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Involvement of Abscisic Acid in Regulating Water Status in *Phaseolus vulgaris* L. during Chilling[1]

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ABSTRACT

During the first hours of chilling, bean (*Phaseolus vulgaris* L., cv Mondragone) seedlings suffer severe water stress and wilt without any significant increase in leaf abscisic acid (ABA) content (P. Vernieri, A. Pardossi, F. Tognoni [1991] Aust J Plant Physiol 18: 25–35). Plants regain turgor after 30 to 40 h. We hypothesized that inability to rapidly synthesize ABA at low temperatures contributes to chilling-induced water stress and that turgor recovery after 30 to 40 h is mediated by changes in endogenous ABA content. Entire bean seedlings were subjected to long-term (up to 6 d) chilling (3°C, 0.2–0.4 kPa vapor pressure deficit, 100 μmol·m^{-2}·s^{-1} photosynthetic photon flux density, continuous fluorescent light). During the first 24 h, stomata remained open, and plants rapidly wilted as leaf transpiration exceeded root water absorption. During this phase, ABA did not accumulate in leaves or in roots. After 24 h, ABA content increased in both tissues, leaf diffusion resistance increased, and plants rehydrated and regained turgor. No osmotic adjustment was associated with turgor recovery. Following turgor recovery, stomata remained closed, and ABA levels in both roots and leaves were elevated compared with controls. The application of ABA (0.1 mM) to the root system of the plants throughout exposure to 3°C prevented the chilling-induced water stress. Excised leaves fed 0.1 mM ABA via the transpiration stream had greater leaf diffusion resistance at 20 and 3°C compared with non-ABA fed controls, but the amount of ABA needed to elicit a given degree of stomatal closure was higher at 3°C compared with 20°C. These findings suggest that endogenous ABA may play a role in ameliorating plant water status during chilling.

The reduction of root water uptake (15) and the "locking-open" phenomenon of stomata (5, 8) causes a marked water deficit in chilling-sensitive species exposed to low, nonfreezing temperatures. ABA is thought to play an important role in the adaptation of plants to environmental stresses. The involvement of ABA in the plant's response to chilling, however, is not yet well understood.

The development of chilling resistance of "hardened" plants is associated with the accumulation of ABA during the hardening treatment (7, 19, 24). Nevertheless, no relationship between ABA content following temperature conditioning and chilling tolerance was found in cucumber cotyledons (11).

The effect of cold protectants such as triadimefon (1) and mefluidide (29) has been attributed to their induction of ABA accumulation. Applications of ABA to sensitive plants before cold treatment reduced chilling injury (9, 14, 17, 19), but ABA supplied at the onset of chilling was ineffective (23). Moreover, ABA accumulation observed in chilled plants was not associated with amelioration of the water status (3, 7, 26, 27, 29). Some authors (4, 5, 7, 8) have ascribed the "stomatal locking-open" to a reverse action of ABA on stomata at chilling temperatures.

Leaf ABA content did not increase in bean (*Phaseolus vulgaris* L. cv Mondragone) seedlings exposed to a short-term (24 h) cold treatment, in spite of the occurrence of a strong water deficit (27). However, Mondragone seedlings were able to regain turgor when the chilling treatment lasted more than 24 h.

This study was undertaken to test the hypothesis that turgor recovery in chilled bean was associated with an accumulation of endogenous ABA that takes longer to occur at lower temperatures. Other experiments were performed with intact plants or excised leaves fed exogenous ABA to study its influence on chilling resistance and stomatal behavior at low temperatures.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Experiments were performed on 15- to 20-d-old bean (*Phaseolus vulgaris* L., cv Mondragone) seedlings with nearly (90%) expanded primary leaves. Seedlings were germinated in perlite and transplanted to perforated plastic pots (8 cm diameter) filled with expanded clay and placed in tanks (6-L capacity, 24 plants per tank) with continuously aerated nutrient solution (27). Seedlings were grown under natural light in a heated glasshouse (22/17°C minimum day/night air temperature and 25–27°C maximum temperature) during the autumn and winter of 1990 to 1991.

Experimental Treatments

Plants were chilled in a growth chamber at 3.0 ± 0.5°C, 0.2 to 0.4 kPa VPD[2], under continuous light (100 μmol·m^{-2}·s^{-1} PPFD from fluorescent tubes). Depending on the experi-

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[2] Abbreviations: VPD, vapor pressure deficit; CH, cycloheximide; DW, dry weight; FW, fresh weight; TW; turgid weight; LDR, leaf diffusion resistance; RWC, relative water content; Ψ_w, water potential; Ψ_o, osmotic potential; Ψ_p, turgor potential.

ment, 40 to 80 plants were transferred 1 h after dawn to a precooled nutrient solution. Controls were maintained at 20.0 ± 0.5°C, 0.8 to 1.0 kPa VPD, in the same light conditions as stressed plants.

Experiments with exogenous ABA were performed by adding (±)-ABA (Sigma Chemical Co.) to the nutrient solution (final concentration 0.1 mM) at the inception of chilling treatment. In other experiments, excised leaves, cut from well-hydrated plants, were fed 0.1 mM (±)-ABA or 0.1 mM CH (Sigma Chemical Co.) through the petiole. Excised leaves were previously maintained with the petiole dipped in distilled water in a growth chamber (20.0 ± 0.5°C, 0.8–1.0 kPa VPD, 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD fluorescent light) for 4 to 5 h; only leaves able to remain fully turgid were used for the experiments.

In other experiments, 14-mm diameter leaf discs were cut from turgid leaves and kept for 12 h at room temperature on moist filter paper in a water-saturated atmosphere to ensure full turgidity. Discs were then stressed by floating them on a mannitol solution (Ψ_o −1.6 MPa) at 20°C and then allowed to recover in distilled water at 20 or 3°C in the dark.

Measurements of Water Relations

Leaf discs (14 mm) punched from intact seedlings were used for RWC and Ψ_w measurement. RWC was calculated according to the equation RWC = 100·(FW − DW)/(TW − DW). TW was determined after floating discs on distilled water at 20°C in dim light for 24 h. DW was measured after oven drying at 75°C for 48 h.

Ψ_w and Ψ_o were determined with a SC-104 thermocouple psychrometer connected to an NT-nanovoltmeter (Decagon Devices, Pullman, WA). Ψ_o was measured on frozen/thawed discs, and Ψ_p was calculated as the difference between Ψ_w and Ψ_o. Ψ_o values were corrected for the apoplastic water diluting the symplastic solution. The apoplastic water volume determined by pressure-volume analysis was 13% (20). Further details concerning RWC and Ψ_w measurements have been reported previously (20).

Stomatal behavior was assessed by measuring abaxial LDR using an automatic diffusion porometer (MK III, Delta-T Devices, Cambridge, UK). The whole porometer was placed in the growth chamber and allowed to equilibrate at 20 or 3°C. We used an operating RH range slightly lower (i.e. 50% at 3°C or 60% at 20°C) than ambient RH to get faster cycling. A calibration curve was constructed at the experimental air temperature and RH using the manufacturer's calibration plate, which provided six diffusion resistances of known value (0.4–22.5 $s \cdot cm^{-1}$). We considered stomata closed when LDR was greater than 25 $s \cdot cm^{-1}$. All experiments were conducted at atmospheric CO_2 concentration.

The rates of plant transpiration and root water uptake were assessed with potometers. Intact, bare-rooted plants were sealed in 80-mL vessels (one plant per vessel) through a rubber bung with a graduated pipette attached. Transpiration rate was determined by the rate of weight loss of the potometer-plant apparatus. Root water uptake was estimated by weighing the nutrient solution used to refill the graduated pipette to the reference point.

Measurement of Electrolyte Leakage

Electrolyte leakage from leaf tissues was determined by measuring the electrical conductivity of distilled water (20 mL) in which leaf discs were incubated for 2 h at room temperature. Electrolyte leakage was expressed as the percentage of the total electrolytes of the same discs after a freeze-thaw cycle.

ABA Analysis

ABA was measured in crude aqueous extracts of leaf discs (20–30 mg FW) or root segments (200–300 mg FW) using a solid-phase radioimmunoassay based on a monoclonal antibody (DBPA1) raised against free (*S*)-ABA (28). Physicochemical methods were used to validate radioimmunoassay results with crude extracts of nonstressed and stressed bean tissues (28).

Statistics

A completely randomized design was adopted. All the experiments were repeated at least three times with similar results. We report data from representative experiments. Data are the mean (±SD) of 6 to 15 replicates. Each plant represented a replicate. Data were analyzed by analysis of variance, and differences among means were determined by LSD.

Because water relations and ABA levels did not change significantly in control plants throughout each experiment, only values for plants at the beginning of the stress treatment are presented.

RESULTS

Effect of Chilling on Leaf Water Relations, Endogenous ABA Levels, and Electrolyte Leakage

During the first 24 h after chilling, plants maintained open stomata, as indicated by low LDR (2–6 $s \cdot cm^{-1}$), and a marked water stress took place (Fig. 1). RWC, Ψ_w, and Ψ_o rapidly decreased, and turgor loss occurred within a few hours. Values in tissues of control plants (LDR = 3 $s \cdot cm^{-1}$; RWC = 92%; Ψ_w = −0.41 MPa; Ψ_o = −0.92 MPa; Ψ_p = 0.51 MPa, at the beginning of the experiment) did not significantly change throughout the whole treatment period. Despite a strong decline in turgor, no significant variations in ABA levels were detected in leaf (1.5–2.3 $nmol \cdot g^{-1}$ DW) or root (0.2–0.3 $nmol \cdot g^{-1}$ DW) tissues of stressed plants.

The second phase (between 24 and 50 h) was characterized by parallel increases in LDR and ABA content in both leaves and roots and rehydration of the plants that regained turgor. After 50 h, RWC, Ψ_w, Ψ_o, and Ψ_p were 79%, −0.74, −0.96, and 0.22 MPa, respectively. We observed a short delay in ABA buildup in roots with respect to that occurring in leaves.

In the third phase (50–144 h), all the measured parameters were fairly constant. We found high LDR (20–25 $s \cdot cm^{-1}$) and ABA content (10–15 $nmol \cdot g^{-1}$ DW in leaves and 1.2–1.5 $nmol \cdot g^{-1}$ DW in roots). The values of RWC (81–85%), Ψ_w (−0.64 to −0.58 MPa), Ψ_o (−0.98 to −0.88 MPa), and Ψ_p (0.3–0.35 MPa) were slightly but significantly lower than prechilling levels.

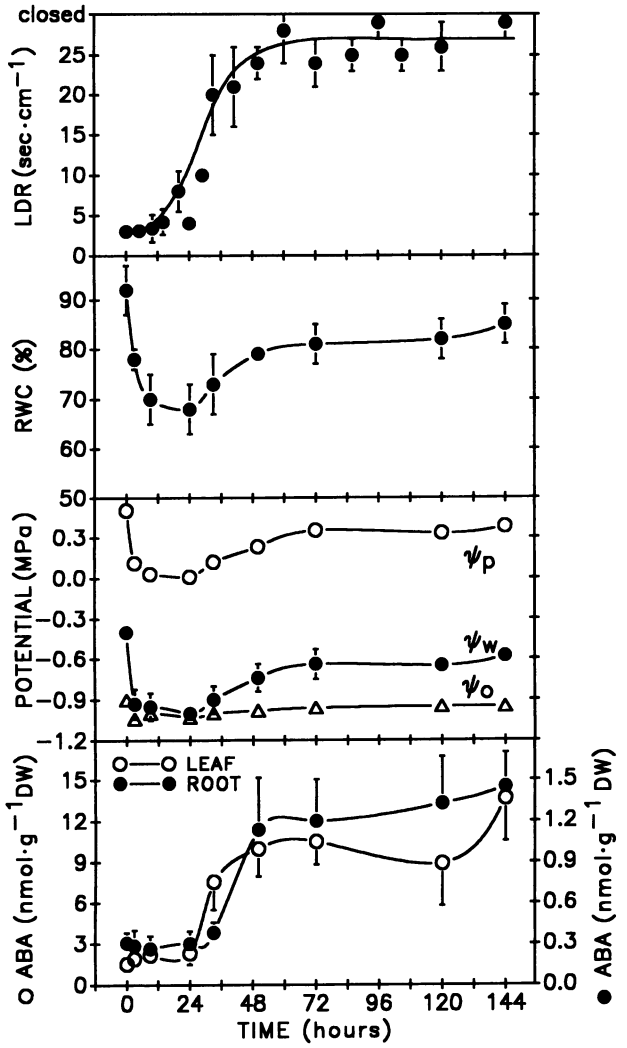

Figure 1. Changes in LDR, RWC, Ψ_w, and leaf and root ABA content of bean seedlings during chilling (3.0 ± 0.5°C, 0.2–0.4 kPa VPD) under continuous light (100 μmol·m^{-2}·s^{-1} PPFD from fluorescent tubes). Values are means (±SD) of 6 to 15 replicates.

Similar results were obtained in all the replicate experiments, with small differences in the starting time of the second phase, which occurred 30 to 40 h after chilling.

Not all chilled plants regained turgor. Depending on the experiment, 10 to 30% of plants did not rehydrate (Fig. 2), even when transferred to nonchilling temperature (data not shown). This phenomenon was not associated with a lack of ABA accumulation because there was no significant difference in leaf (Fig. 2) and root (not shown) ABA content between the plants that did and did not rehydrate.

The effect of chilling on membrane integrity of leaf tissues was evaluated by measuring electrolyte leakage. In chilled plants, solute leakage was high during the first 24 h when RWC was low (Table I). Upon plant rehydration, electrolyte

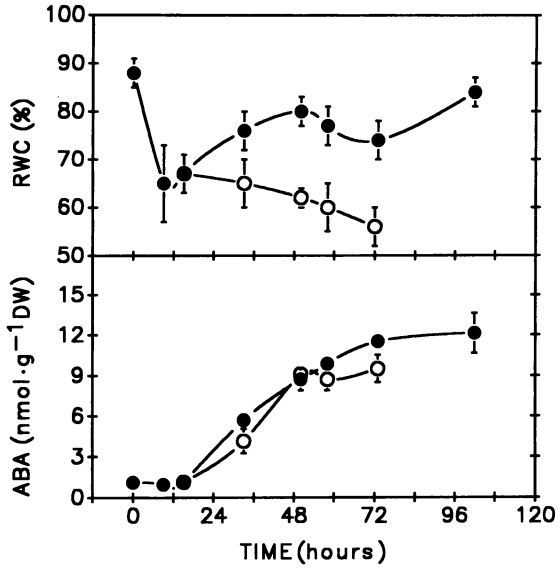

Figure 2. Changes in leaf RWC and leaf ABA content of bean seedlings during chilling (3.0 ± 0.5°C, 0.2–0.4 kPa VPD) under continuous light (100 μmol·m^{-2}·s^{-1} PPFD from fluorescent tubes). Measurements were made on plants that were capable (●) or incapable (O) of rehydrating and regaining turgor. Values are means (±SD) of 6 to 15 replicates.

leakage diminished to values that then remained constant, although somewhat higher than in controls.

Neither dry matter accumulation nor leaf (primary and trifoliate) expansion occurred during the chilling period. Upon transferring to nonchilling temperature (20°C), chilled plants resumed growth but at a slower rate compared with controls (data not shown).

Effect of Chilling on Transpiration and Root Water Absorption

Because of the lower VPD (0.2–0.4 versus 0.8–1.0 kPa at 20°C), the transpiration rate at 3°C (7–64 mg·plant^{-1}·h^{-1})

Table I. *Electrolyte Leakage and RWC in Bean Seedlings during Chilling*

Chilling treatment was performed in a growth chamber at 3.0 ± 0.5°C, 0.2 to 0.4 kPa VPD, under continuous light (100 μmol·m^{-2}·s^{-1} PPFD from fluorescent tubes). Mean values (±SD) of six replicates.

Treatment	Electrolyte Leakage	RWC
	%	%
Nonstressed	14 ± 2	92 ± 5
3 h chilled	25 ± 4	75 ± 5
9 h chilled	50 ± 5	65 ± 5
12 h chilled	47 ± 5	65 ± 4
24 h chilled	40 ± 5	68 ± 3
36 h chilled	35 ± 5	72 ± 3
48 h chilled	21 ± 4	75 ± 4
72 h chilled	22 ± 4	79 ± 3
144 h chilled	22 ± 5	80 ± 4

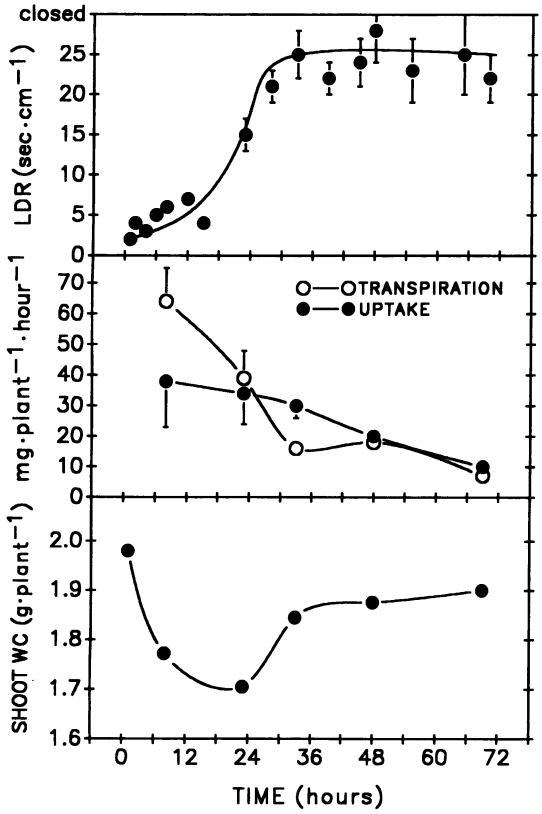

Figure 3. Changes in LDR, plant transpiration, root water uptake, and shoot water content (WC) in bean seedlings during chilling (3.0 ± 0.5°C, 0.2–0.4 kPa VPD) under continuous light (100 μmol·m^{-2}·s^{-1} PPFD from fluorescent tubes). Mean leaf area was 49.5 ± 5.2 cm^2·plant^{-1}. The change in shoot water content was calculated on the basis of cumulated quantities of transpired and absorbed water by the plants. Values are means (±SD) of 6 to 15 replicates.

was markedly abated with respect to the values at 20°C (120–150 mg·plant^{-1}·h^{-1}) (Fig. 3). Chilling also resulted in a reduction of the root water uptake, which ranged from 10 to 38 mg·plant^{-1}·h^{-1} compared with 100 to 150 mg·plant^{-1}·h^{-1} at 20°C.

In the presence of low LDR, the transpiration rate during the first period at 3°C exceeded water uptake. Thereafter, when stomata began to close, transpiration strongly declined to values equal to or even less than the rate of water absorption. During the first 24 h, plants dehydrated, as indicated by the decrease in shoot water content (calculated on the basis of cumulated quantities of transpired and absorbed water). After the initial wilting, plants rehydrated and regained turgor.

Effect of Low Temperature on ABA Catabolism in Stressed Leaf Tissue

Leaf and root ABA content remained high in chilled bean seedlings even after plants recovered from water stress (Fig. 1). We hypothesized that the high ABA content observed in chilled bean after turgor recovery was due to an inhibition of ABA catabolism by low temperatures. To test this possibility, we used a method that previously proved suitable for studying the effect of temperature on ABA metabolism in stressed tissues (22, 27).

Leaf discs were incubated in a mannitol solution (Ψ_w −1.6 MPa) at 20°C for 4 h and then transferred to distilled water at 20 or 3°C. Endogenous ABA content rapidly increased in stressed leaf discs (from 0.9 to 36 nmol·g^{-1} DW after 4 h) (Fig. 4). Upon rehydration, at 20°C, ABA rapidly declined and decreased again to prestress values after 6 h, whereas at 3°C the levels remained almost constant (Fig. 4). These results were not due to differential leaching of ABA from the tissue to the medium or to different water relations of tissue under different thermal conditions. In fact, the amount of ABA released in the medium was proportional to that present in the tissue and, furthermore, upon transferring to distilled water, leaf discs recovered full turgor within 2 h at both temperatures (data not shown).

Effect of Exogenously Applied ABA on Chilling Resistance of Intact Plants

The effect of exogenous ABA was studied in intact plants supplied with 0.1 mM ABA dissolved in the nutrient solution during the chilling period. In ABA-treated plants, the endogenous levels of leaf ABA rapidly increased to three times the initial values after 3 h (Fig. 5). Exogenous ABA triggered a rapid increase in LDR, which was probably responsible for reducing the severity of chilling-induced water stress. During the first 24 h of chilling, ABA-treated plants did not wilt and had higher RWC with respect to nontreated ones. As expected, nontreated plants lost turgor during the first hours of chilling; thereafter, when LDR and ABA levels increased, they regained turgor.

Relationships among RWC, LDR, and Endogenous ABA Levels in Excised Leaves at 20 and 3°C

To characterize the stomatal locking-open in chilled leaves, we investigated the changes in LDR and ABA content in cv Mondragone leaves during dehydration. Leaves from well-hydrated plants were detached, placed on a bench, and

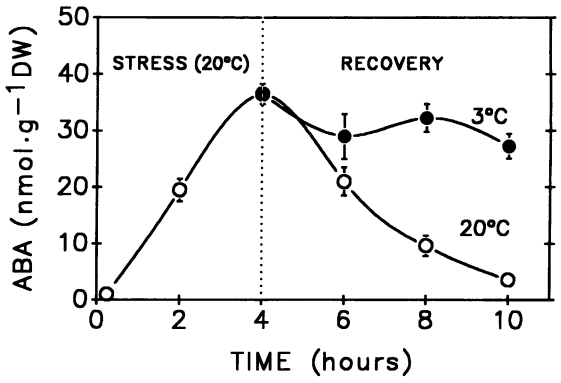

Figure 4. Changes in ABA content in discs of primary leaves of bean incubated in a mannitol solution (Ψ_o −1.6 MPa) at 20°C for 4 h and then transferred to distilled water at 3 or 20°C in the dark. Values are means (±SD) of 6 to 15 replicates.

allowed to lose water at 20°C (0.8–1.0 kPa VPD) or 3°C (0.2–0.4 kPa VPD). At 20°C, a significant reduction of stomatal aperture occurred within only 15 min when RWC was approximately 75%; ABA levels started to increase at 65% RWC (Fig. 6). After 60 min, LDR reached values of approximately 25 $s \cdot cm^{-1}$ with RWC of 60% and ABA content of nearly 19 $nmol \cdot g^{-1}$ DW. On the other hand, at 3°C water stress did not show any detectable effect on ABA content, and LDR remained at the original value (1–3 $s \cdot cm^{-1}$) with an RWC value of 95 to 50% (Fig. 6). A significant increment in LDR (12–14 $s \cdot cm^{-1}$) of chilled leaves was observed only at an RWC of approximately 45% without any detectable increase in ABA content. We attributed this increase in LDR to hydropassive stomatal closure. Passive closure at a high degree of dehydration was also observed at 20°C in detached leaves previously fed 0.1 mM CH for 2 h and then allowed to dehydrate. CH is known to block water stress-induced ABA accumulation and to inhibit active stomatal closure in bean leaves (12). As reported in Table II, CH-fed leaves, which did not accumulate ABA, had low LDR (2–3 $s \cdot cm^{-1}$) despite a severe water deficit, and stomatal closure occurred only at a very low RWC (53%).

Effect of Exogenously Applied ABA on LDR of Excised Leaves at 20 or 3°C

In this experiment, we determined whether ABA has a reverse action on stomata at chilling temperature as proposed by other authors (4, 5, 7, 8).

Leaves were cut and petioles were immersed in water (control) or ABA solution (0.1 mM) at 20 or 3°C. Control and ABA-treated leaves showed no symptoms of water stress at either temperature. LDR (1–3 $s \cdot cm^{-1}$) and endogenous ABA levels (0.9–1.2 $nmol \cdot g^{-1}$ DW) remained unchanged without ABA feeding at both temperatures (data not shown).

In ABA-fed leaves at 20°C, LDR rapidly increased and stomata closed within 7 h (Fig. 7). Stomatal closure occurred at a bulk leaf ABA content of about 58 $nmol \cdot g^{-1}$ DW. At 3°C, a significant increase in LDR of ABA-fed leaves was detected after 7 h, when ABA content was nearly 100 $nmol \cdot g^{-1}$ DW. After 20 h, chilled leaves had an ABA level much higher than nonchilled leaves, but LDR never reached the values of leaves at 20°C. The higher ABA levels in leaves kept at 3°C with respect to those maintained at 20°C might be explained by the inhibition of ABA catabolism at low temperatures (Fig. 4).

DISCUSSION

At 3°C, the disruption of stomatal control of leaf transpiration and the reduced water supply from the roots induced a marked water deficit in cv Mondragone bean seedlings, which rapidly wilted. Because of inhibition of ABA synthesis by low temperatures (27), ABA did not accumulate in roots or leaves during the first hours of chilling. Plants were able to regain turgor when chilling lasted more than 24 h. Such recovery was not observed in all the chilled plants because some plants suffered a nonreversible degree of dehydration (RWC lower than approximately 65%), as was also found by Bagnall et al. (2). There was no significant osmotic adjustment involved in the recovery of turgor.

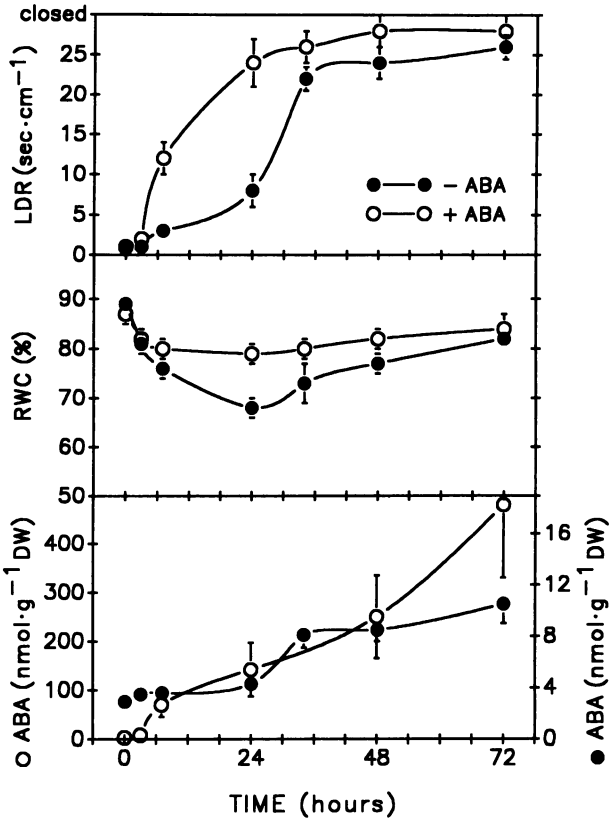

Figure 5. Effect of exogenous ABA on LDR, RWC, and endogenous ABA content of leaf tissues in bean seedlings treated or nontreated with ABA during chilling (3.0 ± 0.5°C, 0.2–0.4 kPa VPD) under continuous light (100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD from fluorescent tubes). Values are means (±SD) of 6 to 10 replicates.

Recovery was due to stomatal closure and the consequent decline in transpiration below the rate of root water absorption. A hydraulic recovery after a few days of impaired water status has also been reported by Markhart et al. (16) in soybean transferred from a 29/23°C to 17/11°C thermoperiod and by Bagnall et al. (2) in root-chilled mung bean. Also, McWilliam et al. (17) observed a recovery of turgor following stomatal closure in bean after several hours of chilling, but endogenous ABA levels were not measured.

The rehydration of chilled bean seedlings was associated with an increase in the endogenous ABA levels in both roots and leaves. This is in contrast with the conclusions by Capell and Dörffling (3) for cucumber and Zhang et al. (29) for corn. Zhang et al. (29) stated that ABA accumulation during chilling did not have any protective effects. Bagnall et al. (2) also suggested that turgor recovery in root-chilled mung bean was not controlled by endogenous ABA, although ABA increased in both roots and leaves before the plants recovered.

The correlation ($r^2 = 0.67$) between LDR and the bulk leaf ABA content (Fig. 8) suggests an effect of ABA accumulation on stomatal behavior, but the reduction in stomatal aperture could be the result of other physiological changes linked to the ABA increase.

As previously reported by other authors (21), LDR of

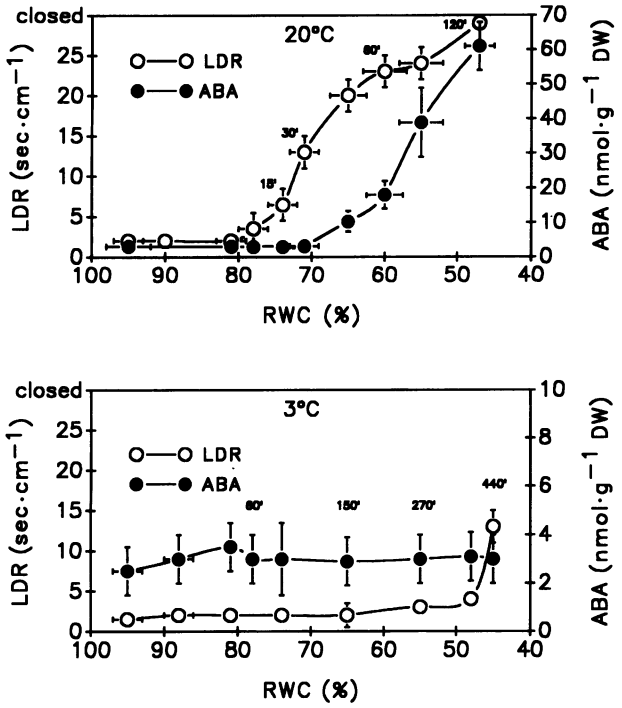

Figure 6. LDR and endogenous ABA content in excised primary leaves of bean as a function of RWC during water loss at 20°C (0.8–1.0 kPa VPD) or 3°C (0.2–0.4 kPa VPD) under continuous light (100 μmol·m^{-2}·s^{-1} PPFD from fluorescent tubes). Values are means (± SD) of six replicates. Figures inside the graph indicate time (minutes) from excision.

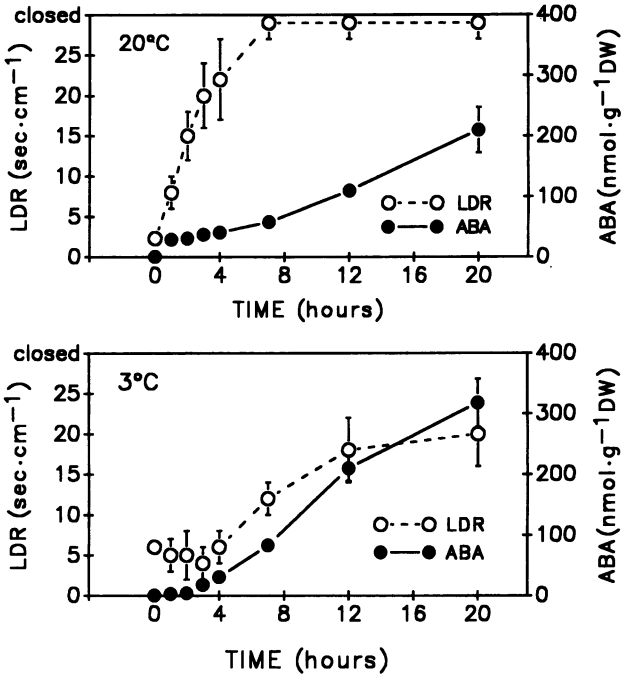

Figure 7. Effect of exogenous ABA on LDR and endogenous ABA content in excised primary leaves of bean at 20°C (0.8–1.0 kPa VPD) or 3°C (0.2–0.4 kPa VPD) under continuous light (100 μmol·m^{-2}·s^{-1} PPFD from fluorescent tubes). Excised leaves were fed 0.1 mM ABA through the petiole. In control leaves (fed distilled water), LDR (1–3 s·cm^{-1}) and endogenous ABA levels (1.5 nmol·g^{-1} DW) remained unchanged at both temperatures. Values are means (± SD) of six replicates.

excised leaves increased before any detectable increase in ABA levels. Existing ABA has to be redistributed in response to water stress to induce the closure of stomata (10, 18). Accordingly, in chilled bean during the first 24 h, when ABA did not accumulate and the degree of dehydration appeared insufficient to induce passive closure of stomata, stomatal locking-open might be caused by inadequate redistribution of leaf ABA. The reduced transpiration stream inside the leaves or some metabolic dysfunctions (e.g. preventing apoplastic pH adjustment for the release of cell ABA into the apoplast) could impair the movement of ABA to the guard cells (18, 21). Low temperatures could diminish stomatal sensitivity to ABA. Physiological processes could be modulated by change in the sensitivity of tissues to hormones more than by the variation of hormone concentrations (25). Indeed, the amount of ABA needed to elicit a given degree of stomata closure was higher at 3°C than at 20°C (Fig. 7), indicating that the apparent sensitivity of stomata to the hormone was

Table II. *Effect of CH on RWC, Endogenous ABA Content, and LDR of Excised Bean Leaves during Water Loss at 20°C (0.8–1.0 kPa VPD) under Continuous Light (100 μmol·m^{-2}·s^{-1} PPFD from Fluorescent Tubes)*

Leaves were previously fed 0.1 mM CH through the petiole for 2 h. Mean values (±SD) of six replicates.

Time (min)	RWC		ABA		LDR	
	−CH	+CH	−CH	+CH	−CH	+CH
	%		nmol·g^{-1} DW		s·cm^{-1}	
0	95 ± 2	96 ± 3	1.8 ± 0.2	1.6 ± 0.4	2.0 ± 0.5	2.5 ± 0.6
5	90 ± 3	89 ± 2	1.7 ± 0.7	1.7 ± 0.3	2.5 ± 0.8	2.2 ± 0.5
10	80 ± 3	79 ± 3	1.7 ± 0.3	1.5 ± 0.1	3.5 ± 1.0	2.5 ± 1.2
20	72 ± 4	70 ± 4	2.0 ± 1.5	1.6 ± 0.2	12.2 ± 2.5	3.0 ± 1.1
50	65 ± 4	62 ± 2	15.0 ± 1.3	1.6 ± 0.8	20.0 ± 3.1	3.2 ± 0.4
75	55 ± 5	52 ± 3	35.2 ± 3.5	1.5 ± 0.3	Closed	Closed

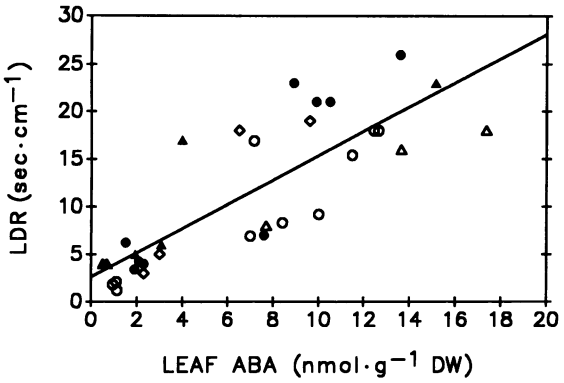

Figure 8. Relationship between LDR and the bulk leaf ABA content in bean seedlings subjected to chilling. Different symbols indicate different runs. Values are means (±SD) of 6 to 15 replicates. Solid line represents fitted linear regression ($y = 2.62 + 1.27x$; $r^2 = 0.67$).

decreased by chilling. Upon recovery of chilled leaves, the increase in ABA levels could lead to stomatal closure by improving its distribution or causing an "autosensitization" of the tissues (21). Thereafter, the high ABA content may have been needed to maintain stomatal closure (18).

Our data suggest an involvement of endogenous ABA in ameliorating the water balance of chilled plants and confirm the beneficial effects of ABA application on increasing chilling tolerance (9, 13, 14, 17, 19). However, our conclusions are in contrast to those by Eamus and colleagues (4, 5, 7, 8), who stated that stomatal locking-open in chilled bean seedlings was due to a reverse action of ABA on stomata at low temperatures. These authors reported that, at 5°C, 0.1 mM ABA caused stomatal opening in excised bean leaves (7, 8). On the contrary, we found that at 3°C LDR of leaves fed 0.1 mM ABA started to increase after 7 h (Fig. 7). A different sensitivity of the plant material could explain this discrepancy, but most likely it derives from the different duration of experimental treatment (Eamus and Wilson [7, 8] monitored the effect of ABA on stomata for only 1–2 h).

Although our results suggest an ABA-induced chilling resistance through its effect on stomata, the ABA accumulation could also increase the hydraulic conductivity of the plants, thus enhancing the rehydration process. The effect of ABA on the hydraulic conductivity is not yet well established (18); nonetheless, exogenous ABA has been reported to increase water transport in chilled roots (13, 14). Because chilling temperatures depress hydraulic conductivities of both roots (14, 15) and leaves (6), an improvement of water transport properties of plant tissues could contribute to rehydration of chilled plants.

To conclude, we have provided evidence indicating an involvement of endogenous ABA in increasing plant tolerance to chilling-induced water stress through its effect on stomata. Work concerning the possible effects of ABA accumulation on water transport in chilled bean seedlings is in progress.

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