Enhancing Arabidopsis Salt and Drought Stress Tolerance by Chemical Priming for Its Abscisic Acid Responses[1]

Gabor Jakab[2]*, Jurriaan Ton[2,3], Victor Flors, Laurent Zimmerli, Jean-Pierre Métraux, and Brigitte Mauch-Mani

Institute of Botany, Biochemistry, University of Neuchâtel, CH–2007 Neuchatel, Switzerland (G.J., J.T., V.F., L.Z., B.M.-M.); Department of Biology, Plant Biology, University of Fribourg, CH–1700 Fribourg, Switzerland (G.J., J.-P.M.); and Departamento de Ciencias Experimentales, Área de Fisiología Vegetal, Universitat Jaume I, 12071 Castellon, Spain (V.F.)

Drought and salt stress tolerance of Arabidopsis (*Arabidopsis thaliana*) plants increased following treatment with the nonprotein amino acid β-aminobutyric acid (BABA), known as an inducer of resistance against infection of plants by numerous pathogens. BABA-pretreated plants showed earlier and higher expression of the salicylic acid-dependent *PR-1* and *PR-5* and the abscisic acid (ABA)-dependent *RAB-18* and *RD-29A* genes following salt and drought stress. However, *non-expressor of pathogenesis-related genes 1* and *constitutive expressor of pathogenesis-related genes 1* mutants as well as transgenic NahG plants, all affected in the salicylic acid signal transduction pathway, still showed increased salt and drought tolerance after BABA treatment. On the contrary, the *ABA deficient 1* and *ABA insensitive 4* mutants, both impaired in the ABA-signaling pathway, could not be protected by BABA application. Our data demonstrate that BABA-induced water stress tolerance is based on enhanced ABA accumulation resulting in accelerated stress gene expression and stomatal closure. Here, we show a possibility to increase plant tolerance for these abiotic stresses through effective priming of the preexisting defense pathways without resorting to genetic alterations.

Plant growth is greatly affected by a combination of environmental stresses such as extreme temperatures, drought, or high salinity. From an agricultural point of view, such stresses are among the most significant factors responsible for substantial and unpredictable losses in crop production. The physiological mechanisms governing the plant responses to salinity and drought show high similarity, suggesting that both stresses must be perceived by the plant cell as deprivation of water. High salt concentrations (most commonly NaCl) in the soil lead to a decrease in water potential, which affects water availability (Hasegawa et al., 2000). In addition to the hyperosmotic shock and the generated subsequent oxidative stress (Borsani et al., 2001), the deleterious consequences of high NaCl concentration in the external solution of plant cells also include ion toxicity and nutrient imbalance (Serrano et al., 1999; Hasegawa et al., 2000; Rodriguez-Navarro, 2000). As sessile organisms, plants had to develop various biochemical and physiological mechanisms to respond and adapt to these stresses and thus acquire stress tolerance. Adaptation to stress has been suggested to be mediated by both preexisting and induced defenses (Bray et al., 2000; Hasegawa et al., 2000; Pastori and Foyer, 2002).

An early response to water stress is the closure of stomatal pores through the action of the phytohormone abscisic acid (ABA). Enhanced ABA levels cause an increase in cytosolic Ca^{2+} concentration and subsequent activation of plasma membrane-localized anion channels (Hamilton et al., 2000; Pei et al., 2000; Zhang et al., 2001; Kohler and Blatt, 2002). This leads to guard cell depolarization, potassium efflux, loss of guard cell turgor and volume, and finally stomatal closure (Blatt, 2000; MacRobbie, 2000; Schroeder et al., 2001). ABA also causes an increase in H_2O_2 production, which serves as a signaling intermediate to promote stomatal closure (Pei et al., 2000; Murata et al., 2001; Schroeder et al., 2001; Zhang et al., 2001).

In response to osmotic stress, many plant species accumulate Pro due to the simultaneous ABA-mediated activation of its biosynthesis and inactivation of its degradation pathways during stress (Hare et al., 1999). Pro, a compatible osmolyte, performs a protective function by scavenging free radicals and regulating redox potential by replenishment of the $NADP^+$ supply (Hasegawa et al., 2000). Many stress signals have been shown to increase the level of ABA indicating that ABA plays an important role in plant stress responses. In addition, some effects of stress conditions can be simulated by applying ABA to plants. For example, there is much overlap in the expression pattern

[1] This work was supported by the National Center of Competence in Research on Plant Survival in Natural and Agricultural Ecosystems (grant to B.M.-M.), the Swiss National Science Foundation (grant nos. 3100–064024 to B.M.-M. and 3100A0–104224/1 to J.-P.M.), and the Agència Valenciana de Ciència i Tecnologia, Generalitat Valenciana, Spain (grant to V.F.).

[2] These authors contributed equally to the paper.

[3] Present address: Section of Phytopathology, Faculty of Biology, Utrecht University, 3584 CA Utrecht, The Netherlands.

* Corresponding author; e-mail gabor.jakab@unine.ch; fax 41–32–718–2201.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.105.065698.

of stress genes after exposure to drought and high salt, or application of ABA. Stress signals and ABA are likely to share common elements in their respective signaling pathways (Leung and Giraudat, 1998; Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000; Finkelstein et al., 2002). However, signaling in response to osmotic stress caused by high salt concentrations or drought seems not to depend solely on ABA. Studies of cis- and trans-acting elements regulating stress gene expression revealed the existence of ABA-independent signaling during water stress (Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000). This was also observed in a number of other studies where the regulation of gene expression in response to drought and salinity was found to involve several signaling systems (Ingram and Bartels, 1996; Bray, 1997; Thomashow, 1999; Fowler and Thomashow, 2002; Seki et al., 2002a, 2002b). Some cold- and drought-inducible (*COR*, *RD*, *KIN*) genes contain a cis-acting element called drought/cold-responsive element that regulates ABA-independent gene induction (Shinozaki and Yamaguchi-Shinozaki, 1996; Thomashow, 1999). On the other hand, gene expression induced by ABA often depends on the presence of another cis-acting element called ABA-responsive element (Shinozaki and Yamaguchi-Shinozaki, 2000; Uno et al., 2000; Finkelstein et al., 2002). A responsive to ABA (RAB)-related gene of Arabidopsis (*Arabidopsis thaliana*), *RAB18*, has been shown to accumulate following drought stress and exogenous application of ABA in wild-type plants (Lang and Palva, 1992). In contrast, ABA-deficient mutants (*aba*; Koornneef et al., 1982) and ABA-insensitive mutants (*abi*; Koornneef et al., 1984) do not show an increase of transcripts of *RAB18* following abiotic stress, and exogenous ABA treatment leads to an increase in *aba* but not *abi* mutants. Another gene, RD29A (responsive to desiccation), however, shows both ABA-independent and ABA-responsive expression because its promoter region contains both drought-responsive element and ABA-responsive element cis-acting elements (Narusaka et al., 2003).

Interestingly, several genes induced transcriptionally by osmotic stress are also part of plant defense responses to wounding and pathogen attack. Expression of peroxidase, *PR-1*, *PR-10*, and osmotin (*PR-5*) is increased by water stress even though the role of these proteins in abiotic stress has not fully been clarified (Zhu et al., 1995; Ingram and Bartels, 1996). During plant pathogen interactions, the expression of the pathogenesis-related (PR) protein genes is induced by the plant hormone salicylic acid (SA), an important regulator of systemic acquired resistance (Sticher et al., 1997). Several studies support a major role of SA in modulating the plant response to several abiotic stresses. SA-treated mustard (*Sinapis alba*) seedlings show an improved thermotolerance and heat acclimation (Dat et al., 1998). In Arabidopsis, endogenous SA accumulation promotes basal thermotolerance but it is not essential for acquired thermotolerance (Clarke et al., 2004). Pretreatment of maize (*Zea mays*) plants with SA induces antioxidant enzymes and leads to increased chilling tolerance (Janda et al., 1999; Kang and Saltveit, 2002). SA treatment also increases the resistance of wheat (*Triticum aestivum*) seedlings to salinity and drought (Shakirova et al., 2003).

The success of plant adaptation to stress depends on an early sensing of the stress followed by an adequate reaction. For instance, plants show a stronger and faster defense response upon exposure to a pathogen, a phenomenon known as priming (Conrath et al., 2002). Priming can also be observed in plants exposed to abiotic stress. If plants have previously undergone an acclimation process, their reaction to the following stress is more successful (Lang and Palva, 1992; Knight et al., 1998).

The nonprotein amino acid β-aminobutyric acid (BABA), a potent inducer of resistance against infection by various pathogens (Jakab et al., 2001), in certain cases exerted its function via priming of SA-dependent defense mechanisms in Arabidopsis (Zimmerli et al., 2000). In other cases, however, BABA acts through potentiation of ABA-dependent signaling pathways (Ton and Mauch-Mani, 2004). Because both pathways could contribute to water stress tolerance, we have tested the drought and salt stress tolerance of Arabidopsis after BABA treatment. In this study we demonstrate that BABA, although rarely found in plants (Jakab et al., 2001), is also able to protect Arabidopsis against abiotic stresses such as drought and high salinity. This protection is based on ABA-dependent but SA-independent defense mechanisms.

RESULTS

BABA Protects Arabidopsis Against Drought and Salt Stress

During previous plant protection experiments by BABA against pathogens, BABA-treated Arabidopsis plants exhibited enhanced drought tolerance. To further investigate this observation drought tolerance of Arabidopsis after treatment with different chemicals has been tested. Depriving Arabidopsis of water leads to desiccation within 1 week, visible as progressive wilting of the plants starting with the older leaves (Fig. 1A, control). However, plants pretreated with BABA (300 μM) looked unaffected at this time (Fig. 1A, BABA) and showed a delayed onset of wilting by several days, presumably due to the reduced rate of water loss (Fig. 1B). Compared to the 70% water loss of control plants, BABA-treated plants lost only 35% of their water content. This protection was comparable to the one induced by ABA (100 μM) and isomer specific because α-aminobutyric acid (AABA) and γ-aminobutyric acid (GABA) failed to induce tolerance. Plants treated with these compounds showed 60% to 70% of water loss, which is comparable to water-treated control plants (Fig. 1B).

We have also tested the potential protective effect of BABA against salt stress, another treatment perceived

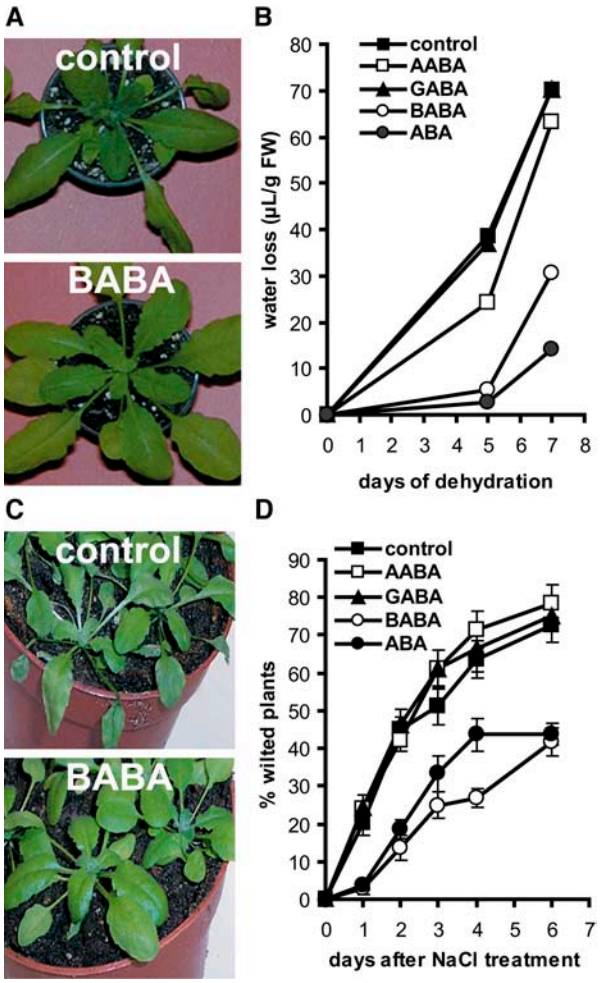

Figure 1. Protective effect of BABA against drought and salinity. A, Symptoms of drought stress in Arabidopsis (accession Col-0). One day prior to stress treatment, plants were pretreated with water (control) or 300 μM BABA. Six-week-old plants were subjected to dehydration. Pictures were taken after 6 d of dehydration. B, Quantification of drought stress in Col-0 plants. One day prior to stress treatment, plants were pretreated with water (control), 300 μM AABA, BABA, GABA, or 100 μM ABA, respectively. Six-week-old plants were subjected to dehydration. Values shown are means of water loss (μL/g fresh weight) in the leaves (10–12 leaves from five to six different plants) after different days of dehydration. Results shown come from a representative experiment that was repeated three times yielding comparable results. C, Symptoms of salt stress in Arabidopsis (accession Col-0). One day prior to stress treatment, plants were pretreated with water (control) or 300 μM BABA. Four-week-old plants were subjected to salt stress by drenching the soil with 3 M NaCl to a final concentration of 300 mM. Pictures were taken 3 d after NaCl application. D, Quantification of salt stress in Col-0 plants. One day prior to stress treatment, plants were pretreated with water (control), 300 μM AABA, BABA, GABA, or 100 μM ABA, respectively. Four-week-old plants were subjected to salt stress by drenching the soil with 3 M NaCl solution to a final concentration of 300 mM. Data shown are means (±SD, n = 50) of the percentage of wilted plants at different days after NaCl treatment.

by plants as water stress. To this end, plants were challenged with high salt concentrations in the soil. Soil drench treatment with 300 mM NaCl caused wilting of almost all Arabidopsis seedlings of the control treatment within 5 d (Fig. 1C, control), whereas the BABA-treated plants showed no symptoms within this timeframe (Fig. 1C, BABA). Pretreatment of the plants with 300 μM BABA, however, reduced the wilting rate by 50% (Fig. 1D). As observed with drought stress, the protection against salinity was isomer specific and comparable to that of ABA-induced tolerance (Fig. 1D). Similar to BABA treatment, ABA treatment reduced the wilting rate to 40% after 6 d of salt application, while following AABA and GABA treatment the wilting rate remained at the same level as in the control (70%–80%; Fig. 1D).

BABA Primes Both SA and ABA Signaling in Arabidopsis Plants during Salt Stress

Previously we have shown that the protective effect of BABA against plant pathogens is based on priming of either SA- or ABA-dependent defense pathways. Because both pathways could be involved in the observed BABA-induced drought and salt stress tolerance, the potential of BABA to trigger priming was tested for both SA- and ABA-dependent defenses using SA- and ABA-inducible marker genes. Upon soil drench treatment with 300 mM NaCl, BABA-treated plants showed activation of the SA-dependent *PR-1* and *PR-5* genes 6 h earlier than noninduced control plants (Fig. 2A). Additionally, BABA-treated plants activated both PR genes at lower salt concentrations than control plants (Fig. 2B). Due to the BABA-potentiated expression of these genes, a low expression was often detected at time point 0 probably caused by small environmental fluctuations during the treatment periods.

To investigate whether BABA also primes for ABA-dependent signals, we quantified the expression of the ABA-responsive *RAB18* and *RD29A* genes. Here again, BABA-treated plants expressed these genes 2 h earlier (Fig. 2A) and at lower salt concentrations than control plants (Fig. 2B). Hence, BABA primes for both SA- and ABA-dependent defense upon salt stress.

BABA-Induced Drought and Salt Tolerance Requires Functional ABA Signaling But No SA Signaling

To determine whether SA- or ABA-dependent signaling is responsible for BABA-induced tolerance, we tested different Arabidopsis genotypes affected in SA- or ABA-dependent signaling. Plants impaired in the SA pathway (*constitutive expressor of pathogenesis-related genes 1* [*cpr1*], *non-expressor of pathogenesis-related genes 1* [*npr1*], and NahG) expressed wild-type levels of BABA-induced tolerance against drought stress (Fig. 3A) and salt stress (Fig. 3B), indicating that SA-dependent signaling is not critical for BABA-induced tolerance to water stress. In a similar manner, ethylene (*ein2*)- and jasmonic acid (*jar1*)-dependent signaling was also found to be dispensable for the

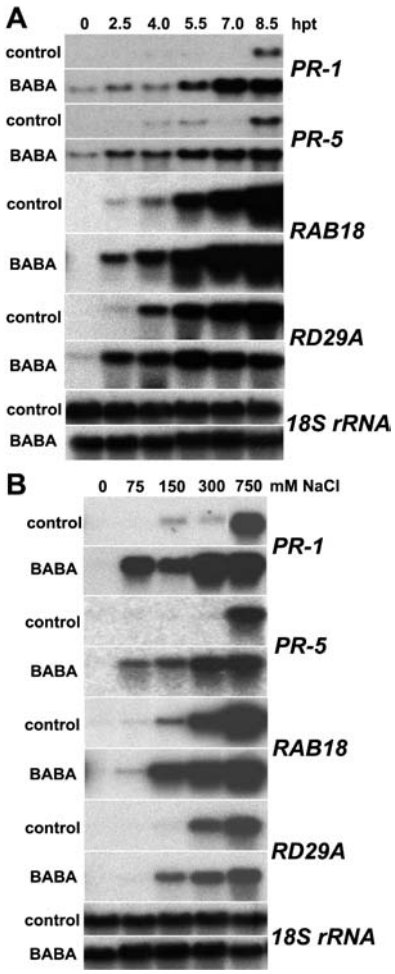

Figure 2. Effect of BABA on salt-inducible expression of ABA-inducible genes (*RD29A* and *RAB18*) and SA-inducible genes (*PR-1* and *PR-5*) in Arabidopsis (Col-0) plants. One day prior to salt application, plants were treated with water (control) or 300 μM BABA. Hybridization with an 18S rRNA-specific probe was used as a loading control. The experiment was performed three times with similar results. A, Four-week-old plants were treated with 300 mM NaCl (final concentration in the soil), and samples were collect at the times indicated on the top of the figure. B, Four-week-old plants were treated with increasing concentrations of NaCl 3 d before harvesting the leaves.

BABA-induced tolerance against drought (Fig. 3A) or high salt (Fig. 3B). The observed delayed onset of desiccation of the *cpr1* and *jar1* mutants is probably due to their overall smaller and more compact phenotype. Conversely, mutants impaired in either ABA biosynthesis (*ABA deficient 1* [*aba1*]) or ABA signaling (*ABA insensitive 4* [*abi4*]) completely lost their ability to react to BABA treatment, demonstrating that BABA-induced water stress tolerance is based on ABA-dependent priming mechanisms (Fig. 3).

BABA Primes ABA Synthesis, Leading to Faster Stomatal Closure But Not to Increased Pro Accumulation

ABA synthesis is important for defense signaling against salt and osmotic stress (Hasegawa et al., 2000). Since an intact ABA-signaling pathway is also necessary for BABA-induced tolerance against drought and salinity (Fig. 3), we have tested whether BABA could prime for ABA accumulation. Our results show that 24 h after salt stress induction plants pretreated with BABA accumulated higher levels of ABA in comparison to control plants, indicating a faster and stronger reaction in BABA-treated plants (Fig. 4A). The accumulation of ABA was as high in BABA-pretreated plants following 75 mM NaCl treatment as in control plants when 150 mM NaCl was applied.

In contrast, Pro accumulation in response to salt stress was not enhanced by BABA treatment, although both BABA-treated and control plants reacted with an increased Pro accumulation (data not shown). Apparently, BABA did not prime the accumulation of this osmolyte.

Another expected effect of primed ABA accumulation was an accelerated stomatal closure under water stress. After transferring Arabidopsis plants from high humidity conditions (relative humidity [RH] = 100%) to low humidity conditions (RH = 60%), BABA-pretreated wild-type plants showed a higher production of ABA during the first day (Fig. 4B) and a faster reduction in stomatal conductance than control plants (Fig. 4C). This accelerated adaptation to low humidity led to enhanced water use efficiency in BABA-treated plants (data not shown). When plants were maintained at low air humidity (RH = 60%), stomatal conductance in BABA-treated plants remained lower during several days (Fig. 4C). The decrease in stomatal conductance ensured elevated water use efficiency in BABA-treated plants, explaining their enhanced tolerance to drought stress.

DISCUSSION

We have shown that Arabidopsis exhibits increased drought and salt stress tolerance following BABA pretreatment. In previous studies we have found that BABA protects Arabidopsis against pathogens through the potentiation of either the SA-dependent defenses or ABA-regulated responses (Zimmerli et al., 2000; Ton and Mauch-Mani, 2004). Expression patterns of marker genes for the SA pathway (*PR-1*, *PR-5*) and the ABA pathway (*RAB18*, *RD29A*) demonstrated that both pathways were also primed by BABA in response to salt stress (Fig. 2). Although ABA is the most important hormone in water stress signal transduction, SA was also reported to be involved in the protection of plants against abiotic stress (Janda et al., 1999; Kang and Saltveit, 2002; Shakirova et al., 2003). The participation of PR proteins in the BABA-induced osmotic stress tolerance appeared possible because induction of PR proteins by water stress and low temperature has been shown in several plant species (Griffith and Ewart, 1995; Yeh et al., 2000). Drought, salt, and cold stress all induce the accumulation of reactive oxygen species such as superoxide, hydrogen peroxide, and

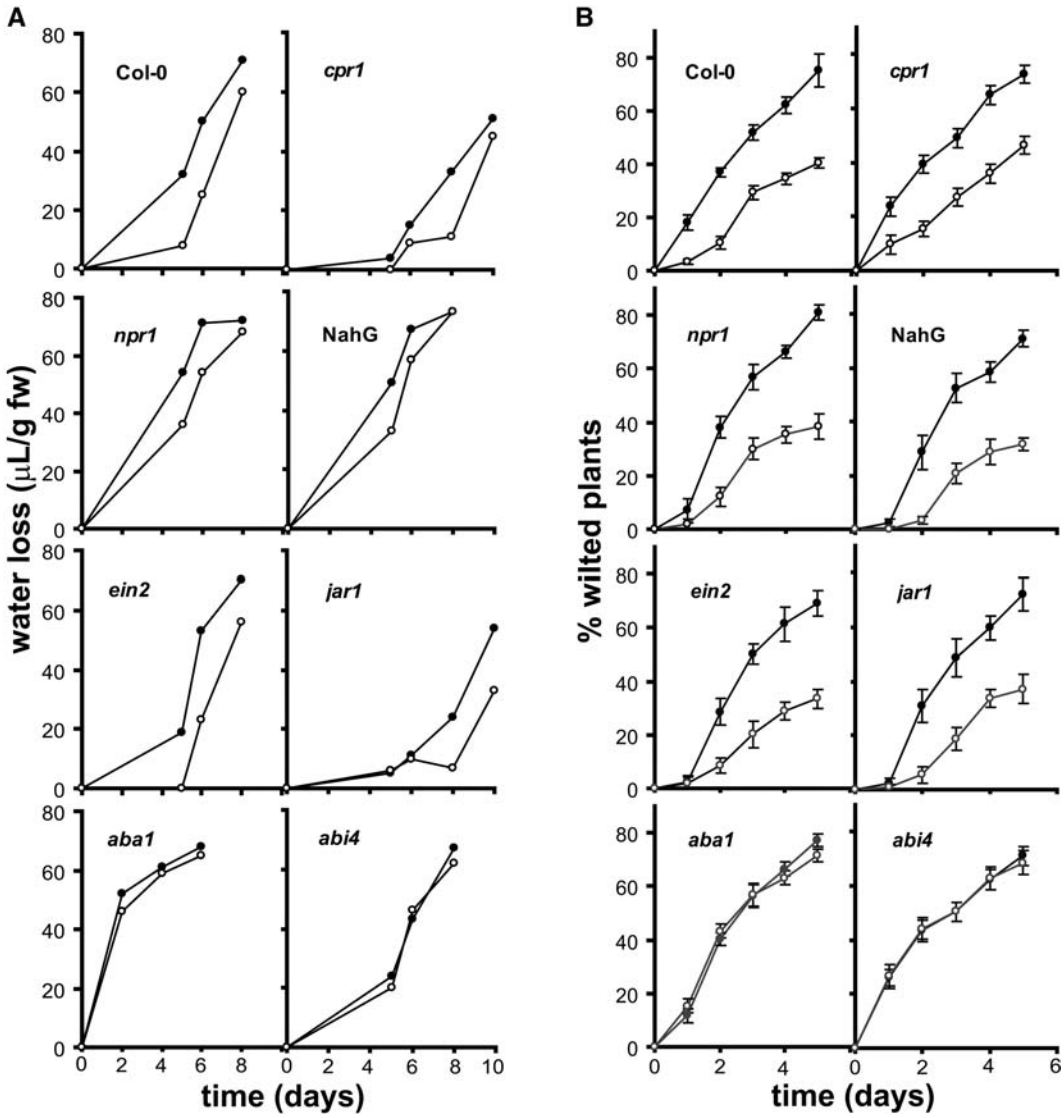

Figure 3. Quantification of drought and salt stress in Arabidopsis. A, Quantification of water loss in 6-week-old Arabidopsis (Col-0, *cpr1-1*, *npr1-1*, NahG, *ein2-1*, *jar1-1*, *aba1-5*, and *abi4-1*) plants after different periods of dehydration. One day prior to the start of dehydration, plants were treated with water (black circles) or 300 μM BABA (white circles). Data shown are the average amounts of water loss in 15 leaves (μL/g fresh weight) collected from five different plants. The experiment was repeated twice with similar results. B, Quantification of wilting in 4-week-old Arabidopsis (Col-0, *cpr1-1*, *npr1-1*, NahG, *ein2-1*, *jar1-1*, *aba1-5*, and *abi4-1*) plants at different days after application of 300 mM NaCl. One day prior to salt application, plants were treated with water (black circles) or 300 μM BABA (white circles). Data shown are means (±SD, $n = 10$) of the percentage wilted plants.

hydroxyl radicals (Van Breusegem et al., 2001). Although it is unclear whether osmotic stress leads to an increased SA level in plants, the observation that osmotic stress and SA activate the same mitogen-activated protein kinase (Hoyos and Zhang, 2000) suggests that osmotic stress signal transduction and SA signal transduction share common components. However, BABA is able to induce drought and salt stress tolerance in Arabidopsis plants independent of functional SA signaling and the observed priming for SA-dependent defense responses are dispensable in BABA-induced protection against water stress.

Although not all genes induced in plant responses to osmotic stress require functional ABA signaling (Shinozaki and Yamaguchi-Shinozaki, 2000), this hormone plays an important role in adaptation to abiotic stress and in regulation of several genes that are thought to be involved in dehydration or salt tolerance. To determine whether the observed priming of the ABA-regulated genes was important in BABA-induced stress tolerance, we have tested different Arabidopsis mutants of this pathway. Mutants impaired in either ABA biosynthesis (*aba1*) or ABA signaling (*abi4*) completely lost their ability to react for

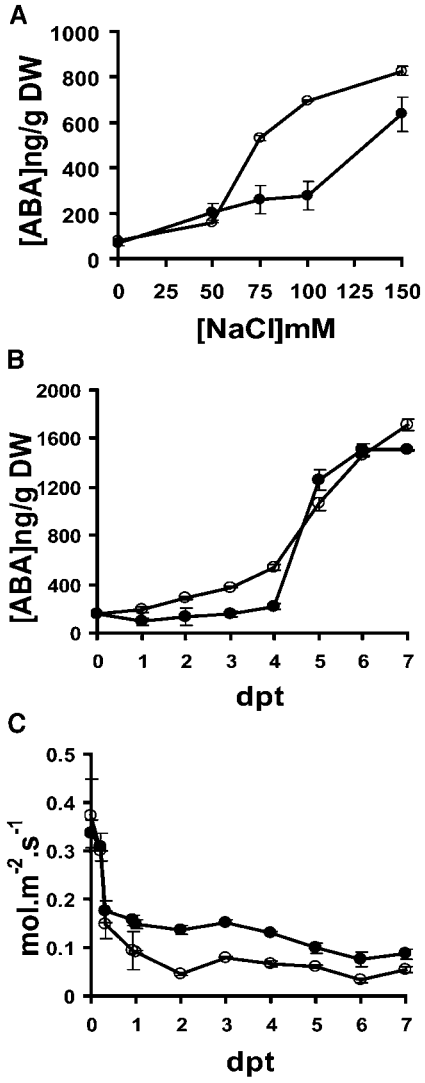

Figure 4. Quantification of ABA accumulation in wild-type Arabidopsis (Col-0) upon exposure to salt stress (A) and decreased humidity (B) and determination of stomatal conductance during water stress (C). A, ABA was quantified in 3-week-old plants subjected to salt stress by drenching the soil with 3 M NaCl solution to a final concentration of 50, 75, 100, and 150 mM of NaCl. Two days prior to stress treatment, plants were treated with water (control, black circles) or 300 μM BABA (white circles), and 24 h after the stress application plants were collected and freeze dried for analysis. Data shown are means (±SD, $n = 15$) of the amount of isolated ABA (ng/g dry weight). B, ABA was quantified in plants subjected to low humidity. Five-week-old plants were kept at 100% relative air humidity for 5 d. Twenty-four hours after treatment with either BABA (300 μM, white circles) or water (control, black circles) plants were transferred to 60% relative air humidity ($T = 0$). Plants were collected at different time points as indicated and freeze dried for analysis. Data shown are means (±SD, $n = 10$) of the amount of isolated ABA (ng/g dry weight). C, Stomatal conductance of water-treated (black symbols) or BABA-treated (300 μM, white symbols) plants during adaptation to low humidity. Five-week-old plants were treated as in B. Stomatal conductance was determined by six consecutive measurements using three leaves per plant at different time points, as indicated. Values shown are means (±SD) of 10 plants per time points.

BABA treatment (Fig. 3). This demonstrates that only priming for ABA signaling is responsible for BABA-induced tolerance to water stress in Arabidopsis. The earlier and faster ABA production, stomatal closure (Fig. 4), and expression of ABA-regulated genes (Fig. 2) lead to an enhanced water use efficiency of the plants without an increase in Pro accumulation.

The rapid reduction of stomatal aperture size under water stress conditions and the enhanced ABA accumulation seem to be the basis for BABA-induced tolerance to drought and salt stress. Besides osmotic stress, salt stress also provokes strong ion toxicity resulting from a fast ion uptake by the plant in response to the disequilibrium in the soil water potential. The continuous accumulation of Cl^- and Na^+ ions may produce toxic effects disrupting metabolic processes (Greenway and Munns, 1980). A protective effect of ABA against ion toxicity, probably due to a decrease of the transpiration rate, has been reported (Amzallag et al., 1990). In contrast to stomatal closure, osmotic adjustment through the synthesis of organic compounds is a long-term adaptation to reduce the osmotic potential in the plant cell (Bray et al., 2000). Therefore we hypothesize that BABA-treated plants do not accumulate more Pro than the water-treated plants because the priming of ABA already leads to a fast osmotic adjustment through a faster stomatal closure, which in return helps to reduce the toxic accumulation of Na^+ and Cl^-. This is supported by findings of Savoure et al. (1997), who studied Pro accumulation in *abi1-1* and *aba1-1* mutants. They observed a reduced accumulation of Pro in the *abi1-1* mutant in response to NaCl that can be explained in part by a protective effect of ABA against ion toxicity. The reduced accumulation of Pro in *aba1-1* mutants, however, may be directly due to the lower ABA content.

Based on experiments with excised roots using concentrations of BABA (1–10 mM) that in our hands proved to be phytotoxic, Essah et al. (2003) proposed that both BABA and also GABA could function as regulators of the proteins involved in the regulation of Na^+ influx into Arabidopsis roots. However, under our experimental conditions (using much lower concentrations of the inducers to avoid phytotoxic side effects) this effect can be excluded, because we observed no protection following GABA treatment.

Due to global climate change, drought and salinity are an increasing problem for agriculture and ecosystems. The resulting abiotic stress is the primary cause of crop loss worldwide and reduces average yields for most crop plants by more than 50% (Bray et al., 2000). Therefore, several plant biotechnology programs have been initiated to increase water stress tolerance in crop plants using genetic engineering and traditional breeding (Xiong and Zhu, 2002; Wang et al., 2003). Here, we present a new concept to meet this objective and protect plants through priming of existing defense mechanisms avoiding manipulation of the genome. Moreover, primed plants do not suffer from costly defense investments (Kasuga et al., 1999; Heil, 2002),

since their defense arsenal is not activated before stress exposure.

MATERIALS AND METHODS

Biological Material

Arabidopsis (*Arabidopsis thaliana*) mutants *npr1-1*, *cpr1-1*, *jar1-1*, *ein2-1*, *aba1-5*, and *abi4-1* (all in ecotype Columbia [Col-0] background) were obtained from X. Dong (Duke University, Durham, NC), P.E. Staswick (University of Nebraska, Lincoln, NE), and the Nottingham Arabidopsis Stock Centre (Loughborough, UK), respectively. A transgenic line of Arabidopsis (Col-0) harboring the *NahG* gene (Delaney et al., 1994) was provided by J. Ryals (Novartis, Research Triangle Park, NC). Arabidopsis wild-type accessions Col-0 and Wassilewskija were obtained from Lehle Seeds. Plants were grown in a steam-sterilized soil mix of commercial potting soil/perlite (3:1) at 22°C day and 18°C night temperature with 8 h light/24 h with a light intensity of 200 μE m^{-2} s^{-1}.

Chemical Treatment

AABA, BABA, GABA, and NaCl (Fluka) were dissolved in water, ABA first in a small volume of ethanol and further diluted by water. The 10-times concentrated solutions were applied as soil drench to obtain the indicated final concentrations in the soil as described earlier (Zimmerli et al., 2000).

Drought Treatment

Drought was induced by stopping to water 5-week-old plants 1 d after BABA treatment by soil drench. Ten to 12 leaves from five to six different plants were removed at the time points indicated. Subsequently, leaves were weighted, incubated in demineralized water for 3 h, and weighed again. The difference in weight was considered as water loss. The time point of BABA treatment is considered day 0.

RNA Extraction and Analysis

RNA was isolated from frozen tissue samples as described previously (Zimmerli et al., 2000). To obtain the gene-specific DNA fragments Arabidopsis cDNA as a template and PCR amplification were used with the following primers: rab18fw, 5′-AACATGGCGTCTTACCAGAA; rab18rev, 5′-CGATTGTTCGAAGCTTAACG; rd29afw, 5′-CCGGTGGGCTTTGGTGAC; and rd29arev, 5′-CTCCTCCGATGCTGCCTTCT.

Determination of Pro and ABA Content

Pro content was measured according to Bates et al. (1973) from lyophilized leaves (0.1 g). ABA analysis was performed according to Gómez-Cadenas et al. (2002). ABA, extracted from 50 mg of lyophilized tissue, was dissolved in 1 mL of MeOH:water (10:90; v/v), filtered, and injected into the HPLC. Analyses were carried out using a Waters (Milford) Alliance 2690 HPLC system with a Nucleosil ODS reversed-phase column (100 × 2 mm i.d.; 5 μm). The chromatographic system was interfaced to a Quatro LC (quadrupole-hexapole-quadrupole) mass spectrometer (Micromass). The Masslynx NT version 3.4 (Micromass) software was used to process the quantitative data from calibration standards and the plant samples. As internal standard, 100 ppb deuterated ABA (dABA, Sigma) was added to the tissue before the homogenization.

Stomatal Conductance Measurements

Five-week-old Arabidopsis plants were transferred to high humidity (by closing the tray with a tightly fitting transparent cover) conditions 5 d before treatment with BABA (300 μM). One day after BABA treatment, the cover was removed and leaves were measured at different time points. Stomatal conductance and water use efficiency were measured with a closed gas-exchange infrared analyzer portable photosynthesis system (LC-PRO+, ADC). Leaves were totally enclosed within a fan-stirred cuvette and maintained under artificial conditions (leaf temperature of 21°C and irradiance of 870 of μmol m^{-2} s^{-1}). Measurements were taken after an adaptation period of 5 min.

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers M90508 (PR-1), M90510 (PR-5), X68042 (RAB18), and D13044 (RD29A).

ACKNOWLEDGMENTS

We thank J. Ryals for the cDNA of *PR-1* and *PR-5*, and A. Gomez-Cadenas for technical support with ABA determination. We are grateful to Felix Mauch for critically reading the manuscript.

Received May 17, 2005; revised June 17, 2005; accepted June 17, 2005; published August 19, 2005.

LITERATURE CITED

Amzallag GN, Lerner HR, Poljakoff-Mayber A (1990) Exogenous ABA as a modulator of the response of sorghum to high salinity. J Exp Bot **41:** 1529–1534

Bates LS, Waldre RP, Teare ID (1973) Rapid determination of free proline for water stress studies. Plant Soil **39:** 205–208

Blatt MR (2000) Cellular signaling and volume control in stomatal movements in plants. Annu Rev Cell Dev Biol **16:** 221–241

Borsani O, Valpuesta V, Botella MA (2001) Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in Arabidopsis seedlings. Plant Physiol **126:** 1024–1030

Bray EA (1997) Plant responses to water deficit. Trends Plant Sci **2:** 48–54

Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to abiotic stresses. *In* W Gruissem, B Buchannan, R Jones, eds, Biochemistry and Molecular Biology of Plants. American Society of Plant Physiologists, Rockville, MD, pp 1158–1249

Clarke SM, Mur LAJ, Wood JE, Scott IM (2004) Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in Arabidopsis thaliana. Plant J **38:** 432–447

Conrath U, Pieterse CM, Mauch-Mani B (2002) Priming in plant-pathogen interactions. Trends Plant Sci **7:** 210–216

Dat JF, Lopez-Delgado H, Foyer CH, Scott IM (1998) Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. Plant Physiol **116:** 1351–1357

Delaney TP, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gutrella M, Kessmann H, Ward E, et al (1994) A central role of salicylic acid in plant disease resistance. Science **266:** 1247–1250

Essah PA, Davenport R, Tester M (2003) Sodium influx and accumulation in Arabidopsis. Plant Physiol **133:** 307–318

Finkelstein RR, Gampala SS, Rock CD (2002) Abscisic acid signaling in seeds and seedlings. Plant Cell **14:** S14–S45

Fowler S, Thomashow MF (2002) Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell **14:** 1675–1690

Gómez-Cadenas A, Pozo OJ, García-Agustín P, Sancho JV (2002) Direct analysis of abscisic acid in crude plant extracts by liquid chromatography-electrospray/tandem mass spectrometry. Phytochem Anal **13:** 228–234

Greenway H, Munns R (1980) Mechanisms of salt tolerance in nonhalophytes. Annu Rev Plant Physiol **31:** 149–190

Griffith M, Ewart KV (1995) Antifreeze proteins and their potential use in frozen foods. Biotechnol Adv **13:** 375–402

Hamilton DW, Hills A, Kohler B, Blatt MR (2000) Ca2+ channels at the plasma membrane of stomatal guard cells are activated by hyperpolarization and abscisic acid. Proc Natl Acad Sci USA **97:** 4967–4972

Hare PD, Cress WA, van Staden J (1999) Proline synthesis and degradation: a model system for elucidating stress-related signal transduction. J Exp Bot **50:** 413–434

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol **51:** 463–499

Heil M (2002) Ecological costs of induced resistance. Curr Opin Plant Biol **5:** 345–350

Hoyos ME, Zhang S (2000) Calcium-independent activation of salicylic acid-induced protein kinase and a 40-kilodalton protein kinase by hyperosmotic stress. Plant Physiol **122:** 1355–1363

Ingram J, Bartels D (1996) The molecular basis of dehydration tolerance in plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 377–403

Jakab G, Cottier V, Toquin V, Rigoli G, Zimmerli L, Metraux J-P, Mauch-Mani B (2001) β-aminobutyric acid-induced resistance in plants. Eur J Plant Pathol **107:** 29–37

Janda T, Szalai G, Tari I, Paldi E (1999) Hydroponic treatment with salicylic acid decreases the effects of chilling injury in maize (Zea mays L.) plants. Planta **208:** 175–180

Kang HM, Saltveit ME (2002) Chilling tolerance of maize, cucumber and rice seedling leaves and roots are differentially affected by salicylic acid. Physiol Plant **115:** 571–576

Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol **17:** 287–291

Knight H, Brandt S, Knight MR (1998) A history of stress alters drought calcium signalling pathways in Arabidopsis. Plant J **16:** 681–687

Kohler B, Blatt MR (2002) Protein phosphorylation activates the guard cell Ca2+ channel and is a prerequisite for gating by abscisic acid. Plant J **32:** 185–194

Koornneef M, Jorna ML, Brinkhorst-van der Swan DLC, Karssen CM (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of Arabidopsis thaliana (L.) Heynh. Theor Appl Genet **61:** 385–393

Koornneef M, Reuling G, Karssen C (1984) The isolation and characterization of abscisic acid-insensitive mutants of Arabidopsis thaliana. Physiol Plant **61:** 377–383

Lang V, Palva ET (1992) The expression of a rab-related gene, rab18, is induced by abscisic acid during the cold acclimation process of Arabidopsis thaliana (L.) Heynh. Plant Mol Biol **20:** 951–962

Leung J, Giraudat J (1998) Abscisic acid signal transduction. Annu Rev Plant Physiol Plant Mol Biol **49:** 199–222

MacRobbie EA (2000) ABA activates multiple Ca(2+) fluxes in stomatal guard cells, triggering vacuolar K(+)(Rb(+)) release. Proc Natl Acad Sci USA **97:** 12361–12368

Murata Y, Pei ZM, Mori IC, Schroeder J (2001) Abscisic acid activation of plasma membrane Ca(2+) channels in guard cells requires cytosolic NAD(P)H and is differentially disrupted upstream and downstream of reactive oxygen species production in *abi1-1* and *abi2-1* protein phosphatase 2C mutants. Plant Cell **13:** 2513–2523

Narusaka Y, Nakashima K, Shinwari ZK, Sakuma Y, Furihata T, Abe H, Narusaka M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Interaction between two cis-acting elements, ABRE and DRE, in ABA-dependent expression of Arabidopsis rd29A gene in response to dehydration and high-salinity stresses. Plant J **34:** 137–148

Pastori GM, Foyer CH (2002) Common components, networks, and pathways of cross-tolerance to stress: the central role of ''redox'' and abscisic acid-mediated controls. Plant Physiol **129:** 460–468

Pei ZM, Murata Y, Benning G, Thomine S, Klusener B, Allen GJ, Grill E, Schroeder JI (2000) Calcium channels activated by hydrogen peroxide mediate abscisic acid signaling in guard cells. Nature **406:** 731–734

Rodriguez-Navarro A (2000) Potassium transport in fungi and plants. Biochim Biophys Acta **1469:** 1–30

Savoure A, Hua XJ, Bertauche N, Van Montagu M, Verbruggen N (1997) Abscisic acid-independent and abscisic acid-dependent regulation of proline biosynthesis following cold and osmotic stresses in Arabidopsis thaliana. Mol Gen Genet **254:** 104–109

Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D (2001) Guard cell signal transduction. Annu Rev Plant Physiol Plant Mol Biol **52:** 627–658

Seki M, Ishida J, Narusaka M, Fujita M, Nanjo T, Umezawa T, Kamiya A, Nakajima M, Enju A, Sakurai T, et al (2002a) Monitoring the expression pattern of around 7,000 Arabidopsis genes under ABA treatments using a full-length cDNA microarray. Funct Integr Genomics **2:** 282–291

Seki M, Narusaka M, Ishida J, Nanjo T, Fujita M, Oono Y, Kamiya A, Nakajima M, Enju A, Sakurai T, et al (2002b) Monitoring the expression profiles of 7000 Arabidopsis genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. Plant J **31:** 279–292

Serrano R, Mulet JM, Rios G, Marquez JA, de Larriona IF, Leube MP, Mendizabal I, Pascual-Ahuir A, Proft M, Ros R, et al (1999) A glimpse of the mechanisms of ion homeostasis during salt stress. J Exp Bot **50:** 1023–1036

Shakirova FM, Sakhabutdinova AR, Bezrukova MV, Fatkhutdinova RA, Fatkhutdinova DR (2003) Changes in the hormonal status of wheat seedlings induced by salicylic acid and salinity. Plant Sci **164:** 317–322

Shinozaki K, Yamaguchi-Shinozaki K (1996) Molecular responses to drought and cold stress. Curr Opin Biotechnol **7:** 161–167

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

Sticher L, Mauch-Mani B, Metraux JP (1997) Systemic acquired resistance. Annu Rev Phytopathol **35:** 235–270

Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. Annu Rev Plant Physiol Plant Mol Biol **50:** 571–599

Ton J, Mauch-Mani B (2004) Beta-amino-butyric acid-induced resistance against necrotrophic pathogens is based on ABA-dependent priming for callose. Plant J **38:** 119–130

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc Natl Acad Sci USA **97:** 11632–11637

Van Breusegem F, Vranova E, Dat JF, Inze D (2001) The role of active oxygen species in plant signal transduction. Plant Sci **161:** 405–414

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta **218:** 1–14

Xiong L, Zhu JK (2002) Molecular and genetic aspects of plant responses to osmotic stress. Plant Cell Environ **25:** 131–139

Yeh S, Moffatt BA, Griffith M, Xiong F, Yang DS, Wiseman SB, Sarhan F, Danyluk J, Xue YQ, Hew CL, et al (2000) Chitinase genes responsive to cold encode antifreeze proteins in winter cereals. Plant Physiol **124:** 1251–1264

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

Zhu B, Chen TH, Li PH (1995) Expression of three osmotin-like protein genes in response to osmotic stress and fungal infection in potato. Plant Mol Biol **28:** 17–26

Zimmerli L, Jakab G, Metraux JP, Mauch-Mani B (2000) Potentiation of pathogen-specific defense mechanisms in Arabidopsis by β-amino-butyric acid. Proc Natl Acad Sci USA **97:** 12920–12925