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The Tomato DELLA Protein PROCERA Acts in Guard Cells to Promote Stomatal Closure

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Plants employ stomatal closure and reduced growth to avoid water deficiency damage. Reduced levels of the growth-promoting hormone gibberellin (GA) lead to increased tolerance to water deficit, but the underlying mechanism is unknown. Here, we show that the tomato (*Solanum lycopersicum*) DELLA protein PROCERA (PRO), a negative regulator of GA signaling, acts in guard cells to promote stomatal closure and reduce water loss in response to water deficiency by increasing abscisic acid (ABA) sensitivity. The loss-of-function *pro* mutant exhibited increased stomatal conductance and rapid wilting under water deficit stress. Transgenic tomato overexpressing constitutively active stable DELLA proteins (S-*della*) displayed the opposite phenotype. The effects of S-*della* on stomatal aperture and water loss were strongly suppressed in the ABA-deficient mutant *sitiens*, indicating that these effects of S-*della* are ABA dependent. While DELLA had no effect on ABA levels, guard cell ABA responsiveness was increased in S-*della* and reduced in *pro* plants compared with the wild type. Expressing S-*della* under the control of a guard-cell-specific promoter was sufficient to increase stomatal sensitivity to ABA and to reduce water loss under water deficit stress but had no effect on leaf size. This result indicates that DELLA promotes stomatal closure independently of its effect on growth.

INTRODUCTION

Water deficit has a marked impact on plant development and productivity, as expressed by the suppression of growth, flowering, and fruit development (Zhu, 2002; Chaves et al., 2003; Munns and Tester, 2008). Plants have adopted various strategies to cope with drought, including maintaining their water status by rapid stomatal closure and altered growth and development. Both rapid stomatal movement and integrated growth plasticity involve long-distance communication between different organs, which is primarily mediated by the stress-related hormone abscisic acid (ABA; Munns, 2002; Sachs, 2005). Accumulating evidence also suggests that reduced gibberellin (GA) activity promotes plant tolerance to water deficit stress (Magome et al., 2004, 2008; Li et al., 2012; Nir et al., 2014; Colebrook et al., 2014).

GA-dependent responses are inhibited by nuclear proteins known as DELLAs, which interact with and regulate numerous transcription factors (Locascio et al., 2013). DELLAs, which are a subgroup of the GRAS transcription factor family, can transactivate transcription, but they lack a DNA binding domain (Hirano et al., 2012). When GA levels increase, this hormone binds to its receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1), which in turn interacts with DELLA. This complex binds to SCF E3 ubiquitin ligase via a specific F-box protein, and DELLA is polyubiquitinated and then degraded by the 26S proteasome to relieve GA responses (Harberd et al., 2009; Hauvermale et al., 2012).

The DELLA N-terminal region, which contains the conserved DELLA and VHYNP motifs (Locascio et al., 2013), interacts with GID1 to form the GID1-GA-DELLA complex (Murase et al., 2008). The C-terminal region includes the GRAS domain and plays a major role in repressing GA responses (Sun et al., 2012). Various gain-of-function, dominant mutations affecting the N-terminal region of DELLA interfere with GA-induced DELLA degradation by blocking the integration of DELLA into the GID1-GA complex (Harberd et al., 2009). These mutant proteins are stable (S-*della*) and, therefore, constitutively active. By contrast, null and loss-of-function, recessive mutations in the DELLA genes lead to a constitutive GA response phenotype (Sun and Gubler, 2004; Weston et al., 2008; Harberd et al., 2009). Tomato (*Solanum lycopersicum*) has only one DELLA gene, called *PROCERA* (*PRO*) (Jasinski et al., 2008; Livne et al., 2015). Tomato plants homozygous for the weak loss-of-function *pro* mutation exhibit increased GA responses but also retain some responsiveness to GA (George Jones, 1987; Jupe et al., 1988; Van Tuinen et al., 1999; Bassel et al., 2008; Fleishon et al., 2011). Recently, we identified a GA-insensitive, null mutant (*pro*$^{\Delta GRAS}$), with a mutation that likely truncates the protein prior to the GRAS domain (Livne et al., 2015). We found that seeds of this mutant are highly sensitive to desiccation and proposed that the loss of DELLA activity in seeds reduces ABA-induced desiccation tolerance.

Osmotic stress suppresses GA levels and signaling activity (Achard et al., 2006; Wang et al., 2008; Colebrook et al., 2014), which, in turn, promotes tolerance to the abiotic stresses, including drought (Magome et al., 2004; Achard et al., 2006; Shan et al., 2007; Li et al., 2012; Colebrook et al., 2014). While the mechanism of this stress tolerance is not fully understood, indirect effects on transpiration due to decreased plant size (Magome et al., 2008; Achard

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et al., 2006), activation of various stress-related genes (Wang et al., 2008; Tuna et al., 2008), and suppression of reactive oxygen species accumulation (Achard et al., 2008) have been suggested. We recently showed that reducing GA levels in tomato by overexpressing *GA METHYLTRANSFERASE1* increased tolerance to water deficit stress (Nir et al., 2014). The transgenic plants maintained higher leaf water content for a longer period under water deficit conditions than the wild type due to reduced whole-plant transpiration. While the reduced transpiration was associated with smaller leaves, we noted that the stomatal aperture of the transgenic plants was reduced, raising the possibility that GA may also regulate transpiration directly in guard cells. This hypothesis is also in line with the previously reported role for GA as a positive regulator of stomatal opening (Santakumari and Fletcher, 1987; Göring et al., 1990).

In this study, we investigated the role of guard cell GA signaling in regulating transpiration by examining the responses of guard cell to water deficiency in DELLA mutants and transgenic tomato plants expressing S-*della* proteins. DELLA affected the guard cell's response to ABA and promoted stomatal closure in response to soil water deficit. Moreover, while increasing DELLA activity in guard cells alone did not affect leaf size, it was sufficient to reduce stomatal aperture and delay the negative effects of water limitation. These results suggest that DELLA increases the sensitivity of guard cells to ABA, leading to earlier stomatal closure.

RESULTS

The Tomato *pro* Mutant Exhibits Increased Transpiration

To examine DELLA's effect on plant water status, we tested the response of the DELLA loss-of-function tomato mutant *pro* (Bassel et al., 2008) to water deficit stress. Control (M82) and *pro* seedlings were grown until they produced six expanded leaves, after which irrigation was stopped to induce dehydration. Before the onset of the water deficit treatment, all plants were irrigated to saturation. After 4 d, nonirrigated *pro* plants began to wilt, while control plants remained turgid (Figure 1A). Four days after the initiation of the drought treatment, the relative water content (RWC) of leaves was reduced in *pro* by ~30% and in M82 by ~10% (Figure 1B).

We then examined whether the rapid water loss in *pro* plants was caused by higher stomatal conductance and increased transpiration. Stomatal conductance measured at 10 AM in irrigated *pro* plants was ~50% higher than that measured in M82 (Figure 1C). We monitored whole-plant transpiration in irrigated plants growing in a greenhouse using an array of load cells (lysimeters; see Methods) that simultaneously followed the daily weight loss of each plant. The daily transpiration rate (normalized to plant weight to eliminate the effect of plant size) of *pro* plants was significantly higher than that measured for M82 plants (Figure 1D). Microscopy analysis of imprints taken from the abaxial leaf epidermis of irrigated *pro* and M82 plants revealed a much larger stomatal pore area in *pro* (72 μm^2) versus M82 plants (37 μm^2; Figure 1E). *pro* stomata were slightly larger than those of M82 and their density was similar (Supplemental Figure 1). These results suggest that the rapid water loss observed in *pro* resulted from increased stomatal pore area, which in turn led to increased stomatal conductance and transpiration.

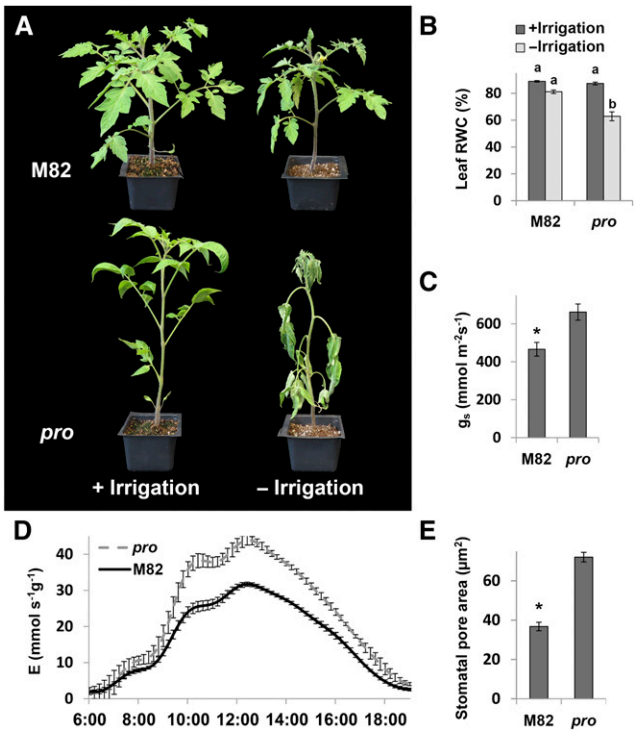

Figure 1. The Tomato DELLA Loss-of-Function *pro* Mutant Exhibits Rapid Water Loss under Water Deficit Conditions.

M82 and *pro* were grown until they produced six leaves before irrigation was stopped.

(A) Representative plants grown under continuous irrigation (+irrigation) or after 4 d of limited water conditions (-irrigation).

(B) Average leaf RWC of control M82 and *pro* plants grown with or without irrigation for 4 d. Values are means of five replicates (five leaflets taken from the fourth leaf below the apex from five different plants) ± SE. Each set of letters above the columns represents significant differences between respective treatments (Tukey-Kramer HSD, $P < 0.05$).

(C) Stomatal conductance (g_s) of the fourth leaf below the apex, as measured, with a porometer, at 10 AM. Values are means of eight measurements from eight plants ±SE.

(D) Whole-plant transpiration (*E*) over the course of 12 h (6 AM to 6 PM). M82 and *pro* plants were placed on the lysimeter system and pot (pot + soil + plant) weight was measured every 3 min. To eliminate the effect of plant size on transpiration rate, the rate of plant water loss was normalized to plant weight. Values are means of eight plants ± SE.

(E) Stomatal pore area (μm^2) of the fourth leaf below the apex of irrigated M82 and *pro* plants, measured at 10 AM. Values are means of ~100 measurements (stomata) ± SE. Asterisks in **(C)** and **(E)** denote a significant difference (Student's *t* test, $P < 0.05$).

Increased DELLA Activity Reduces Stomatal Conductance

We next aimed to determine how increased DELLA activity affects plant water status. To this end, transgenic tomato plants overexpressing the *Arabidopsis thaliana* S-*della* protein RGAΔ17 (Livne et al., 2015), which lacks the DELLA domain and is stable in the presence of GA (Dill et al., 2001), were subjected to water deficit conditions. Relatively weak, semidwarf $35S_{pro}$:*rgaΔ17* lines were used to minimize the effect of plant size. After 5 d without

irrigation, the control plants began to wilt, while the transgenic plants remained turgid (Figure 2A). At this same time point, leaf RWC was reduced by 25% in M82 plants but remained unchanged in *35S$_{pro}$:rgaΔ17* (Figure 2B). After 10 d without irrigation, *35S$_{pro}$:rgaΔ17* plants wilted (Figure 2A). After 14 d without irrigation, the plants were rehydrated and the recovery process was monitored. M82 plants failed to recover (Figure 2A), but *35S$_{pro}$:rgaΔ17* plants fully recovered, and only small necrotic lesions were noted on several leaves. Irrigated *35S$_{pro}$:rgaΔ17* plants displayed significantly lower whole-plant transpiration rates compared with M82 plants (Figure 2C). Since *35S$_{pro}$:rgaΔ17* had smaller leaflets (Supplemental Figure 2A), the reduced transpiration may have been the result of lower stomata counts per leaf. However, stomatal density was higher in *35S$_{pro}$:rgaΔ17* leaflets (Supplemental Figure 2B) and the total number of stomata per leaf and per plant was similar between the two genotypes (Supplemental Figure 2C). *35S$_{pro}$:rgaΔ17* stomata were only slightly smaller than those of M82 (Supplemental Figure 2D), but their pore area (25 μm^2) was much smaller as compared with M82 (42 μm^2; Figure 2D).

We measured stomatal conductance in M82 and *35S$_{pro}$:rgaΔ17* during the drought treatment. Control and *35S$_{pro}$:rgaΔ17* plants with seven expanded leaves were exposed to water deficit conditions (see above). The stomatal conductance of the fourth leaf below the apex was measured each day at noon using a porometer. At the same time, the soil relative volumetric water content (VWC) was determined using a soil moisture sensor. Stomatal conductance was lower in *35S$_{pro}$:rgaΔ17* plants throughout the experiment (Figure 2E). *35S$_{pro}$:rgaΔ17* stomata closed when VWC reached ~40% and M82 stomata only closed when VWC reached ~20%. These results suggest that high DELLA activity increases the sensitivity of stomata to reduced soil water content.

We also generated transgenic tomato plants overexpressing *proΔ17*, a tomato S-*della* mutant gene. All transgenic lines presented a dwarf phenotype and resistance to GA_3 treatment (Supplemental Figure 3A). The transgenic plants displayed significantly lower daily whole-plant transpiration that M82 plants (normalized to plant weight; Supplemental Figure 3C). In addition, the transgenic plants maintained higher leaf water content for a longer period under water deficit conditions (see below; Figure 4). Under normal irrigation, stomatal pore area at noon was much smaller in *35S$_{pro}$:proΔ17* versus M82 plants (Supplemental Figure 3D), but stomatal size was similar (Supplemental Figure 3B).

The findings that *35S$_{pro}$:rgaΔ17* and *35S$_{pro}$:proΔ17* plants have reduced transpiration, increased leaf water content under water deficit stress, and smaller stomatal pore area than the wild type raised the possibility that DELLA proteins act in guard cells to reduce water loss under both irrigation and water deficit conditions by promoting stomatal closure. To confirm the expression of *PRO* and *proΔ17* in guard cells, we extracted RNA from isolated guard cells (Supplemental Figure 4A). qRT-PCR analysis revealed the expression of *PRO* in M82 guard cells and showed ~60-fold higher expression level of *proΔ17* in transgenic guard cells versus M82 (Supplemental Figure 4B).

To determine the contribution of leaf size to water loss, we generated transactivated tomato plants expressing a *GFP-proΔ17* fusion under the control of the *FILAMENTOUS FLOWER* (*FIL*) promoter (*FIL$_{pro}$>>GFP-proΔ17*). We used the transgenic line *FIL$_{pro}$:LhG4* as a driver (Shani et al., 2010) and *OP:GFP-proΔ17* as a responder line. The *FIL* promoter is active during a limited developmental window in leaf primordia and initiating leaflets, but not later during leaf expansion or in mature leaves (Lifschitz et al., 2006). The expression of GFP-pro*Δ17* in young leaf primordia and initiating leaflets of *FIL$_{pro}$>>GFP-proΔ17* plants was confirmed by confocal

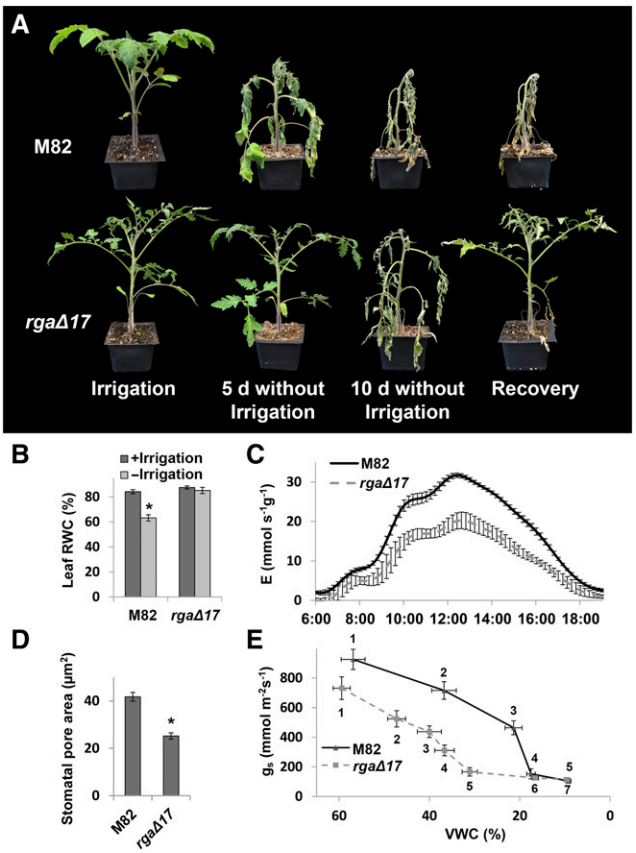

Figure 2. Overexpression of the Arabidopsis S-*della* Gene, *rgaΔ17*, in Tomato Plants Improves Water Status under Water Deficit Stress.

Control M82 and transgenic *35S$_{pro}$:rgaΔ17#4* (*rgaΔ17*) plants were grown until they produced five expanded leaves before irrigation was stopped.

(A) Representative plants grown under continuous irrigation or for 5 or 10 d without irrigation. After 14 d without irrigation, plants were rehydrated and their recovery was monitored.

(B) Average leaf RWC in control M82 and *rgaΔ17* plants, grown with or without irrigation for 5 d. Values are means of five replicates (five leaflets taken from the fourth leaf below the apex from five different plants) ± SE.

(C) Whole-plant transpiration (*E*) over the course of 12 h (6 AM to 6 PM). M82 and *rgaΔ17* plants were placed on the lysimeter system and pot weight was measured every 3 min. The rate of plant water loss was normalized to plant weight. Values are means of eight plants ± SE.

(D) Stomatal pore area of the third leaf below the apex of irrigated control and *35S$_{pro}$:rgaΔ17* plants, measured at 10 AM. Values are means of ~100 measurements (stomata) ± SE. Asterisks in **(B)** and **(D)** denote a significant difference (Student's *t* test, $P < 0.05$).

(E) Stomatal conductance (g_s) of the fourth leaf below the apex under water deficit conditions in M82 and *rgaΔ17* plants. At the same time, soil relative VWC was measured using the EC-5 soil moisture sensor. Measurements were taken once a day at noon, starting on the first day after the cessation of irrigation. Numbers above (for M82) and below (for *rgaΔ17*) the lines indicate the day of measurement. Values are means of eight measurements taken from eight different plants ± SE.

microscopy (Supplemental Figure 5A). However, in epidermal peels from mature *FIL$_{pro}$>>GFP-pro∆17* leaves, no GFP signal was detected in pavement or guard cells (Supplemental Figure 5B). As expected, the transactivated plants featured normal stem length but smaller leaflets than the wild type (Figures 3A and B; Supplemental Figure 5C). When subjected to water deficit conditions, M82 and *FIL$_{pro}$>>GFP-pro∆17* plants wilted at the same time (after 5 d) and exhibited similar RWC at this time (Figures 3A and 3C). In addition, stomatal pore area was similar in irrigated *FIL$_{pro}$>>GFP-pro∆17* and M82 plants (Figure 3D). Stomatal density in *FIL$_{pro}$>>GFP-pro∆17* was higher than in M82, but the total number of stomata per plant was similar (Supplemental Figures 5D and 5E). These results indicate that the reduced leaf size in S-*della*-overexpressing plants is not the main determinant of reduced transpiration.

DELLA Activity Promotes the Stomatal Response to ABA

The shortage of water and loss of turgor induce ABA biosynthesis (McAdam and Brodribb, 2016), which, in turn, promotes stomatal closure. The stomatal aperture of the ABA-deficient tomato mutant *sitiens* (*sit*) is larger than in wild-type plants (Tal, 1966). Pores in *pro* stomata were larger than those of wild-type plants, resembling those of *sit* (Figures 4B and 4C; Supplemental Figure 6). Therefore, we investigated the possible interaction between DELLA and ABA in the regulation of stomatal movement. To this end, *35S$_{pro}$:pro∆17* was introgressed from one of the transgenic M82 lines into *sit*. qRT-PCR analysis confirmed that the transgene was expressed in both M82 and *sit* (Supplemental Figure 7). *sit* leaves were smaller and stems were shorter than those of M82 (Figure 4A). Overexpression of *pro∆17* reduced the leaf size and stem length of both M82 and *sit*, but the effects were greater in the *sit* background. After 1 d without irrigation, *sit* and *35S$_{pro}$:pro∆17 sit* began to wilt, whereas M82 only began to wilt after 4 d. Even after 7 d without irrigation, *35S$_{pro}$:pro∆17* M82 plants were turgid (Figure 4A). After 7 d under water deficit conditions, we resumed irrigation and scored recovery 10 d later. *35S$_{pro}$:pro∆17* did not show any sign of damage, and M82 exhibited minor damage in one or two leaves. In contrast, *sit* and *35S$_{pro}$:pro∆17 sit* leaves failed to recover (Figure 4A). Microscopy analysis of imprints of the abaxial leaf epidermis of irrigated M82, *35S$_{pro}$:pro∆17*, *sit*, and *35S$_{pro}$:pro∆17 sit* plants revealed widely opened stomata in *sit* and *35S$_{pro}$:pro∆17 sit* and small pores in *35S$_{pro}$:pro∆17* (Figures 4B and 4C; Supplemental Figure 8). These results indicate that the effect of S-*della* on stomatal aperture under irrigation and under water deficit conditions is ABA dependent. In addition, the results show that the effect of DELLA on plant size is not linked to its effect on stomatal closure.

The suppression of the effect of S-*della* on stomatal closure by *sit* suggested that DELLA either promotes ABA production or affects downstream ABA-associated responses. To test and distinguish between these possibilities, we first analyzed the levels of ABA in irrigated versus water-deprived M82, *pro*, and

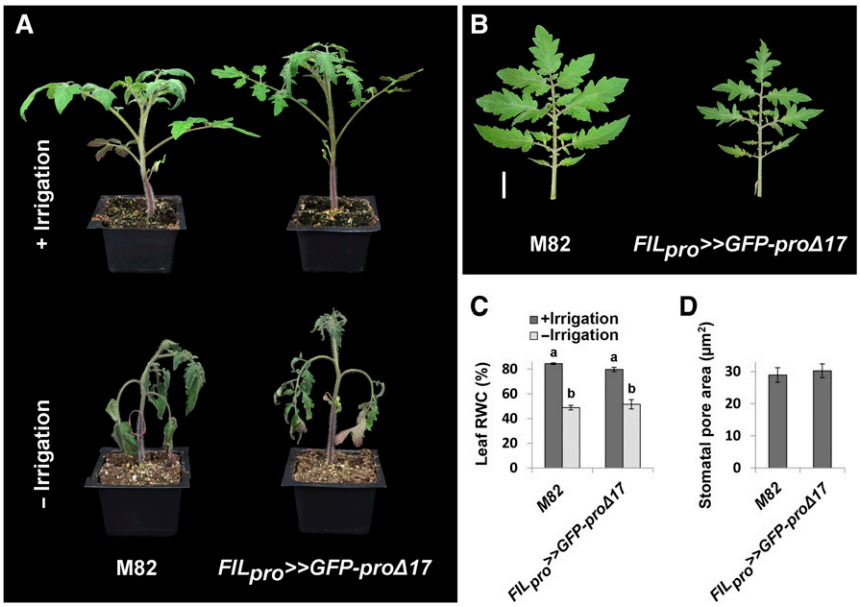

Figure 3. Reducing the Leaflet Size by *FIL$_{pro}$>>GFP-pro∆17* Has No Effect on Water Loss.

Control and transgenic *FIL$_{pro}$>>GFP-pro∆17* were grown until they produced five expanded leaves before irrigation was stropped.
(A) Representative plants grown under continuous irrigation or subjected to 5 d of without irrigation.
(B) Fourth leaf below the apex of control M82 and *FIL$_{pro}$>>GFP-pro∆17*. Bar = 2 cm.
(C) Average leaf RWC in control M82 and *FIL$_{pro}$>>GFP-pro∆17* plants grown with irrigation or subjected to 5 d of water deficit conditions. Values are means of five replicates (five leaflets taken from the fourth leaf below the apex from five different plants) ± SE. Each set of letters above the columns represents significant differences between respective treatments (Tukey-Kramer HSD, $P < 0.05$).
(D) Mean stomatal pore area of the fourth leaf below the apex of irrigated control and *FIL$_{pro}$>>GFP-pro∆17*. Values are means of ~100 measurements (stomata) ± SE.

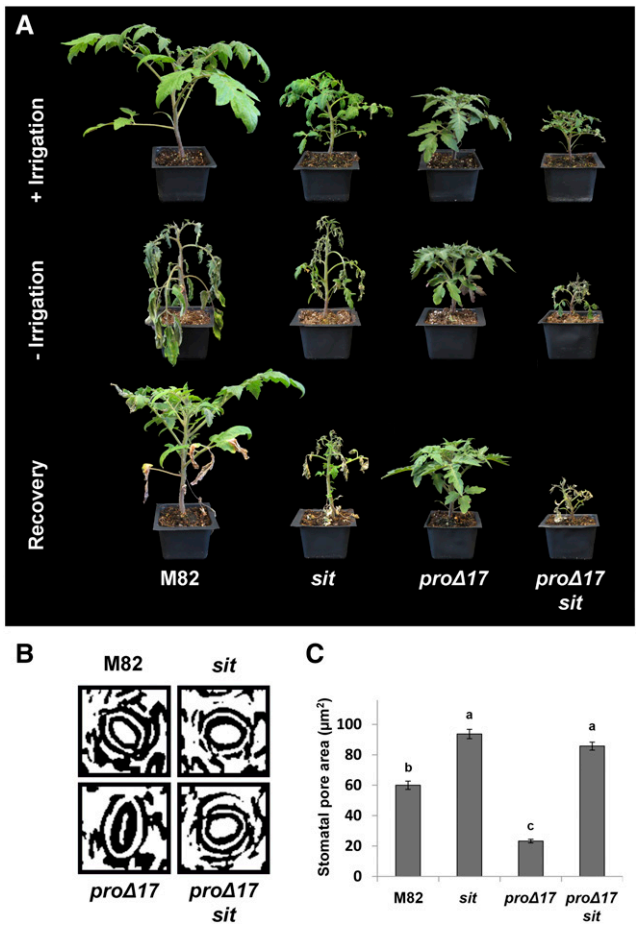

Figure 4. ABA Is Required for the Enhanced DELLA-Dependent Stomatal Closure.

(A) Representative M82, *sit*, $35S_{pro}$:*proΔ17* (*proΔ17*), and $35S_{pro}$:*proΔ17* plants in the *sit* background (*proΔ17 sit*) grown under a normal irrigation regime (+irrigation) or without irrigation (-irrigation) for 7 d. After 7 d without irrigation, the plants were rehydrated and recovery was assessed after 10 d.
(B) Representative stomata of the lines described in **(A)**. Images (microscopic analysis) of imprints of the abaxial leaf epidermal layer of the fourth leaf below the apex collected at 11 AM from irrigated plants.
(C) Stomatal pore area of the fourth leaf below the apex measured at 11 AM. Values are means of ~100 measurements (stomata) ± SE. Each set of letters above the columns represents significant differences between respective treatments (Tukey-Kramer HSD, $P < 0.05$).

$35S_{pro}$:*proΔ17* leaves. In the water deficit treatment, a similar soil VWC was maintained for all plants by adding small amounts of water to the pots that dried faster. All plants started to wilt 8 d after the beginning of the treatment, and leaves were collected for ABA analysis 1 d later. At this time, the soil VWC of all plants reached 40%. Under irrigation, no significant differences in ABA content were recorded between the different lines (Figure 5A). While the water deficit treatment increased ABA levels in all lines, these levels were lower in $35S_{pro}$:*proΔ17* leaves than in M82 or *pro* leaves. This may have been the result of feedback inhibition induced by higher ABA activity (Liu et al., 2016). These results suggest that DELLA does not promote ABA accumulation in tomato leaves.

To evaluate whether DELLA affects stomatal response to ABA, we treated peeled abaxial epidermal strips taken from M82, *pro* and $35S_{pro}$:*rgaΔ17* leaves with different ABA concentrations and monitored stomatal closure. Incubation with 10 μM ABA reduced stomatal aperture in M82 by 30%, in *pro* by 24%, and in $35S_{pro}$:*rgaΔ17* by 50% (Figure 5B). Application of 100 μM reduced stomatal aperture in M82 by 55%, in *pro* by 38%, and in $35S_{pro}$:*rgaΔ17* by 75%. In addition, a stronger stomatal response to ABA was observed in $35S_{pro}$:*proΔ17* (60% stomatal closure in $35S_{pro}$:*proΔ17* and 30% in M82; Figure 5C), but not in FIL_{pro}>>*GFP-proΔ17* epidermal peels (Supplemental Figure 9). These results indicate that DELLA activity affects the response of the guard cells to ABA. In these experiments, no significant difference in the pore area between untreated M82 and S-*della* stomata was detected. It is possible that the buffer used in this experiment to force stomatal opening in the epidermal peels masked the effect of DELLA in the mock treatment.

In addition to its effect on stomatal closure, ABA also promotes gene expression (Hubbard et al., 2010). We analyzed the expression of two tomato ABA-responsive genes (González-Guzmán et al., 2014), *DELTA 1-PYRROLINE-5-CARBOXYLATE SYNTHASE* (*SlP5CS1*; Solyc06g019170) and *RESPONSIVE TO ABA18* (*SlRAB18*; Solyc02g084850), in isolated M82 and $35S_{pro}$:*proΔ17* guard cells. The expression of both genes was significantly higher in $35S_{pro}$:*proΔ17* guard cells than in M82 (Figures 5D and 5E), suggesting that S-*della* constitutively promotes ABA responses in guard cells.

We then analyzed H_2O_2 accumulation, one of the most immediate responses to ABA in guard cells (Pei et al., 2000), following exposure of peeled abaxial epidermal strips to ABA. H_2O_2 was detected with the fluorescent dye 2',7'-dichlorofluorescin (H2DCF; Zhang et al., 2001). $35S_{pro}$:*proΔ17* guard cells displayed a significantly stronger response to ABA than those of M82. Conversely, *pro* guard cells exhibited less of a response than the wild type (Figure 5E; Supplemental Figure 10).

To determine whether DELLA activity affects stomatal movement typically induced by reduced apoplastic osmotic potential, we incubated abaxial leaf epidermal strips of M82, *pro*, and $35S_{pro}$:*proΔ17* plants in 50 mM sorbitol to induce stomatal closure (Yuan et al., 2014). Sorbitol induced similar levels of stomatal closure (~45%) in all examined lines (Figure 5F), suggesting that DELLA activity is not involved in ABA-independent stomatal closure.

To determine if DELLA affects guard cells in a cell-autonomous manner, we examined the effects of exclusive expression of *proΔ17* in guard cells on responses to water deficiency. In tomato plants, the promoter of *KST1* (a K^+ channel gene) is active exclusively in guard cells (Kelly et al., 2013). Transgenic $KST1_{pro}$:*LhG4* plants were crossed with *OP:GFP-proΔ17* plants to transactivate *proΔ17* expression in guard cells. The phenotype of the transactivated plants was indistinguishable from that of M82 (Figures 6A and 6C). Confocal microscopy analysis detected the expression of GFP only in guard cells (Figure 6B). After 7 d under water deficit conditions, M82 plants wilted but $KST1_{pro}$>>*GFP-proΔ17* plants remained turgid (Figure 6C). At this same time point, leaf RWC was reduced by 30% in M82 plants but remain unchanged in the transactivated $KST1_{pro}$>>*GFP-proΔ17* leaves (Figure 6D). Stomatal pore area in the third leaf below the apex of

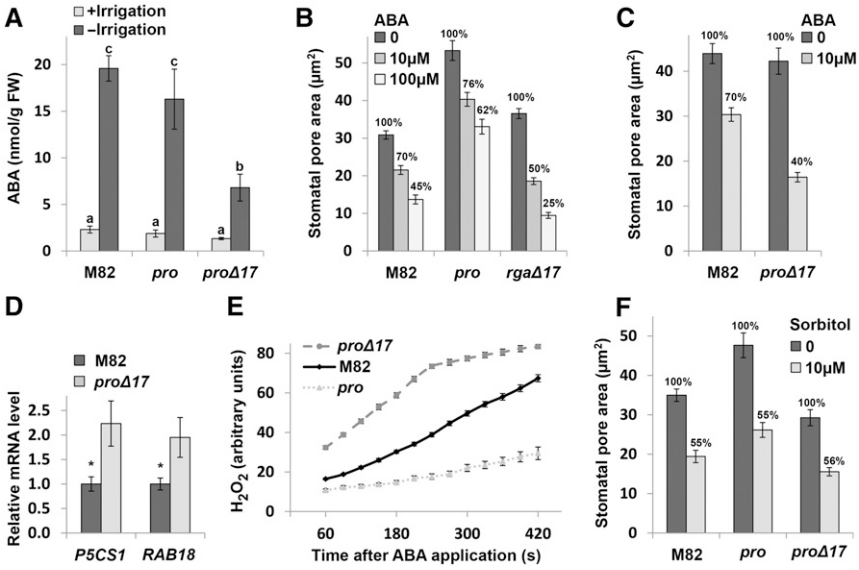

Figure 5. DELLA Activity Promotes the Response of Guard Cells to ABA.

(A) ABA content of the fourth leaf below the apex of M82, *pro*, and *35S$_{pro}$:proΔ17* (*proΔ17*) plants grown with irrigation or subjected to water deficit conditions. For the water deficit treatment, leaves from all lines were collected when soil VWC reached 40%, and all were collected at the same time of day. Values are means of three replicates, taken from three plants ± SE. Each set of letters above the columns represents significant differences between the respective treatments (Tukey-Kramer HSD, $P < 0.05$).

(B) Epidermal strips from leaf number 4 of M82, *pro*, and *35S$_{pro}$:rgaΔ17* were first incubated in stomatal opening buffer and then treated with 0, 10, or 100 μM ABA for 40 min and stomatal pore area was microscopically assessed. Values are means of ~100 measurements (stomata) ± SE. Percentage of stomatal pore area with respect to the mock treatment is presented for each line above the bars.

(C) Epidermal strips, taken from leaf number 4 of M82 and *35S$_{pro}$:proΔ17* (*proΔ17*) were incubated under light in stomatal opening buffer for 2 h and then treated with 10 μM ABA (or mock) for 40 min before stomatal pore area was microscopically assessed. Values are means of ~100 stomata ± SE. Asterisk denotes a significant difference (Student's *t* test, $P < 0.05$).

(D) Expression of ABA-responsive genes in isolated guard cells. qRT-PCR analyses of *SlP5CS1* (Solyc06g019170) and *SlRAB18* (Solyc02g084850) expression. RNA was extracted from isolated M82 and *35S$_{pro}$:proΔ17* guard cells. Values are means of three biological replicates (each containing RNA extracted from guard cells isolated from five leaves of independently grown plant) ± SE. Asterisk denotes a significant difference (Student's *t* test, $P < 0.05$). The experiment was repeated twice and yielded similar results.

(E) Quantification of H_2O_2 accumulation (DCF fluorescent signal) in guard cells of M82, *pro*, and *35S$_{pro}$:proΔ17*. Epidermal strips were taken from leaf number 4 of M82, *35S$_{pro}$:proΔ17*, and *pro*, immersed for 10 min in 50 μM H_2DCF-DA and then transferred to 10 μM ABA solution; the fluorescent signal was detected microscopically every 30 s after the application of ABA. The intensity of the fluorescent signals was quantified using ImageJ software. Values are means of five different images (each containing ~15 stomata) ± SE. The experiment was repeated three times and yielded similar results each time.

(F) Stomatal response to sorbitol treatment. Epidermal strips, taken from leaf number 4 of M82, *pro*, and *35S$_{pro}$:proΔ17* plants were incubated under light in stomatal opening buffer for 2 h and then transferred to 50 μM sorbitol solution (or mock) for 30 min, before stomatal pore area was analyzed microscopically. Values are means of ~100 measurements (stomata) ± SE.

irrigated *KST1$_{pro}$>>GFP-proΔ17* plants was smaller than that of M82 leaves (Figure 6E) and *KST1$_{pro}$>>GFP-proΔ17* guard cells exhibited increased response to ABA (Figure 6F). These results suggest that exclusive expression of DELLA in guard cell is sufficient to promote stomatal closure. The results also demonstrate that DELLA can affect stomatal closure and transpiration without affecting leaf size.

Since DELLA proteins are transcriptional regulators, S-*della* may affect ABA sensitivity in guard cells by transcriptionally regulating ABA signaling component(s). To start addressing this possibility, we analyzed the expression of several ABA signaling genes in isolated M82 and *35S$_{pro}$:proΔ17* guard cells. These included all putative ABA receptor genes *Pyrabactin Resistance1* (*PYR1*)/*Regulatory Component of ABA Receptor* (*RCAR*) (*SlPYR1*), *SlPYR1-Like 2-1* (*SlPYL2-1*), *SlPYL2-2*, *SlPYL4-1*, *SlPYL4-2*, *SlPYL4-3*, *SlPYL6-1*, *SlPYL6-2*, *SlPYL6-3*, *SlPYL-8-1*, *SlPYL8-2*, *SlPYL9-1*, and *SlPYL9-2*, the ABA signaling inhibitor phosphatase type 2C (SlPP2C), subclass III SnRK2 kinase *Open Stomata 1* (SlOST1), and NADPH oxidase (homolog of the Arabidopsis RBOH1) (*SlRBOH1*). It should be noted that the names of these tomato genes do not necessarily reflect their relatedness to the Arabidopsis genes. qRT-PCR analysis revealed ~2-fold higher expression of the ABA receptor genes *SlPYR1* and *SlPYL8*-1 in *35S$_{pro}$:proΔ17* compared with M82 (Figure 7A). We then analyzed the expression of these two genes in *pro*. The expression of both genes was downregulated in *pro* compared with M82, although these changes were significant only for *SlPYR1* (Student's *t* test, $P < 0.05$; Figure 7B). DELLA activity had no effect on the expression of all other receptor genes and not on *SlPP2C*, *SlOST1*-like gene, and *SlRBOH1* (Figure 7C).

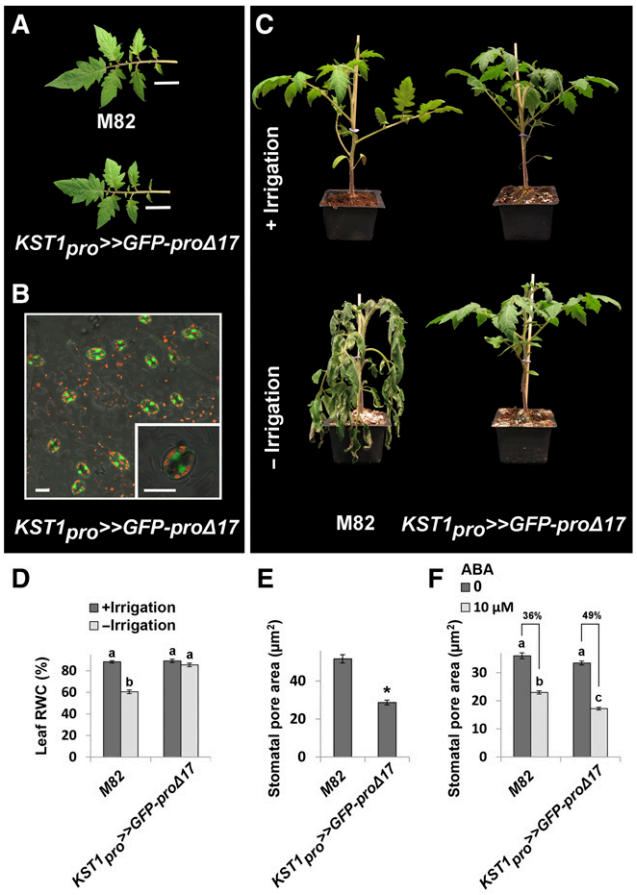

Figure 6. Expressing S-*della* in Guard Cells Only, Promotes Stomatal Sensitivity to ABA and Reduces Water Loss under Water Deficit Conditions.

(A) Leaf number 3 of M82 and *KST1*$_{pro}$*>>GFP-pro*Δ*17* plants. Bar = 2 cm.
(B) Microscopy analysis of the GFP signals in *KST1*$_{pro}$*>>GFP-pro*Δ*17* leaves.
(C) Irrigated (+Irrigation) and water deficit-treated (-Irrigation) for 7 d M82 and *KST1*$_{pro}$*>>GFP-pro*Δ*17* plants. Bar = 20 μm.
(D) Average leaf RWC in M82 and *KST1*$_{pro}$*>>pro*Δ*17* grown with irrigation or subjected to 7 d without irrigation. Values are means of five replicates (five leaflets taken from the fourth leaf below the apex from five different plants) ± SE. Each set of letters above the columns represents significant differences between respective treatments (Tukey-Kramer HSD, $P < 0.05$).
(E) Stomatal pore area in leaf number 3 of M82 and *KST1*$_{pro}$*>>pro*Δ*17*, measured at 11 AM. Values are means of ~100 measurements (stomata) ± SE. Asterisk denotes a significant difference (Student's *t* test, $P < 0.05$).
(F) Epidermal strips (leaf no. 4) were incubated under light in stomatal opening buffer for 2 h and then treated with 10 μM ABA or mock for 40 min before stomatal pore area was microscopically assessed. Values are means of ~100 measurements (stomata) ± SE.

DISCUSSION

Various abiotic stresses, including water deficit, promote DELLA stability and activity via the suppression of GA biosynthesis (Colebrook et al., 2014). The accumulated DELLA induces transcriptional reprogramming, which leads to growth suppression and improves plant adaptation to the changing environment (Achard et al., 2008; Colebrook et al., 2014). Here, we show that the DELLA protein PRO acts in guard cells to promote tolerance to water deprivation via a mechanism independent of its effect on plant growth. Reduced DELLA activity in the tomato *pro* mutant increased stomatal conductance and transpiration and suppressed its response to water deficiency and ABA treatment. On the other hand, overexpression of S-*della* reduced stomatal conductance and transpiration and promoted stomatal closure under mild water deficit stress.

Studies in Arabidopsis have found that DELLA proteins promote ABA biosynthesis in seeds (Zentella et al., 2007; Piskurewicz et al., 2008). Furthermore, Lim et al. (2013) showed that the Arabidopsis DELLA protein GAI interacts in seeds with the ABA signaling components ABA-INSENSITIVE3 (ABI3) and ABI5, and Ariizumi et al. (2013) showed that seeds of the Arabidopsis *della* gain-of-function mutant *gai-1* are more sensitive to ABA than the wild type. Taken together, these studies suggest that DELLAs affect both ABA biosynthesis and signaling in Arabidopsis seeds.

The expression of S-*della* in the ABA-deficient mutant *sit* failed to promote stomatal closure and tolerance to water deprivation, indicating that this effect of S-*della* is ABA dependent. While ABA levels were unaffected in *pro* and S-*della* plants, high DELLA activity increased the response of guard cells to ABA and low DELLA activity reduced this response, suggesting that DELLA affects stomatal movement by increasing sensitivity to ABA. This increased response to ABA could explain the earlier stomatal closure during drought found in plants overexpressing S-*della*. The gradual exposure of plants to increasing intensities of water deficiency leads to gradual increases in ABA levels (Du et al., 2013); under these conditions, enhanced sensitivity to ABA should lead to earlier stomatal closure.

We also found that expression of the ABA-responsive genes *SlP5CS1* and *SlRAB18* (González-Guzmán et al., 2014) was higher in S-*della* guard cells than in the wild type. This suggests that the DELLA-mediated increase in ABA sensitivity in guard cells is not limited to ABA-induced stomatal movement and that DELLA also promotes ABA-induced transcriptional activity (Hubbard et al., 2010). The results also indicate that overexpression of S-*della* constitutively promotes ABA activity in guard cells regardless of water availability, leading to smaller stomatal aperture and increased expression of ABA-responsive genes under both irrigation and water deficit stress. It is unlikely that DELLA also affects stomatal movement in an ABA-independent manner, for example, by affecting leaf structure or cell wall elasticity (Marshall and Dumbroff, 1999). DELLA activity did not influence sorbitol-induced stomatal closure, which involves an ABA-independent pathway. The site of DELLA-driven regulation of stomatal movement is likely guard cells, as shown by the exclusive expression of S-*della* in guard cells, which was sufficient to increase their sensitivity to ABA and promote tolerance to water deficit stress.

Since DELLAs are transcription regulators, it is unclear yet how they affect ABA sensitivity to promote rapid cytosolic responses, such as stomatal movement. Perhaps DELLAs affect the transcription of component(s) in the ABA signaling machinery. ABA binds to its receptors, PYR1/PYL/RCAR, which triggers a conformational change within the receptor, thereby increasing its

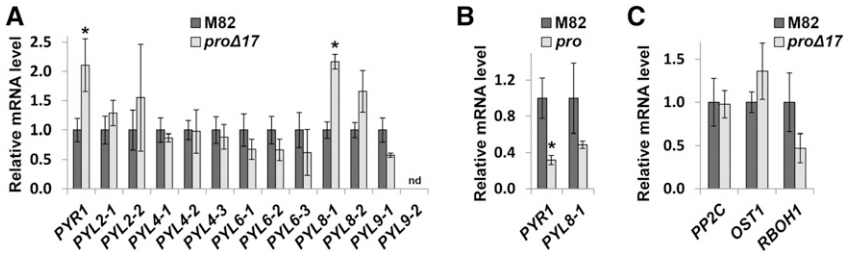

Figure 7. DELLA Activity Promotes the Expression of Two ABA Receptor Genes in Guard Cells.

(A) qRT-PCR analyses of all putative tomato ABA receptor genes in M82 and *35S$_{pro}$:proΔ17* guard cells. RNA was extracted from isolated guard cells. Values are means of three biological replicates (each containing RNA extracted from guard cells isolated from five leaves of independently grown plant) ± SE. Asterisk denotes a significant difference (Student's *t* test, $P < 0.05$). The experiment was repeated twice and yielded similar results. nd, not detected.
(B) qRT-PCR analyses of *SlPYR1* and *SlPYL8-2* expression in M82 and *pro* guard cells. RNA was extracted from isolated guard cells. Values are means of three biological replicates (each containing RNA extracted from guard cells isolated from five leaves of independently grown plant) ± SE. Asterisk denotes a significant difference (Student's *t* test, $P < 0.05$). The experiment was repeated twice and yielded similar results.
(C) qRT-PCR analyses of *SlPP2C*, *SlOST1*, and *SlRBOH* (*NADPH OXIDASE*) expression in M82 and *35S$_{pro}$:proΔ17* guard cells. RNA was extracted from isolated guard cells. Values are means of three biological replicates (each containing RNA extracted from guard cells isolated from five leaves of independently grown plant) ± SE. The experiment was repeated twice and yielded similar results.

affinity to the signaling suppressor phosphatases PP2C. Inhibition of PP2C activity by the activated receptor releases downstream SnRK2 kinases to phosphorylate downstream proteins, such as transcription factors, ion channels, and NADPH oxidase, which in turn regulate gene transcription and stomatal movement (Hubbard et al., 2010; Kim et al., 2010; Cai et al., 2017). The effect of DELLA activity on ABA-induced H_2O_2 accumulation (reduced and increased response in *pro* and S-*della*, respectively) suggests that DELLA affects an early event(s) in the ABA response pathway, upstream or at the level of NADPH oxidase. This impact may be mediated by enhanced (e.g., PYR/PYL receptors, SnRK2 kinases, or NADPH oxidase) or attenuated (e.g., PP2C) transcription of components in the ABA signaling pathway. The expression of two genes encoding ABA receptors, *SlPYR1* and *SlPYL8-1*, was up-regulated in S-*della* and reduced in *pro* guard cells compared with the wild type. Other receptor and ABA signaling components were not affected by DELLA. While these results support the hypothesis that the effect of DELLA on ABA sensitivity is mediated by a transcriptional effect on these two ABA receptor genes, further research is required to address this possibility. Although increased sensitivity to ABA is our preferred hypothesis, we cannot exclude the possibility that DELLA affects ABA uptake into guard cells via transcriptional regulation of ABA transporter genes (Kang et al., 2010).

Our findings, as well as earlier studies, suggest that DELLAs play a role in plant adaptation to water deficit stress. It has been previously suggested that water deficiency reduces GA concentrations (Colebrook et al., 2014) and thereby increases DELLA accumulation. Water deficit also induces ABA accumulation, which in turn stabilizes DELLA (Achard et al., 2006; Jiang and Zhang, 2002; Endo et al., 2008; Guo et al., 2014). We propose that DELLA plays a role in both early and long-term responses to water deficit stress. Our model (Figure 8) suggests that DELLA increases the sensitivity of guard cells to ABA by promoting the transcription of specific ABA receptor genes. The increased sensitivity to ABA promotes ABA-induced stomatal closure. These actions protect plants from transient and/or mild water deficit stress. In the case of persistent drought, DELLA accumulates to high levels and suppresses growth. The inhibition of plant growth further reduces transpiration and water loss and redirects the available energy to protection and adaptation processes (Achard et al., 2008).

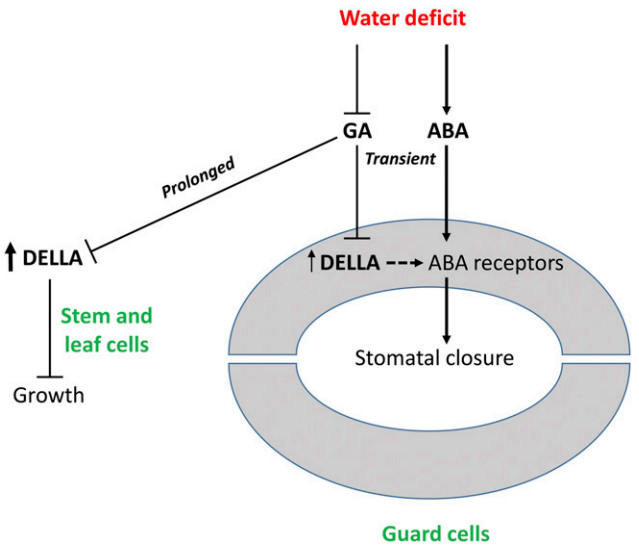

Figure 8. Hypothetical Model of the Mechanism by Which DELLA Promotes Plant Tolerance to Water Deficit Stress.

Under transient water deficit, GA biosynthesis is suppressed, leading to DELLA accumulation. The accumulated DELLA in guard cells promotes, directly or indirectly, the transcription of two ABA receptor genes (*SlPYR1* and *SlPYL8-2*). It is possible that the increased expression of these two receptor genes is responsible for the enhanced sensitivity to ABA, which leads to rapid stomatal closure. Under prolonged drought, GA levels are low and DELLA accumulates to high levels, leading to growth suppression. Both stomatal closure and growth suppression protect plants from short and long drought episodes. Dashed line indicates the hypothetical link suggested by our study.

METHODS

Plant Materials and Growth Conditions

Tomato (*Solanum lycopersicum*) M82 (*sp/sp*) plants were used throughout this study. The *pro* allele was in the M82 background (Fleishon et al., 2011; Livne et al., 2015) and the *sit* allele, originally in 'Alisa Craig' (Harrison et al., 2011), was backcrossed three times to M82. All plants were grown in a growth room set to a photoperiod of 12/12-h night/days, light intensity (cool-white bulbs) of ~250 μmol m^{-2} s^{-1}, and 25°C. In other experiments, plants were grown in a greenhouse under natural daylength conditions, at 700 to 1200 μmol m^{-2} s^{-1} and 18 to 29°C.

DNA Constructs, the Transactivation System, and Plant Transformation

Truncated *PRO* (*proΔ17*) was generated using PCR-based "overlap extention" mutagenesis (Ho et al., 1989). *proΔ17* was inserted into the pART7 vector downstream to the *35S* promoter into the *Kpn*I and *Bam*HI sites. The *proΔ17* coding sequence was also inserted into the pART7-GFP vector into the *Kpn*I and *Bam*HI sites to create *GFP-proΔ17*. This construct was then cloned downstream of Operator array (OP) sequences from *Escherichia coli* into the *Kpn*I and *Bam*HI sites to create *OP:GFP-proΔ17*. To specifically express *GFP-proΔ17* in guard cells or in leaf primordia, the LhG4 transactivation system (Moore et al., 1998) with the *KST1* and *FIL* promoters, respectively, was used. The *KST1* promoter was inserted into the pBJ36 vector, upstream of *LhG4*, in the *Sal*I and *Pst*I sites to create $KST1_{pro}$*:LhG4*. $KST1_{pro}$*:LhG4* and FIL_{pro}*:LhG4* (Plesch et al., 2001; Shani et al., 2010) were used as driver lines and *OP:GFP-proΔ17* as the responder line. The cross between these lines generated the transactivated lines $KST1_{pro}$*>>GFP-proΔ17* and FIL_{pro}*>>GFP-proΔ17*. The constructs were subcloned into the pART27 binary vector and introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation. The constructs were transferred into M82 cotyledons using transformation and regeneration methods described by McCormick (McCormick, 1991). Kanamycin-resistant T0 plants were grown and at least four independent transgenic lines were selected and self-pollinated to generate homozygous transgenic lines.

RNA Extraction and cDNA Synthesis

Total RNA was isolated from tomato seedlings or leaves from mature plants. RNA extraction and cDNA synthesis were performed as previously described (Nir et al., 2014). Frozen tissues were ground, resuspended in guanidine HCl, and combined with phenol/chloroform. The samples were mixed by vortexing for 30 s, and after 30 min, they were centrifuged at 4°C for 45 min. Ethanol (100%) and 1 M acetic acid were added, and the samples were mixed and stored overnight at −80°C. NaAc (3 M) was added and the samples were washed with cold 70% ethanol. SuperScript II reverse transcriptase (18064014; Invitrogen) and 3 μg of total RNA was used to synthesize cDNA according to the manufacturer's instructions.

qRT-PCR Analysis

qRT-PCR analysis was performed using an Absolute Blue qPCR SYBR Green ROX Mix (AB-4162/B) kit (Thermo Fisher Scientific). Reactions were performed using a Rotor-Gene 6000 cycler (Corbett Research). A standard curve was obtained using dilutions of the cDNA sample. The expression was quantified using Corbett Research Rotor-Gene software. Three independent technical repeats were performed for each sample. Relative expression was calculated by dividing the expression level of the examined gene by that of *TUBULIN*. Gene-to-*TUBULIN* ratios were then averaged and are presented as the proportion of the control treatment, which was set to a value of 1. All primer sequences are presented in Supplemental Table 1.

Measurements of Stomatal Pore Area, Length, and Density

Stomatal pore area and density were determined using the rapid imprinting technique (Geisler et al., 2000). This approach allowed us to reliably and simultaneously score hundreds of stomata from each experiment. Briefly, light-body vinylpolysiloxane dental resin (eliteHD+; Zhermack Clinical) was attached to the abaxial side of the leaf, dried for ~1 min, and then removed. The resin epidermal imprints were covered with transparent nail polish, which was removed once it dried and served as a mirror image of the resin imprint. The nail polish imprints were placed on glass cover slips and photographed under a model 1M7100 bright-field inverted microscope (Zeiss) with a mounted Hitachi HV-D30 CCD camera. Stomatal images were later analyzed to determine aperture size using the ImageJ software (http://rsb.info.nih.gov/ij/) fit-line tool. A microscopy ruler (Olympus) was used for size calibration.

Measurement of Leaf Area

Total leaf area was measured using a model Li 3100 leaf area meter (LI-COR Biosciences).

Measurement of Stomatal Pore Area in Detached Epidermis

Abaxial epidermal strips were peeled and the detached layer was incubated in stomatal opening buffer (Wigoda et al., 2006) for 2 h in the light (400 μmol m^{-2} s^{-1}). The strips were then transferred to fresh stomatal opening buffer, with or without ABA. After 40 min, the strips were placed on glass cover slips and photographed under a bright-field inverted microscope and images were analyzed as above.

Detection of H_2O_2 in Guard Cells

Abaxial epidermal strips were peeled and floated on stomatal opening buffer (Wigoda et al., 2006) for 2 h in the light (400 μmol m^{-2} s^{-1}). The strips were then transferred to stomatal opening buffer supplemented with 50 μM H_2DCF-DA (Sigma-Aldrich) for 10 min and then transferred to glass cover slips containing ABA dissolved in stomatal opening buffer (or mock). Images were taken using a fully motorized epifluorescence inverted microscope (Olympus-IX8 Cell-R) equipped with a 12-bit CCD camera (Orca-AG; Hamamatsu). The images were later analyzed using ImageJ software. Fluorescence was visualized with an excitation wavelength of 485 nm at low power (1.5%) and an emission wavelength of 530 nm.

Isolation of Guard Cells

Guard cells from tomato leaves were isolated according to Araújo et al. (2011) with some modifications. Briefly, 20 g of fully expanded leaves without the veins were ground twice in a blender in 100 mL cold distilled water, each time for 1 min. The blended mixture was poured onto a 200-μm mesh (Sefar) to remove broken mesophyll and epidermal cells. The remaining epidermal peels were rinsed thoroughly with deionized water. The peels were then transferred into 10-mL buffer (Araújo et al., 2011) containing the enzyme Cellulysin cellulase from *Trichoderma viride* (Calbiochem) and digested for 1 h at a shaking speed of 150 rpm. This enzymatic treatment digests pavement cells, but not guard cells (Wang et al., 2011). The digested material was poured again onto a 200-μm mesh placed in a tube and rinsed thoroughly with digestion buffer (without the enzyme). To remove residues of buffer and cell particles, the tubes were centrifuged at 4°C for 5 min at 2200 rpm. Samples of digested epidermal strips were stained with neutral red, and cell vitality was examined microscopically (Supplemental Figure 4).

RWC Determination

Leaf RWC was measured as follows: Fresh leaf weight (FW) was measured immediately after leaf detachment. Leaves were then soaked for 8 h in 5 mM $CaCl_2$ at room temperature in the dark, and the turgid weight (TW) was

recorded. Dry weight (DW) was recorded after drying the leaves at 70°C. RWC was calculated as (FW− DW)/(TW − DW) × 100 (Sade et al., 2009).

Stomatal Conductance and RWC

Stomatal conductance was determined using a SC-1 Leaf Porometer (Decagon Devices). VWC was measured using the 5TM soil moisture and temperature sensor, combined with the ProCheck interface reader (Decagon Devices).

Whole-Plant Transpiration Measurements

Whole-plant transpiration rates were determined using an array of lysimeters placed in the greenhouse (Plantarry 3.0 system; Plant-DiTech), as described in detail by Halperin et al. (2017). Briefly, plants in 4L pots were grown under semicontrolled temperature conditions (20–28°C day and 12–16°C night), natural daylength, and light intensity of ~1000 μmol m^{-2} s^{-1}. Each pot was placed on a temperature-compensated load cell with digital output (Vishay Tedea-Huntleigh) and sealed to prevent evaporation from the surface of the growth medium. The weight output of the load cells was monitored every 3 min. Transpiration rate was normalized to plant weight.

GFP Analysis

GFP signals in guard cells were detected with a model SP8 confocal laser scanning microscope (Leica Microsystems), with a 488-nm excitation laser line and a 505- to 525-nm emission filter.

Hormone Treatments

GA_3 and ABA (Sigma-Aldrich) were applied to plants by spraying. To evaluate the sensitivity of guard cells to ABA, epidermal peels were incubated for 30 min in a solution containing different concentrations of ABA and then the stomata were examined microscopically.

ABA Analysis

ABA extraction and analysis were performed as previously described (Lashbrooke et al., 2016). Briefly, 40 to 100 mg of ground frozen plant tissue was extracted at −20°C with methanol/water/formic acid containing stable isotope-labeled internal standards of ABA. Abscisates were purified by solid phase extraction and detected by ultraperformance liquid chromatography-electrospray tandem mass spectrometry (Waters) operated in MRM mode. Quantification was performed against an external calibration curve using analyte/internal standard peak ratios.

Accession Numbers

Sequence data from this article can be found in the Sol Genomics Network (https://solgenomics.net/) under the following accession numbers: *PRO*, Solyc11g011260; *TUBULIN*, Solyc04g077020; *SlP5CS1*, *Solyc06g019170*; *SlRAB18*, Solyc02g084850; *SlPYR1*, Solyc06g061180; *SlPYL2-1*, Solyc12g095970; *SlPYL2-2*, Solyc08g065410; *SlPYL4-1*, Solyc06g050500; *SlPYL4-2*, Solyc10g085310; *SlPYL4-3*, Solyc10g076410; *SlPYL6-1*, Solyc03g095780; *SlPYL6-2*, Solyc05g052420; *SlPYL6-3*, Solyc09g015380; *SlPYL-8-1*, Solyc01g095700; *SlPYL8-2*, Solyc03g007310; *SlPYL9-1*, Solyc08g082180; *SlPYL9-2*, Solyc12g055990; *SlPP2C*, Solyc07g062970; *SlOST1*, Solyc01g108280; and *SlRBOH1*, Solyc08g081690.

Supplemental Data

Supplemental Figure 1. Stomatal size and density in M82 and pro plants.

Supplemental Figure 2. Overexpression of rgaΔ17 in tomato plants reduces leaf size and increases stomatal density.

Supplemental Figure 3. Overexpression of the tomato stable DELLA (S-*della*), proΔ17, reduces stomatal pore area and transpiration.

Supplemental Figure 4. PRO and *proΔ17* expression in guard cells.

Supplemental Figure 5. Expressing *proΔ17* under the control of the *FIL* promoter reduces leaf size and increases stomatal density.

Supplemental Figure 6. Abaxial leaf epidermal tissues of control M82, *pro*, and *sit* plants.

Supplemental Figure 7. qRT-PCR analysis of *proΔ17* in *sit*.

Supplemental Figure 8. Stomata in leaf no. 4 of M82, *sit*, $35S_{pro}$:*proΔ17*, and $35S_{pro}$:*proΔ17* in the *sit* background.

Supplemental Figure 9. Stomatal response of FIL_{pro}>>*GFP-proΔ17* to ABA treatment.

Supplemental Figure 10. H_2O_2 accumulation (DCF fluorescent signal) in M82, pro, and $35S_{pro}$:*proΔ17* guard cells following ABA application.

Supplemental Table 1. Primers used in this study.

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AUTHOR CONTRIBUTIONS

D.W., I.N., and N.O designed the research plan. I.N., H.S., and I.P. performed the research. A.A contributed analytic tools. I.N., H.S., I.P., A.A., and D.W. analyzed data. I.N., N.O., and D.W. wrote the article.

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REFERENCES

Achard, P., Cheng, H., De Grauwe, L., Decat, J., Schoutteten, H., Moritz, T., Van Der Straeten, D., Peng, J., and Harberd, N.P. (2006). Integration of plant responses to environmentally activated phytohormonal signals. Science **311:** 91–94.

Achard, P., Renou, J.-P., Berthomé, R., Harberd, N.P., and Genschik, P. (2008). Plant DELLAs restrain growth and promote survival of adversity by reducing the levels of reactive oxygen species. Curr. Biol. **18:** 656–660.

Araújo, W.L., et al. (2011). Antisense inhibition of the iron-sulphur subunit of succinate dehydrogenase enhances photosynthesis and growth in tomato via an organic acid-mediated effect on stomatal aperture. Plant Cell **23:** 600–627.

Ariizumi, T., Hauvermale, A.L., Nelson, S.K., Hanada, A., Yamaguchi, S., and Steber, C.M. (2013). Lifting DELLA repression of Arabidopsis seed germination by nonproteolytic gibberellin signaling. Plant Physiol. **162:** 2125–2139.

Bassel, G.W., Mullen, R.T., and Bewley, J.D. (2008). Procera is a putative DELLA mutant in tomato (*Solanum lycopersicum*): effects on the seed and vegetative plant. J. Exp. Bot. **59:** 585–593.

Cai, S., et al. (2017). Evolutionary conservation of ABA signaling for stomatal closure. Plant Physiol. **174:** 732–747.

Chaves, M.M., Maroco, J.P., and Pereira, J.S. (2003). Understanding plant responses to drought - from genes to the whole plant. Funct. Plant Biol. **30:** 239–264.

Colebrook, E.H., Thomas, S.G., Phillips, A.L., and Hedden, P. (2014). The role of gibberellin signalling in plant responses to abiotic stress. J. Exp. Biol. **217:** 67–75.

Dill, A., Jung, H.S., and Sun, T.P. (2001). The DELLA motif is essential for gibberellin-induced degradation of RGA. Proc. Natl. Acad. Sci. USA **98:** 14162–14167.

Du, Y.-L., Wang, Z.-Y., Fan, J.-W., Turner, N.C., He, J., Wang, T., and Li, F.-M. (2013). Exogenous abscisic acid reduces water loss and improves antioxidant defence, desiccation tolerance and transpiration efficiency in two spring wheat cultivars subjected to a soil water deficit. Funct. Plant Biol. **40:** 494–506.

Endo, A., et al. (2008). Drought induction of Arabidopsis 9-cis-epoxycarotenoid dioxygenase occurs in vascular parenchyma cells. Plant Physiol. **147:** 1984–1993.

Fleishon, S., Shani, E., Ori, N., and Weiss, D. (2011). Negative reciprocal interactions between gibberellin and cytokinin in tomato. New Phytol. **190:** 609–617.

Geisler, M., Nadeau, J., and Sack, F.D. (2000). Oriented asymmetric divisions that generate the stomatal spacing pattern in arabidopsis are disrupted by the too many mouths mutation. Plant Cell **12:** 2075–2086.

Göring, H., Koshuchowa, S., and Deckert, C. (1990). Influence of gibberellic acid on stomatal movement. Biochem. Physiol. Pflanz. **186:** 367–374.

Guo, W., Cong, Y., Hussain, N., Wang, Y., Liu, Z., Jiang, L., Liang, Z., and Chen, K. (2014). The remodeling of seedling development in response to long-term magnesium toxicity and regulation by ABA-DELLA signaling in Arabidopsis. Plant Cell Physiol. **55:** 1713–1726.

González-Guzmán, M., et al. (2014). Tomato PYR/PYL/RCAR abscisic acid receptors show high expression in root, differential sensitivity to the abscisic acid agonist quinabactin, and the capability to enhance plant drought resistance. J. Exp. Bot. **65:** 4451–4464.

Halperin, O., Gebremedhin, A., Wallach, R., and Moshelion, M. (2017). High-throughput physiological phenotyping and screening system for the characterization of plant-environment interactions. Plant J. **89:** 839–850.

Harberd, N.P., Belfield, E., and Yasumura, Y. (2009). The angiosperm gibberellin-GID1-DELLA growth regulatory mechanism: how an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. Plant Cell **21:** 1328–1339.

Harrison, E., Burbidge, A., Okyere, J.P., Thompson, A.J., and Taylor, I.B. (2011). Identification of the tomato ABA-deficient mutant sitiens as a member of the ABA-aldehyde oxidase gene family using genetic and genomic analysis. Plant Growth Regul. **64:** 301–309.

Hauvermale, A.L., Ariizumi, T., and Steber, C.M. (2012). Gibberellin signaling: a theme and variations on DELLA repression. Plant Physiol. **160:** 83–92.

Hirano, K., Kouketu, E., Katoh, H., Aya, K., Ueguchi-Tanaka, M., and Matsuoka, M. (2012). The suppressive function of the rice DELLA protein SLR1 is dependent on its transcriptional activation activity. Plant J. **71:** 443–453.

Ho, S.N., Hunt, H.D., Horton, R.M., Pullen, J.K., and Pease, L.R. (1989). Site-directed mutagenesis by overlap extension using the polymerase chain reaction. Gene **77:** 51–59.

Hubbard, K.E., Nishimura, N., Hitomi, K., Getzoff, E.D., and Schroeder, J.I. (2010). Early abscisic acid signal transduction mechanisms: newly discovered components and newly emerging questions. Genes Dev. **24:** 1695–1708.

Jasinski, S., Tattersall, A., Piazza, P., Hay, A., Martinez-Garcia, J.F., Schmitz, G., Theres, K., McCormick, S., and Tsiantis, M. (2008). PROCERA encodes a DELLA protein that mediates control of dissected leaf form in tomato. Plant J. **56:** 603–612.

Jiang, M., and Zhang, J. (2002). Water stress-induced abscisic acid accumulation triggers the increased generation of reactive oxygen species and up-regulates the activities of antioxidant enzymes in maize leaves. J. Exp. Bot. **53:** 2401–2410.

George Jones, M. (1987). Gibberellins and the procera mutant of tomato. Planta **172:** 280–284.

Jupe, S.C., Causton, D.R., and Scott, I.M. (1988). Cellular basis of the effects of gibberellin and the pro gene on stem growth in tomato. Planta **174:** 106–111.

Kang, J., Hwang, J.U., Lee, M., Kim, Y.Y., Assmann, S.M., Martinoia, E., and Lee, Y. (2010). PDR-type ABC transporter mediates cellular uptake of the phytohormone abscisic acid. Proc. Natl. Acad. Sci. USA **107:** 2355–2360.

Kelly, G., Moshelion, M., David-Schwartz, R., Halperin, O., Wallach, R., Attia, Z., Belausov, E., and Granot, D. (2013). Hexokinase mediates stomatal closure. Plant J. **75:** 977–988.

Kim, T.-H., Böhmer, M., Hu, H., Nishimura, N., and Schroeder, J.I. (2010). Guard cell signal transduction network: advances in understanding abscisic acid, CO_2, and Ca^{2+} signaling. Annu. Rev. Plant Biol. **61:** 561–591.

Lashbrooke, J.G., Cohen, H., Levy-Samocha, D., Tzfadia, O., Panizel, I., Zeisler, V., Massalha, H., Stern, A., Trainotti, L., Schreiber, L., Costa, F., and Aharoni, A. (2016). MYB107 and MYB9 homologs regulate suberin deposition in Angiosperms. Plant Cell **28:** 2097–2116.

Li, J., et al. (2012). Tomato SlDREB gene restricts leaf expansion and internode methylation and chromatin patterning elongation by downregulating key genes for gibberellin biosynthesis. J. Exp. Bot. **63:** 695–709.

Lifschitz, E., Eviatar, T., Rozman, A., Shalit, A., Goldshmidt, A., Amsellem, Z., Alvarez, J.P., and Eshed, Y. (2006). The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli. Proc. Natl. Acad. Sci. USA **103:** 6398–6403.

Lim, S., Park, J., Lee, N., Jeong, J., Toh, S., Watanabe, A., Kim, J., Kang, H., Kim, D.H., Kawakami, N., and Choi, G. (2013). ABA-insensitive3, ABA-insensitive5, and DELLAs Interact to activate the expression of SOMNUS and other high-temperature-inducible genes in imbibed seeds in Arabidopsis. Plant Cell **25:** 4863–4878.

Liu, S., Li, M., Su, L., Ge, K., Li, L., Li, X., Liu, X., and Li, L. (2016). Negative feedback regulation of ABA biosynthesis in peanut (*Arachis hypogaea*): a transcription factor complex inhibits AhNCED1 expression during water stress. Sci. Rep. **6:** 37943.

Livne, S., Lor, V.S., Nir, I., Eliaz, N., Aharoni, A., Olszewski, N.E., Eshed, Y., and Weiss, D. (2015). Uncovering DELLA-independent gibberellin responses by characterizing new tomato procera mutants. Plant Cell **27:** 1579–1594.

Locascio, A., Blázquez, M.A., and Alabadí, D. (2013). Genomic analysis of DELLA protein activity. Plant Cell Physiol. **54:** 1229–1237.

Magome, H., Yamaguchi, S., Hanada, A., Kamiya, Y., and Oda, K. (2004). dwarf and delayed-flowering 1, a novel Arabidopsis mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor. Plant J. **37:** 720–729.

Magome, H., Yamaguchi, S., Hanada, A., Kamiya, Y., and Oda, K. (2008). The DDF1 transcriptional activator upregulates expression of a gibberellin-deactivating gene, GA2ox7, under high-salinity stress in Arabidopsis. Plant J. **56:** 613–626.

Marshall, J.G., and Dumbroff, E.B. (1999). Turgor regulation via cell wall adjustment in white spruce. Plant Physiol. **119:** 313–320.

McAdam, S.A.M., and Brodribb, T.J. (2016). Linking turgor with ABA biosynthesis: implications for stomatal responses to vapor pressure deficit across land plants. Plant Physiol. **171:** 2008–2016.

McCormick, S. (1991). Transformation of tomato with Agrobacterium tumefaciens. In Plant Tissue Culture Manual, H. Linclsey, ed (Dordrecht, The Netherlands: Kluwer Academic Publishers), pp. 1–9.

Moore, I., Gälweiler, L., Grosskopf, D., Schell, J., and Palme, K. (1998). A transcription activation system for regulated gene expression in transgenic plants. Proc. Natl. Acad. Sci. USA **95:** 376–381.

Munns, R. (2002). Comparative physiology of salt and water stress. Plant Cell Environ. **25:** 239–250.

Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. **59:** 651–681.

Murase, K., Hirano, Y., Sun, T.P., and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature **456:** 459–463.

Nir, I., Moshelion, M., and Weiss, D. (2014). The Arabidopsis gibberellin methyl transferase 1 suppresses gibberellin activity, reduces whole-plant transpiration and promotes drought tolerance in transgenic tomato. Plant Cell Environ. **37:** 113–123.

Pei, Z.M., Murata, Y., Benning, G., Thomine, S., Klüsener, B., Allen, G.J., Grill, E., and Schroeder, J.I. (2000). Calcium channels activated by hydrogen peroxide mediate abscisic acid signalling in guard cells. Nature **406:** 731–734.

Piskurewicz, U., Jikumaru, Y., Kinoshita, N., Nambara, E., Kamiya, Y., and Lopez-Molina, L. (2008). The gibberellic acid signaling repressor RGL2 inhibits Arabidopsis seed germination by stimulating abscisic acid synthesis and ABI5 activity. Plant Cell **20:** 2729–2745.

Plesch, G., Ehrhardt, T., and Mueller-Roeber, B. (2001). Involvement of TAAAG elements suggests a role for Dof transcription factors in guard cell-specific gene expression. Plant J. **28:** 455–464.

Sachs, T. (2005). Auxin's role as an example of the mechanisms of shoot/root relations. Plant Soil **268:** 13–19.

Sade, N., Vinocur, B.J., Diber, A., Shatil, A., Ronen, G., Nissan, H., Wallach, R., Karchi, H., and Moshelion, M. (2009). Improving plant stress tolerance and yield production: is the tonoplast aquaporin SlTIP2;2 a key to isohydric to anisohydric conversion? New Phytol. **181:** 651–661.

Santakumari, M., and Fletcher, R.A. (1987). Reversal of triazole-induced stomatal closure by gibberellic acid and cytokinins in *Commelina benghalensis*. Physiol. Plant. **71:** 95–99.

Shan, D.-P., Huang, J.-G., Yang, Y.-T., Guo, Y.-H., Wu, C.-A., Yang, G.-D., Gao, Z., and Zheng, C.-C. (2007). Cotton GhDREB1 increases plant tolerance to low temperature and is negatively regulated by gibberellic acid. New Phytol. **176:** 70–81.

Shani, E., Ben-Gera, H., Shleizer-Burko, S., Burko, Y., Weiss, D., and Ori, N. (2010). Cytokinin regulates compound leaf development in tomato. Plant Cell **22:** 3206–3217.

Sun, T.-P., and Gubler, F. (2004). Molecular mechanism of gibberellin signaling in plants. Annu. Rev. Plant Biol. **55:** 197–223.

Sun, X., Jones, W.T., and Rikkerink, E.H. (2012). GRAS proteins: the versatile roles of intrinsically disordered proteins in plant signalling. Biochem. J. **442:** 1–12.

Tal, M. (1966). Abnormal stomatal behavior in wilty mutants of tomato. Plant Physiol. **41:** 1387–1391.

Tuna, A.L., Kaya, C., Dikilitas, M., and Higgs, D. (2008). The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. Environ. Exp. Bot. **62:** 1–9.

Van Tuinen, A., Peters, A.H.L.J., Kendrick, R.E., Zeevaart, J.A.D., and Koornneef, M. (1999). Characterisation of the procera mutant of tomato and the interaction of gibberellins with end-of-day far-red light treatments. Physiol. Plant. **106:** 121–128.

Wang, C., Yang, A., Yin, H., and Zhang, J. (2008). Influence of water stress on endogenous hormone contents and cell damage of maize seedlings. J. Integr. Plant Biol. **50:** 427–434.

Wang, R.-S., Pandey, S., Li, S., Gookin, T.E., Zhao, Z., Albert, R., and Assmann, S.M. (2011). Common and unique elements of the ABA-regulated transcriptome of Arabidopsis guard cells. BMC Genomics **12:** 216.

Weston, D.E., Elliott, R.C., Lester, D.R., Rameau, C., Reid, J.B., Murfet, I.C., and Ross, J.J. (2008). The Pea DELLA proteins LA and CRY are important regulators of gibberellin synthesis and root growth. Plant Physiol. **147:** 199–205.

Wigoda, N., Ben-Nissan, G., Granot, D., Schwartz, A., and Weiss, D. (2006). The gibberellin-induced, cysteine-rich protein GIP2 from *Petunia hybrida* exhibits in planta antioxidant activity. Plant J. **48:** 796–805.

Yuan, F., et al. (2014). OSCA1 mediates osmotic-stress-evoked Ca^{2+} increases vital for osmosensing in Arabidopsis. Nature **514:** 367–371.

Zentella, R., Zhang, Z.-L., Park, M., Thomas, S.G., Endo, A., Murase, K., Fleet, C.M., Jikumaru, Y., Nambara, E., Kamiya, Y., and Sun, T.-P. (2007). Global analysis of della direct targets in early gibberellin signaling in Arabidopsis. Plant Cell **19:** 3037–3057.

Zhang, X., Zhang, L., Dong, F., Gao, J., Galbraith, D.W., and Song, C.P. (2001). Hydrogen peroxide is involved in abscisic acid-induced stomatal closure in *Vicia faba*. Plant Physiol. **126:** 1438–1448.

Zhu, J.K. (2002). Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. **53:** 247–273.