (Fig. 5*C*), suggesting that *pRD29A::PYL9* lines have an increased ability to mobilize sucrose from senescing leaves.

Core ABA Signaling Promotes Growth Inhibition and the Expression of Cuticular Wax Biosynthesis Genes. To help plants survive extreme environmental conditions, ABA promotes growth inhibition and dormancy (10). The *pRD29A::PYL9* lines showed a stronger seed dormancy and growth inhibition than the Col-0 WT under ABA treatment (Fig. S9 *A*–*C*), whereas the seed dormancy and rosette growth of *pyl8-1pyl9* and *snrk2.2/3/6* were less sensitive to ABA treatment (Fig. S9 *A*, *D*, and *E*). The *pRD29A::PYL9* rice lines also showed a more severe growth inhibition than the ZH11 WT in response to the ABA treatment (Fig. S9*F*). These results indicated that PYL9 promotes, through the core ABA signaling pathway, ABA-induced seed dormancy and growth inhibition of buds.

ABA promotes stomatal closure to reduce water loss. To protect plants from nonstomatal water loss, ABA induces the accumulation of cuticular wax by up-regulating wax biosynthetic genes (33). The *3-ketoacyl-CoA synthetase 2*, *ECERIFERUM 1*, *lipid transfer protein 3*, and *wax ester synthase 1* were more strongly induced after ABA treatment in *pRD29A::PYL9* lines than in the WT (Fig. S9*G*). Furthermore, the expression of wax biosynthetic genes was reduced in both *abi1-1* and *snrk2.2/3/6* than in those of the WT in either the absence or presence of ABA (Fig. S9*H*). These results are consistent with PYL9 promotion of ABA-induced wax biosynthesis through the core ABA signaling pathway. The accumulation of cuticular wax may be especially relevant in very young leaves, where stomata have not developed fully.

Discussion

To escape extreme environmental conditions, plants use a dormancy phase to survive. The two major forms of dormancy are seeds and dormant buds. These forms of dormancy are determined genetically and affected by environmental changes (23). ABA increases plant survival in extreme drought by inducing short-, such as stomatal closure, and long-term responses, such as senescence and abscission, and different forms of dormancy (2, 10, 23). Plants close stomata in response to drought by producing ABA, which is a rapid response that blocks most water loss and gains time for long-term responses to be established. Plants developed an important long-term defense against limited water by favoring water consumption in only newly developed organs and eventually, inducing strong dormancy in meristems or buds. A nonobvious part of this defense in its early stages is the premature senescence and/or abscission of old organs (Fig. S10), which are easily mistaken as drought sensitivity.

Leaf senescence and abscission are forms of programmed cell death. They occur slowly and are associated with efficient transfer of nutrients from the senescing leaves to the developing or storage parts of plants (34). Promotion of leaf senescence and abscission by ABA is a long-term response that allows survival of extreme drought conditions. By selection of the best transgenic survivors of extreme drought conditions, our study reveals that ABA mediates survival by promoting leaf senescence through the ABA receptor PYL9 and other PYLs, PP2C coreceptors, SnRK2 protein kinases, and ABFs and RAV1 transcription factors (Fig. S10). ABFs and RAV1 are positive regulators of ABA-induced leaf senescence (Fig. 4) (31) and overall survival. Phosphorylation of ABFs and RAV1 by SnRK2s is important for their functions in ABA-induced leaf senescence (Fig. 4) and increased survival (15). When phosphorylated by SnRK2s, RAV1 and ABFs increase the expression of NAC transcription factors through ABRE motifs and/or RAV1-binding motifs (Fig. S8*C*) (8). These NAC transcription factors promote the expression of downstream *SAG*s, which in turn, control leaf senescence (5–7, 34). The *SAG*s are involved in transcription regulation, protein modification and degradation, macromolecule degradation, transportation, antioxidation, and autophagy (35). The association of senescing leaves with provision of nutrients to sink tissues during drought suggests that drought survival and leaf senescence are linked by ABA signaling. This common connection through the core ABA pathway finally uncovers the underlying molecular mechanism of drought- and ABA-induced leaf senescence and its association with the ability to survive extreme drought. It must be remembered that many injury responses to drought may resemble senescence symptoms but are mediated by separate signaling pathways.

ABA promotes dormancy and growth inhibition through core ABA signaling (25). Seeds can live for many years in a deep dormancy, escaping extreme environmental conditions. Many plants, especially perennials, have a bud-to-bud lifecycle in addition to a seed-to-seed cycle. Bud dormancy is less extreme and more flexible than seed dormancy. Perennial plants can temporarily cease meristematic activity in response to the inconsistent or unusual timing of unfavorable environmental conditions (23). ABA accumulates in polar apical buds during short-day conditions, which may contribute to growth suppression and maintenance of dormancy (36). Of 146 *BRC1*-dependent *bud dormancy genes* that are putatively involved in shade-induced axillary bud dormancy, 78 are regulated during senescence (35, 37, 38). Strikingly, master positive regulators of senescence, such as *ORE1*, *AtNAP*, and *MAX2*/*ORE9*, are also up-regulated during bud dormancy, suggesting that bud dormancy is coordinated with leaf senescence to contribute to stress resistance. Most of these bud dormancy genes contain a CACGTGt motif in their promoters (37), which is recognized by ABA-related basic region-leucine zipper (b-ZIP) transcription factors (39).

Water flows from tissues with higher water potential to those with lower water potential. During drought stress, the young sink tissues but not senescing leaves can steadily decrease their water potential through osmotic adjustment, which ensures that water flows to these sink tissues (Fig. 5*B*). Under drought conditions, senescence of sources is, however, accompanied by growth inhibition and dormancy or paradormancy (23) in sinks, which elevate the osmolyte concentration in sinks (passive osmotic adjustment). Because the water potential of the atmosphere is extremely low under drought conditions, a relatively sealed plant surface is required to limit nonstomatal water loss. Sealing of the plant surface requires the accumulation of cuticular wax (33). ABA up-regulates wax biosynthesis genes through the core ABA signaling pathway (Fig. S9 *G* and *H*). The promotion of wax biosynthesis by ABA in

buds entering dormancy may also contribute to the improved survival of *pRD29A::PYL9* transgenic lines under drought conditions.

Taken together, our data and previous findings suggest that the ABA core signaling pathway plays a crucial role in survival of extreme drought by promoting stomatal closure, growth inhibition, bud dormancy, and leaf senescence. The ABA-induced dormancy-related genes and the ABA-induced senescence-related genes are largely the same genes, which are simultaneously regulated. Senescence occurs in source tissue and leads to death, whereas dormancy occurs in sink tissue and maintains life. This combination of death and life is similar to a triage strategy, and it is consistent with plant survival and therefore, species persistence during episodes of extreme environmental conditions during evolution.

Our research has generated drought-resistant *pRD29A::PYL9* transgenic plants from a large-scale screening of transgenic lines and illustrated the mechanism and important role of ABA-induced leaf senescence under severe drought stress. In both *Arabidopsis* and rice in extreme drought conditions, the *pRD29A::PYL9* transgenic lines exhibited reduced transpirational water loss, accelerated leaf senescence, reduced cell membrane damage, reduced oxidative damage, increased water use efficiency, and finally, increased survival rates. In addition to being more efficient than the *35S* promoter for engineering drought-resistant transgenic plants, the *RD29A* promoter lacks undesirable phenotypes, including retarded growth under normal growth conditions. The enhanced drought survival of *pRD29A::PYL9* transgenic plants can be further enhanced by the external application of ABA or its analogs. The combined use of *pRD29A::PYL9* transgenic plants and applications of ABA or its analogs represents an effective way to protect crops from severe drought stress.

Materials and Methods

Details are provided in *SI Materials and Methods*, including plasmid constructs, plant materials and growth conditions, transient expression assays in *Arabidopsis*, drought-stress treatments, measurement of photosynthesis parameters and water loss, measurement of electrolyte leakage, determination of hydrogen peroxide level and activities of antioxidant enzymes, measurement of chlorophyll content, osmotic potential measurements, tandem affinity purification, Northern blot and real-time PCR assay, Y2H assays, and sequence comparison.

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