Available online at www.sciencedirect.com
SCIENCE DIRECT®
Plant Science 165 (2003) 671–679
PLANT SCIENCE
www.elsevier.com/locate/plantsci

Involvement of abscisic acid in leaf and root of maize (*Zea mays* L.) in avoiding chilling-induced water stress

Ricardo Aroca [a,1], Paolo Vernieri [b], Juan José Irigoyen [a], Manuel Sánchez-Díaz [a,*], Franco Tognoni [b], Alberto Pardossi [b]

[a] *Departamento de Fisiología Vegetal, Universidad de Navarra, C/Irunlarrea s/n 31008 Pamplona, Spain*
[b] *Dipartimento di Biologia delle Piante Agrarie, Università degli Studi di Pisa, Vialle delle Piagge 23, 56124 Pisa, Italy*

Received 29 November 2002; received in revised form 2 June 2003; accepted 2 June 2003

Abstract

In the present study, we investigated the role of abscisic acid (ABA) on chilling tolerance of maize. Two maize genotypes differing in chilling sensitivity (Z7 tolerant and Penjalinan sensitive) were subjected to chilling (5 °C, 12 h photoperiod, 150 μmol m^{-2} s^{-1} PPFD) for 3 days under two relative humidity (RH) regimes (60 or 100% RH). Some plants were exogenously treated 24 h before chilling with ABA (100 μM). As expected, high humidity (100% RH) or ABA pre-treatment prevented the leaf water deficit induced by chilling at 60% RH in chilling sensitive Penjalinan plants. ABA pre-treatment improved chilling tolerance of Penjalinan plants, mainly by decreasing leaf conductance and by increasing root water flow. At the leaf level, we found a relationship between ABA content and chilling tolerance in both maize genotypes. No relationship between ABA content and leaf conductance was found. Moreover, during chilling, no differences on leaf conductance between the two genotypes were observed, probably indicating that the different water stress suffered by the two genotypes could be linked to differences in the root water uptake. The rise in leaf ABA content during chilling was independent of the leaf water status, so it must be induced by the low temperature per se, and after a longer cold exposure also by the vapour pressure deficit (VPD) (a higher VPD allows more ABA accumulation). At the root level, we did not observe a relationship between the root hydraulic acclimation to chilling and the root ABA content. Z7 plants chilled at 60% RH had the same root ABA content as those which were chilled at 100% RH and as Penjalinan plants; however, the former showed a higher root hydraulic conductance. The rise in the root ABA content in Z7 plants followed the same pattern as observed in the leaves. In Penjalinan plants, the rise in root ABA content was linked only to low temperatures per se, since it increased in the same way in plants chilled under 60 or 100% RH.

Keywords: Abscisic acid; Acclimation; Chilling; Leaf conductance; Maize; Root hydraulic conductance

1. Introduction

It is well documented that low temperature causes shoot water deficit in thermophilic crops such as maize [1–6]. The water deficit is caused by a reduction in the root water uptake greater than in the leaf transpiration rate during chilling [1,2,5]. Among the maize genotypes, there are some differences in the degree of chilling sensitivity. Chilling tolerant genotypes suffer less water deficit during chilling than the sensitive ones [1,3–6]. It is well known that tolerant maize genotypes are capable of closing stomata more fully and faster than the sensitive ones [1,5,6]. Recently, we found that a tolerant variety (Z7, from Central Europe) was capable of increasing its root hydraulic conductance during chilling, while a sensitive one (Penjalinan, from Indonesia) decreased it [1]. These results point out that the root hydraulic conductance is also involved in maize chilling tolerance.

Abscisic acid (ABA) is considered to be one of the most important hormones involved in the plant response to cold stress [3–12]. Thus, it is clear that ABA

* Corresponding author. Tel.: +34-948-42-5600; fax: +34-948-42-5649.
E-mail address: msanchez@unav.es (M. Sánchez-Díaz).
[1] Present address: Division of Biological Science, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0116, USA.

0168-9452/03/$ - see front matter
doi:10.1016/S0168-9452(03)00257-7

contributes to cold induced freezing tolerance in *Arabidopsis thaliana* by the expression of several genes [10,11]. Moreover, ABA application induced freezing tolerance in *Arabidopsis* [12] and chilling tolerance in several sensitive species [7–9]. The protective mechanism of ABA against chilling stress is linked to its capacity for inducing stomatal closure [5,6,9]. However, ABA has also other functions during chilling, i.e. the induction of antioxidant enzymes [7] and the modulation of polyamine levels [8]. On the other hand, it is known that the exogenous application of ABA increases the root hydraulic conductance in some cases, although this effect depends on plant species, temperature and nutrient status [13–20].

Root water uptake and root hydraulic conductance depend to a large extent on leaf transpiration rate [21]. At the same time, the rise in leaf ABA content during chilling is reduced by high humidity [3,22]. However, to our knowledge, there is no information about the influence of high humidity on the root hydraulic conductance and root ABA content during chilling in maize.

Here we report data of the root ABA content and root hydraulic properties of two maize genotypes differing in chilling sensitivity [1] during chilling (5 °C, 12-h photoperiod, 150 μmol m^{-2} s^{-1} PPFD) under 60 or 100% relative humidity (RH) regimes. Also, some plants were acclimated to chilling by exogenous ABA application. Since root response to chilling can be associated with the vapour pressure deficit (VPD) and leaf water status [21,23], we also studied these parameters and leaf ABA content.

2. Materials and methods

2.1. Plant material and experimental design

Two maize (*Zea mays* L.) genotypes differing in chilling sensitivity were used: Z7 (tolerant, from Central Europe) and Penjalinan (sensitive, from Indonesia) [1,5,20]. Seeds of both genotypes were surface disinfected for 10 min in a 0.02% (w/v) $HgCl_2$ solution and washed three times with distilled water. Seeds of both genotypes were germinated in wet expanded clay at 25 °C. After 5 days of sowing, seedlings were grown hydroponically in 10-l plastic tanks (24 plants per tank), filled with continuously aerated full-strength Hoagland's solution in a growth chamber, environmental conditions were: air temperature 25±1 °C, RH 60% (corresponding to 1.1 kPa VPD), 12-h photoperiod, 150 μmol m^{-2} s^{-1} PPFD provided by fluorescent lamps. At 11th day, seedlings were transferred to a cold chamber (5±0.5 °C and the same light conditions of the warm growth chamber) for 3 days. During chilling treatment, plants of both genotypes were subjected to two different RH regimes (60%, corresponding to 0.3 kPa VPD or 100%, saturated atmosphere). One hundred percent RH was obtained by covering hydroponic tanks where plants were grown with plastic sheets [23]. At the same time, 24 plants of each genotype were treated 24 h before chilling with (+/−)—*cis*, *trans*—ABA (final concentration 100 μM; A.G. Scientific Inc., GA, USA). The ABA was first dissolved in 1 ml 70% ethanol and then was added to the nutrient solution or diluted in 1 l of distilled water and sprayed on the leaves [9]. These plants were only chilled at 60% RH. Therefore, we had three groups of chilled plants for each genotype: plants chilled at 60% RH, plants chilled at 100% RH and plants pre-treated with ABA and chilled at 60% RH. All the measurements were made at 0, 6, 30 and 54 h of chilling treatment in non ABA pre-treated plants and at 0 and 54 h of chilling in ABA pre-treated plants.

2.2. Leaf relative water content (RWC)

The leaf water status was assayed by measuring the RWC [24]. Leaf samples were weighed (fresh weight) immediately after harvesting, then placed in a water vapour-saturated vial at 5 °C for 48 h and weighed (turgid weight). The samples were dried in an oven at 80 °C for 48 h and their dry weights were determined. RWC was calculated by the following equation: (fresh weight − dry weight)/(turgid weight − dry weight) × 100.

2.3. Transpiration rate and leaf conductance

The leaf transpiration and the leaf conductance were assayed by the gravimetric method. Intact, bare-root plants were sealed in 50 ml vessels (one plant per vessel) through a rubber bung. Transpiration rate was determined by the rate of weight loss of the vessel during 1 and 3 h in control and chilled plants, respectively. The leaf conductance was calculated from the transpiration rate and VPD as described by Pearcy et al. [25]. Total leaf area of each plant was determined by an image analysis system (WinDIAS, Delta-T Devices, Cambridge, UK).

2.4. Root hydraulic properties

Root hydraulic properties were determined in roots exuding at atmospheric pressure [1]. Plants were cut below the first leaf and a pipette was attached to the root stump with a flexible silicone tube. Control and chilled plants were allowed to exude at 25 °C. Free exuded sap was collected with a syringe after 1 or 3 h from control and chilled plants, respectively (the first 30 min of exuded sap was discarded). Exuded sap and nutrient solution osmolalities were measured by a cryoscopic osmometer (Osmomat O30, Gonotec GmbH, Berlin, Germany). Osmotic root hydraulic

conductance was calculated by the following equation: $J_v = \sigma \times L_o \times |\Delta\Psi_s|$, where J_v is the root free exuded sap flow rate, σ the osmotic reflection coefficient, L_o the osmotic root hydraulic conductance and $\Delta\Psi_s$ the osmotic potential gradient between nutrient solution and the exuded sap. Since we could not determine σ under our experimental conditions, we expressed osmotic root hydraulic conductance as $\sigma \times L_o$, and called this parameter composite root hydraulic conductance (CRHC) [1,26]. The measurements of free exuded sap of chilled plants at 25 °C was used as an indicator of healthy roots, since the exuded sap was undetectable at 5 °C [1].

2.5. Abscisic acid (ABA) determination

Leaf (80–100 mg FW) and root (200–400 mg FW) samples were collected, weighed, frozen immediately in liquid nitrogen, then thawed and extracted with distilled water (water:tissue ratio 20:1 v/w) for 16 h at 4 °C in the dark. Quantitative analysis was performed on crude aqueous extracts using a solid-phase radioimmunoassay (RIA) based on a monoclonal antibody (DBPA1) raised against free (S)-ABA, as previously described by Vernieri et al. [27]. Procedures to validate the efficiency of the extraction method and the RIA results using DBPA1 monoclonal antibody on crude extracts of maize tissues were previously described in detail [28,29].

2.6. Statistical analysis

Means ± standard error (S.E.) of six to ten samples of each parameter are shown. The significance of the difference between means was assayed using unpaired *t*-test.

3. Results

3.1. Chilling effects at shoot level

We used the leaf RWC to evaluate the water deficit induced by chilling (Fig. 1A). The only plants that presented leaf water deficit after 54 h of chilling treatment were Penjalinan (sensitive genotype) plants chilled at 60% RH without the ABA pre-treatment. However, Penjalinan plants chilled at 100% RH or pre-treated with ABA and chilled at 60% RH, did not suffer water deficit (Fig. 1A). None of the three treatments on the Z7 (tolerant genotype) plants showed a decrease in RWC (Fig. 1A).

Since the VPD at 100% RH is equal to zero, we only measured the leaf transpiration rate in control and 60% RH chilled plants. Under control conditions (25 °C, 0 h), the transpiration rate of Z7 plants was higher than that of Penjalinan's (0.67 ± 0.06 and 0.45 ± 0.04, respectively) and the ABA pre-treatment did not cause any significant effect (Fig. 1B). Chilling caused a decrease in the transpiration rate in both genotypes. However, the decrease was more pronounced in Z7 plants than in Penjalinan's (82 and 77%, respectively after 6 h of chilling), although the final rate was the same in both genotypes. No significant changes in leaf transpiration were observed in either of the two genotypes during chilling treatment (Fig. 1B). After 54 h of chilling, the ABA pre-treated plants of both genotypes had significantly lower transpiration rates than non pre-treated ones (Fig. 1B).

Under our experimental conditions, control and chilled plants were subjected to different VPD (1.1 and 0.3 kPa, respectively). In order to understand if the observed decrease in leaf transpiration by chilling treatment was due to stomatal closure, we measured the leaf conductance (see Section 2). Penjalinan plants did not change their leaf conductance during chilling treatment and had a tendency to increase it (from 48 ± 4 mmol m^{-2} s^{-1} at control temperature to 69 ± 13 mmol m^{-2} s^{-1} after 30 h of chilling treatment) (Fig. 1C). On the contrary, Z7 plants tended to decrease their conductance during chilling (from 71 ± 6 mmol m^{-2} s^{-1} at control temperature to 46 ± 5 mmol m^{-2} s^{-1} after 30 h of chilling treatment) (Fig. 1C). However, no significant differences in leaf conductance values were observed between the two genotypes (Fig. 1C).

Under control conditions (0 h) no differences in leaf ABA content between the two genotypes were observed (Fig. 1D). The ABA pre-treatment caused an increase in leaf ABA content in both genotypes, being higher in Z7 plants (Fig. 1D). After 30 h of chilling, Z7 plants significantly increased their leaf ABA contents at both humidity regimes. After that, only the Z7 plants chilled at 60% RH showed an increase on the leaf ABA content, while the ones chilled at 100% RH showed no changes (Fig. 1D). In Penjalinan plants, leaf ABA content only increased after 54 h of chilling in both humidity regimes, although the increase was higher in the plants chilled at 60% RH (Fig. 1D). In each genotype and after 54 h of chilling, the plants chilled at 60% RH (pre-treated with ABA or not) had the same leaf ABA content (Fig. 1D). However, the ABA level was higher in Z7 than in Penjalinan.

3.2. Chilling effects at root level

Under control conditions (25 °C, 0 h), no differences in any of the root hydraulic parameters (free exuded sap flow, J_v; osmotic gradient between nutrient solution and exuded sap, $\Delta\Psi_s$; CRHC) were observed between the two genotypes (Fig. 2A–C). The ABA pre-treatment caused a decrease of J_v and CRHC and an increase in $\Delta\Psi_s$ and root ABA content in both genotypes (Fig. 2A–D). After 6 h of chilling, J_v decreased drastically in all

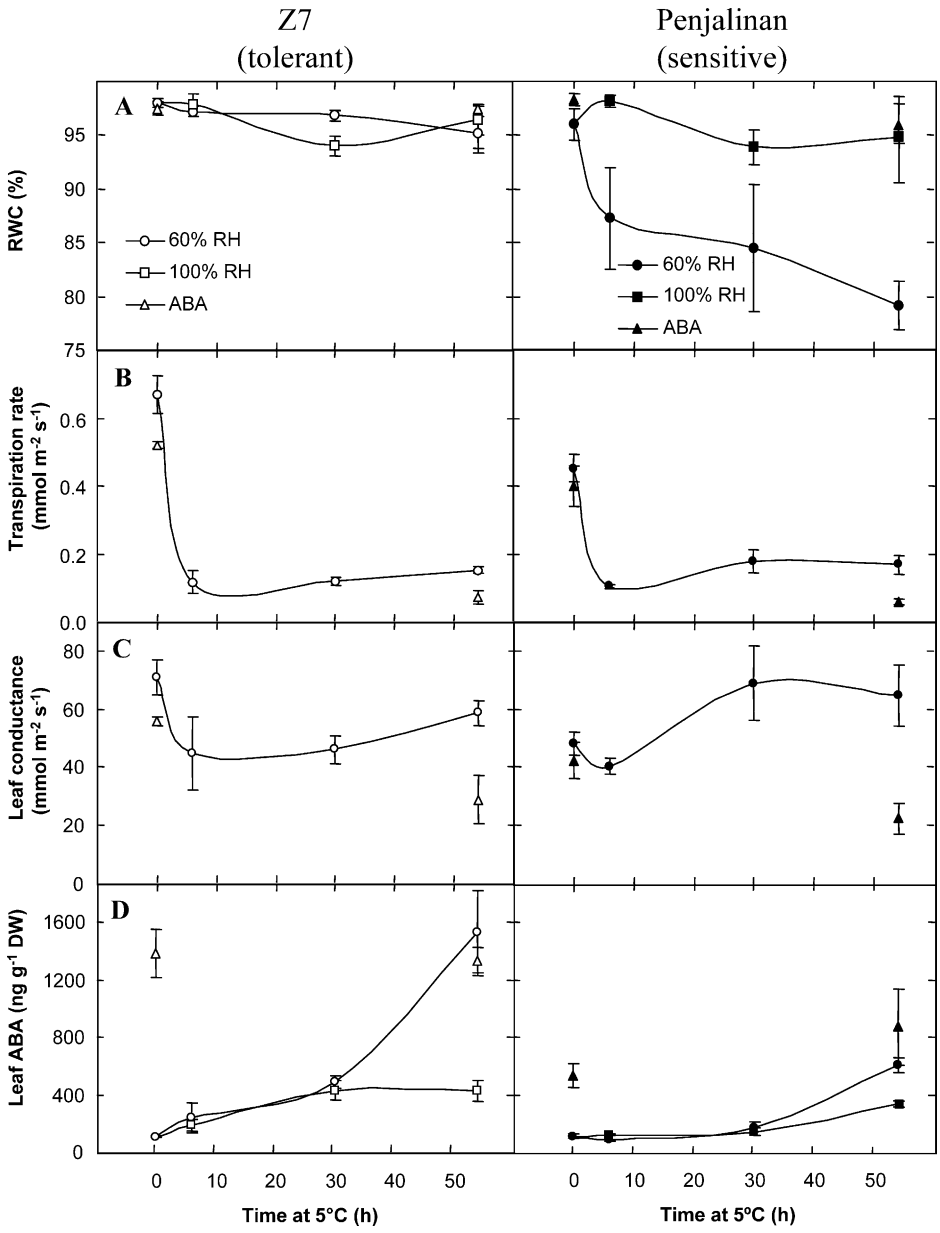

Fig. 1. Leaf RWC (A), leaf transpiration rate (B), leaf conductance (C), and leaf ABA content (D) of two maize genotypes differing in chilling sensitivity: Z7 (tolerant, left panels) and Penjalinan (sensitive, right panels) grown at 25 °C (0 h) then subjected to 5 °C for 3 days at 60% (circles) or 100% (squares) RH. Plants pre-treated with ABA are indicated by triangles. Symbols represent the Mean ± S.E. of six to ten plants.

plant groups. This decrease was greater in 60% RH chilled plants than in those chilled at 100% RH, and no differences were observed between the genotypes (Fig. 2A). At the same time, all chilled plants decreased their $\Delta\Psi_s$, except Z7 plants chilled at 60% RH (Fig. 2B). Therefore, only 60% RH chilled plants of both genotypes decreased their CRHC (Fig. 2C).

After 30 and 54 h of chilling, the Z7 plants chilled at 60% RH recovered their J_v partially, decreased their $\Delta\Psi_s$, and increased their CRHC (Fig. 2A–C). On the contrary, Z7 plants chilled at 100% RH did not change their J_v, $\Delta\Psi_s$ and CRHC during chilling (Fig. 2A–C). On the other hand, Penjalinan plants at both humidity regimes tended to decrease their J_v and CRHC during chilling. However, the Penjalinan plants chilled at 100% RH showed the same CRHC than control plants after 54 h of chilling, while those plants chilled at 60% RH decreased their CRHC down to 7% (Fig. 2A and C).

ABA pre-treated chilled plants of both genotypes had higher $\Delta\Psi_s$ than non pre-treated plants after 54 h of chilling (Fig. 2B). However, the effect of ABA treatment on J_v and CRHC differed in the two genotypes. In Z7

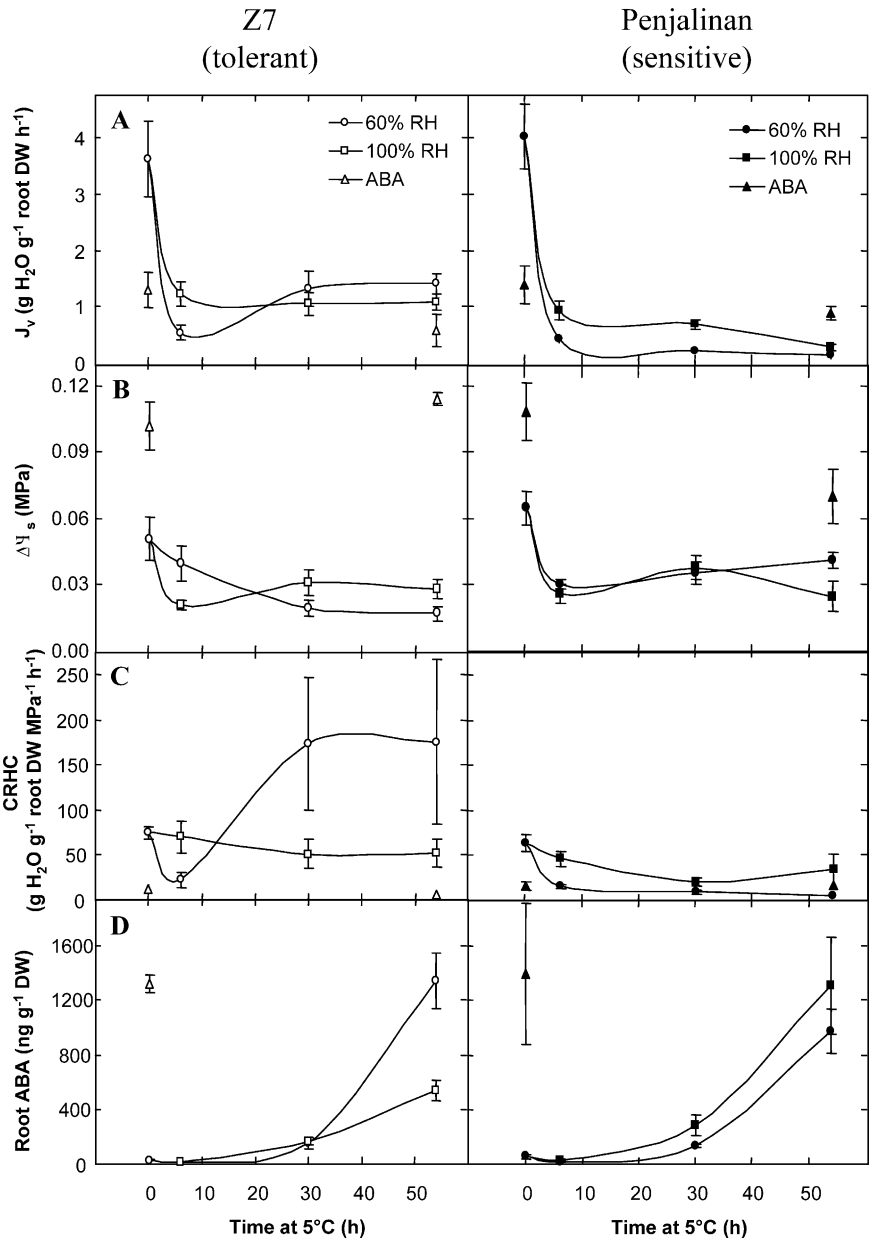

Fig. 2. Free exuded sap flow rate (J_v, A), osmotic gradient between exuded sap and nutrient solution ($\Delta\Psi_s$, B), CRHC (C), and root ABA content (D) of two maize genotypes differing in chilling sensitivity: Z7 (tolerant, left panels) and Penjalinan (sensitive, right panels). The values of ABA for chilled pre-treated plants with ABA have not been include because they are out of scale (6199 ± 633 and 4438 ± 660 ng g^{-1} DW in Z7 and Penjalinan, respectively). Otherwise as for Fig. 1.

plants, ABA caused a decrease in both parameters (J_v and CRHC) but in Penjalinan it caused an increase in J_v and a decrease in CHRC (Fig. 2A and C).

The root ABA content increased at the same level after 30 h of chilling treatment in both genotypes and humidity regimes (Fig. 2D). After 54 h of chilling, the root ABA content increased to the same level in all chilled plants, except those Z7 plants chilled at 100% RH, in which the increase was lower (Fig. 2D). The root ABA content of chilled plants (54 h) and pre-treated with ABA were 6199 ± 633 and 4438 ± 660 ng g^{-1} DW in Z7 and Penjalinan, respectively (data not shown in Fig. 2D).

4. Discussion

It is known that the different degree of chilling sensitivity among maize genotypes is linked with the capacity to avoid water deficit induced by chilling [1,3–6]. In our experimental conditions, Penjalinan plants chilled under 60% RH suffered greater water deficit than

Z7 plants under the same humidity conditions (Fig. 1A). These results confirm that Penjalinan is more sensitive to chilling than Z7 [1,5,20,30]. Moreover, chilled Penjalinan plants pre-treated with ABA did not suffer any water deficit (Fig. 1A), confirming that ABA is effective in avoiding the water deficit induced by chilling [9].

The water deficit induced by chilling is caused by the imbalance between the water loss by the leaves and the water uptake by the roots. Commonly, the reduction of the water uptake is more pronounced than the reduction of the leaf transpiration, which even increases sometimes [1,2,5]. Under our chilling conditions, since the VPD was reduced almost four times (see Section 2) it is quite likely that the water flowed through the root mainly by the cell-to-cell pathway [21]. The cell-to-cell pathway can be estimated by measuring the exuded sap flow of plants (J_v) exuding at atmospheric pressure [1,31]. Using a potometer system in our previous study [1], we observed that during chilling Penjalinan plants reduced the root water uptake more rapidly than the leaf transpiration and that the decrease in root water uptake was correlated with a decrease in J_v measured at 25 °C. Therefore, the measurement of J_v at 25 °C can be used as an estimation of the behaviour of the root water uptake during chilling [1]. After 54 h of chilling at 60% RH, the decrease in leaf transpiration rate was 78 and 62%, respectively in Z7 and in Penjalinan, while the decrease in J_v was 61 and 98%, respectively (Fig. 1B, Fig. 2A). This imbalance in Penjalinan plants chilled at 60% RH should be the cause of the observed water deficit (Fig. 1A). Therefore, the chilling tolerance among maize genotypes is not only linked with the capacity to close the stomata during chilling but it is also linked with the capacity to maintain the root water uptake. Care must be taken in extrapolating J_v values to the real values of the root water uptake, since the former only serves as an indicator [1]. At the same time, the final value of leaf conductance was the same for the two genotypes (Fig. 1C). Therefore, as proposed by Duchoslav and Fracheboud [20], the different degree of water deficit observed between the two genotypes could be the result of differences in root water uptake.

There is no agreement whether the rise in leaf ABA during chilling is associated with chilling per se or with water deficit induced by chilling. Thus, Pardossi et al. [9] and Capell and Dörffling [22] found that in *Phaseolus vulgaris* L. and in *Cucumis sativus* L. the rise in leaf ABA is associated with a chilling-induced water deficit. On the other hand, Starck et al. [32] found that in tomato the rise in ABA is caused by chilling temperature per se. In maize, it has been suggested that both factors (temperature and water deficit) could be involved [4]. After 30 h of chilling, the Z7 plants at both RH increased their leaf ABA content. For longer exposure to chilling, only the Z7 plants chilled at 60% RH continued increasing their leaf ABA content. At the same time, no change in the water status was observed (Fig. 1). Therefore, during the first 30 h of chilling treatment, the rise observed in leaf ABA content in Z7 plants must be caused by low temperature per se. However, the following increase (at longer chilling exposure) could be caused by a greater VPD, because it occurred only in chilled plants at 60% RH. The relationship between increased VPD and accumulation of ABA during chilling has also been found by Capell and Dörffling in cucumber cotyledons [22]. The same behaviour was observed in Penjalinan plants, but to a lower extent and with slower response. Although Penjalinan plants chilled at 60% RH suffered from water deficit, the increase in leaf ABA content was also observed in plants chilled at 100% RH (Fig. 1D), in which no water deficit was observed (Fig. 1A). Moreover, although Penjalinan suffered more from water deficit than Z7, the leaf ABA content was less. Differences in the ABA content observed by our group could be caused by a different metabolism of the ABA delivered from xylem or a different re-export rate from the leaves [33–35]. This also indicates that there is no simple relationship between chilling-induced water deficit and ABA accumulation.

In earlier studies, a relationship was found between the rise in leaf ABA content and a decrease in leaf conductance during chilling [9,30]. However, recently Wilkinson et al. [36] have found that in short-term exposure to cold temperature (1 h), stomatal closure is related to an increase in apoplastic calcium uptake by guard cells, without any change in the ABA content. The same experiments might be done in maize to clarify the short-term response of stomatal closure to low temperature. Nevertheless, this fact does not indicate that ABA is not involved in longer cold treatments. In fact, we observed that Z7 plants accumulated more leaf ABA after 6 h of chilling and that leaf conductance also decreased faster than in Penjalinan plants (Fig. 1C and D) as previously found by Capell and Dörffling [30], although the final values were the same. However, after 54 h of chilling, chilled plants of both genotypes pre-treated with ABA had the same leaf ABA content than non pre-treated plants, but they showed a lower leaf conductance (Fig. 1C and D). This fact can be explained by assuming that ABA pre-treatment increases the stomatal sensitivity to ABA, as in Ackerson's previous work in cotton plants [37].

Some plant species have the ability to acclimate their root hydraulic conductance after some hours of exposure to low temperature [23,26,38,39]. In the present work, we observed such acclimation in Z7 maize plants (Fig. 2C) as in Aroca et al. [1]. Since ABA can rise the root hydraulic conductance in many cases [13–20], we tested the possibility that an increase in root ABA content could be correlated with the root hydraulic acclimation to chilling stress.

In control conditions, ABA treatment caused a decrease in J_v and CHRC in both genotypes (Fig. 2A and C) in agreement with some previous reports [40,41]. However, most of the work reported an increase in root hydraulic conductivity induced by ABA application [15–17,19]. Apparently, the effect of ABA on the root hydraulic conductance depends on many factors. Thus Collins and Morgan [18] found that the stimulation of J_v by ABA was dependent on temperature in maize, being the effect greater at 20 °C. This temperature dependence has been found in other species [13,14]. On the other hand, ABA effect is also genotype dependent [20]. These authors found that ABA increased J_v in Z7 genotype but not in Penjalinan. Finally, the effect of ABA on the root hydraulic conductance is also time-dependent [15,17,40]. In fact Fiscus [40] found that ABA had three separate responses on *Phaseolus* root systems: an initial increase in the water flux volume, a long-term increase in the total ion flux, and a long-term decrease in the hydraulic conductance. We also observed a marked increase in $\Delta\Psi_S$ and a decrease in J_v and CRHC (Fig. 2B and C). Some authors have found an increase of the root hydraulic conductance by ABA, although they studied short time effect (from 1 to 6 h) [15,17,19]. In longer time effect, Ludewig et al. [17] also found a decrease in J_v 24 h after of addition of ABA in sunflower plants. They argued that this could be due to the formation of substances in the medium that counteracted the incremental effect of ABA (see [17]). Such discrepancies between ABA and root hydraulic conductance suggest the need to perform more studies to understand the effect of ABA on root hydraulic conductance.

After 30 h of chilling, Z7 plants under both humidity regimes had the same root ABA content, but those which were chilled at 60% RH had higher hydraulic conductance (Fig. 2C and D). After 54 h of chilling, the Penjalinan plants chilled at 100% RH showed values of CRHC equal to control plants, while the plants which were chilled at 60% RH showed a decrease in CRHC down to 7% control values (Fig. 2C). Nevertheless, both of them had the same root ABA content (Fig. 2D). Finally, after 54 h of chilling, Z7 plants at 60% RH and Penjalinan plants at both humidity regimes had the same root ABA content, but the former had a higher root hydraulic conductance (Fig. 2C and D). Therefore, we did not find a relationship between root ABA content and root hydraulic conductance. Similar results have been found recently by Vernieri et al. [23] in *P. vulgaris*.

The free exuded sap flow (J_v) of chilled Penjalinan plants pre-treated with ABA was greater than in non pre-treated ones (Fig. 2A). Thus, ABA pre-treatment induced chilling tolerance in Penjalinan plants also by increasing root water flow. Such increase in exuded sap flow was mainly caused by a higher $\Delta\Psi_S$ (Fig. 2B). The same effect of ABA decreasing the xylem osmotic potential has been found previously [40,41], although other authors have found the contrary [13,18,39]. In Z7, the effect of ABA improving chilling tolerance could not be seen, quite likely because Z7 is already tolerant [1,5,20,30].

Vernieri et al. [23] have demonstrated recently that the rise in the root ABA content during chilling in *P. vulgaris* is caused by a signal from the water-stressed leaves. However, we did not find a relationship between leaf water status and the rise in the root ABA content in maize: chilled Penjalinan plants at both humidities regimes had the same increase in the root ABA content, and Z7 plants chilled at 60% RH increased their root ABA content without any change in their leaf water status (Fig. 1A, Fig. 2D). In Penjalinan plants, it is plausible that the rise in root ABA content was caused by chilling per se. In the case of Z7, the plants chilled at 60% RH had higher levels of root ABA content than those chilled at 100% RH (Fig. 2D). Therefore, as it happens in the leaves, there may be at least two mechanisms involved in increasing the ABA levels in the root: chilling per se and increased VPD during chilling (since more VPD causes more ABA accumulation).

Like Dörffling and co-workers [3,4,30] described, we found a relationship between leaf ABA content and maize chilling tolerance (Fig. 1D). However, there was no relationship at the root level (Fig. 2D).

In summary, we found that under our experimental conditions root water uptake could be more important than leaf conductance to avoid the water deficit induced by chilling. At the same time, there was a relationship between leaf ABA content and chilling tolerance, but not with leaf conductance. At the root level we did not observe a relationship between ABA content, root hydraulic acclimation and chilling tolerance. Chilling induced an increase in leaf and root ABA contents that was related to chilling per se and to VPD in the tolerant genotype, and only to chilling per se in the sensitive one. Finally, no relationship between leaf water status, root hydraulic acclimation and root ABA content has been observed.

Acknowledgements

The work was supported in part by MURST (Italy) and MCYT (Spain)-Concerted Action Italy-Spain (HI2000-124). R. Aroca was recipient of a grant from Asociación de Amigos de la Universidad de Navarra. The authors wish to thank Dr J. Leipner from ETH (Zurich) for providing maize seeds. The authors would like to thank A. Verhagen for correcting the English and the anonymous referees for their valuable comments in improving the manuscript.

References

[1] R. Aroca, F. Tognoni, J.J. Irigoyen, M. Sánchez-Díaz, A. Pardossi, Different root low temperature response of two maize genotypes differing in chilling sensitivity, Plant Physiol. Biochem. 39 (2001) 1067–1073.

[2] J.J. Irigoyen, J. Pérez de Juan, M. Sánchez-Díaz, Drought enhances chilling tolerance in a chilling-sensitive maize (*Zea mays* L.) variety, New Phytol. 134 (1996) 53–59.

[3] F. Janowiak, K. Dörffling, Chilling of maize seedlings: changes in water status and abscisic acid content in ten genotypes differing in chilling tolerance, J. Plant Physiol. 147 (1996) 582–588.

[4] F. Janowiak, B. Maas, K. Dörffling, Importance of abscisic acid for chilling tolerance of maize seedlings, J. Plant Physiol. 159 (2002) 635–643.

[5] J. Pérez de Juan, J.J. Irigoyen, M. Sánchez-Díaz, Chilling of drought-hardened and non-hardened plants of different chilling-sensitive maize lines. Changes in water relations and ABA contents, Plant Sci. 122 (1997) 71–79.

[6] Z. Ristic, G. Yang, A. Sterzinger, L. Zhang, Higher chilling tolerance in maize is not always related to the ability for greater and faster abscisic acid accumulation, J. Plant Physiol. 153 (1998) 154–162.

[7] T.K. Prasad, M.D. Anderson, C.R. Stewart, Acclimation, hydrogen peroxide, and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings, Plant Physiol. 105 (1994) 619–627.

[8] T.-M. Lee, H.-S. Lur, C. Chu, Role of abscisic acid in chilling tolerance of rice (*Oryza sativa* L.) seedlings. II. Modulation of free polyamine levels, Plant Sci. 126 (1997) 1–10.

[9] A. Pardossi, P. Vernieri, T. Tognoni, Involvement of abscisic acid in regulating water status in *Phaseolus vulgaris* L. during chilling, Plant Physiol. 100 (1992) 1243–1250.

[10] R.S. Pearce, Molecular analysis of acclimation to cold, Plant Growth Regul. 29 (1999) 47–126.

[11] I. Tamminen, P. Mäkelä, P. Heino, E.T. Palva, Ectopic expression of *ABI3* gene enhances freezing tolerance in response to abscisic acid and low temperature in *Arabidopsis thaliana*, Plant J. 25 (2001) 1–8.

[12] V. Lång, E. Mäntyla, B. Welin, B. Sundberg, E.T. Palva, Alterations in water status, endogenous abscisic acid content, and expression of rab18 gene during the development of freezing tolerance in *Arabidopsis thaliana*, Plant Physiol. 104 (1994) 1341–1349.

[13] H. BassiriRad, J.W. Radin, Temperature-dependent water and ion transport properties of barley and sorghum roots II. Effects of abscisic acid, Plant Physiol. 99 (1992) 34–37.

[14] M.G. Pitman, U. Lüttge, A. Läuchli, E. Ball, Action of abscisic acid on ion transport as affected by root temperature and nutrient status, J. Exp. Bot. 25 (1974) 147–155.

[15] E. Hose, E. Steudle, W. Hartung, Abscisic acid and hydraulic conductivity of maize roots: a study using cell- and root-pressure probes, Planta 211 (2000) 874–882.

[16] A. Sauter, S.R. Abrams, W. Hartung, Structural requirements of abscisic acid (ABA) and its impact on water flow during radial transport of ABA analogues through maize roots, J Plant Growth Regul. 21 (2002) 50–59.

[17] M. Ludewig, K. Dörffling, H. Seifert, Abscisic acid and water transport in sunflowers, Planta 175 (1988) 325–333.

[18] J.C. Collins, M. Morgan, The influence of temperature on the abscisic acid stimulated water flow from excised maize roots, New Phytol. 84 (1980) 19–26.

[19] J. Zhang, X. Zhang, J. Liang, Exudation rate and hydraulic conductivity of maize roots are enhanced by soil drying and abscisic acid treatment, New Phytol. 131 (1995) 329–336.

[20] S. Duchoslav, Y. Fracheboud, Influence of chilling stress on the water status of maize seedlings, in: K. Dörffling, B. Brettschneider, H. Tantau, K. Pithan (Eds.), Crop Development for the Cool and Wet Regions of Europe, European Commission, ECSP-ECC-EAEC, Brussels, 1994, pp. 617–623.

[21] E. Steudle, C.A. Peterson, How does water get through roots, J. Exp. Bot. 49 (1998) 775–788.

[22] B. Capell, K Dörffling, Low temperature-induced changes of abscisic acid contents in barley and cucumber leaves in relation to their water status, J. Plant Physiol. 135 (1989) 571–575.

[23] P. Vernieri, A. Lenzi, M. Figaro, F. Tognoni, A. Pardossi, How the roots contribute to the ability of *Phaseolus vulgaris* L. to cope with chilling-induced water stress, J. Exp. Bot. 52 (2001) 2199–2206.

[24] P.E. Weatherley, Studies in the water relations of the cotton plant. I. The field measurements of water deficit in leaves, New Phytol. 49 (1950) 81–87.

[25] R.W. Pearcy, E.D. Schulze, R. Zimmermann, Measurement of transpiration and leaf conductance, in: R.W. Pearcy, J. Ehleringer, H.A. Mooney, P.W. Rundel (Eds.), Plant Physiological Ecology. Field Methods and Instrumentation, Chapman and Hall, London, 1989, pp. 137–160.

[26] J. Bigot, J. Boucaud, Effects of Ca-signalling inhibitors on short-term cold-acclimation of hydraulic conductivity in roots of *Brassica rapa* plants, J. Plant Physiol. 157 (2000) 7–12.

[27] P. Vernieri, A. Pardossi, F. Tognoni, Influence of chilling and drought on water relations and abscisic acid accumulation in bean, Aust. J. Plant Physiol. 18 (1991) 25–35.

[28] A. Bochicchio, P. Vernieri, S. Puliga, F. Balducci, C. Vazzana, Acquisition of desiccation tolerance by isolated maize embryos exposed to different conditions: the questionable role of endogenous abscisic acid, Physiol. Plant. 91 (1994) 615–622.

[29] M. Sturaro, P. Vernieri, P. Castiglioni, G. Binelli, G. Gavazzi, The rea (red embryonic axis) phenotype describes a new mutation affecting the response of maize embryos to abscisic acid and osmotic stress, J. Exp. Bot. 47 (1996) 755–762.

[30] B. Capell, K. Dörffling, Genotype-specific differences in chilling tolerance of maize in relation to chilling-induced changes in water status and abscisic acid accumulation, Physiol. Plant. 88 (1993) 638–646.

[31] J. Gibbs, D.W. Turner, W. Armstrong, K. Sivasithamparam, H. Greenway, Response to oxygen deficiency in primary maize roots. II. Development of oxygen deficiency in the stele has limited short-term impact on radial hydraulic conductivity, Aust. J. Plant. Physiol. 25 (1998) 759–763.

[32] Z. Starck, D. Choluj, H. Gawronska, The effect of drought hardening and chilling on ABA content in xylem sap and ABA delivery rate from root of tomato plant, Acta Physiol. Plant. 20 (1998) 41–48.

[33] S. Pekic, R. Stikic, L. Tomljanovic, V. Andjelkovic, M. Ivanovic, S.A. Quarrie, Characterization of maize lines differing in leaf abscisic acid content in the field. I. Abscisic acid physiology, Ann. Bot. 75 (1995) 67–73.

[34] J. Zhang, W. Jia, D.-P. Zhang, Re-export and metabolism of xylem -delivered ABA in attached maize leaves under different transpirational fluxes and xylem ABA concentrations, J. Exp. Bot. 48 (1997) 1557–1564.

[35] W. Jia, J. Zhang, Comparison of exportation and metabolism of xylem-delivered ABA in maize leaves at different water status and xylem sap pH, Plant Growth Regul. 21 (1997) 43–49.

[36] S. Wilkinson, A.L. Clephan, W.J. Davies, Rapid low temperature-induced stomatal closure occurs in cold-tolerant *Commelina communis* leaves but not in cold-sensitive tobacco leaves, via a mechanism that involves apoplastic calcium but not abscisic acid, Plant Physiol. 126 (2001) 1566–1578.

[37] R.C. Ackerson, Stomatal response of cotton to water stress and abscisic acid as affected by water stress history, Plant Physiol. 65 (1980) 455–459.
[38] A. Fennell, A.H. Markhart, Rapid acclimation of root hydraulic conductivity to low temperature, J. Exp. Bot. 49 (1998) 879–884.
[39] J. Bigot, J. Boucaud, Effects of synthetic plant growth retardants and abscisic acid on root functions of *Brassica rapa* plants exposed to low root-zone temperature, New Phytol. 139 (1998) 255–265.
[40] E.L. Fiscus, Effects of abscisic acid on the hydraulic conductance and the total ion transport through Phaseolus root systems, Plant Physiol. 68 (1981) 169–174.
[41] A.H. Markhart, E.L. Fiscus, A.W. Naylor, P.J. Kramer, Effect of abscisic acid on root hydraulic conductivity, Plant Physiol. 64 (1979) 611–614.