Different root low temperature response of two maize genotypes differing in chilling sensitivity

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Abstract – Here we report on the root hydraulic properties of intact and excised root systems of two maize genotypes differing in chilling sensitivity (Z7, tolerant and Penjalinan, sensitive) subjected for 3 d to 5 °C. When root hydraulic conductance (L) was measured under a hydrostatic force using an excised root system in a pressure chamber, an initial decrease of L was observed in both genotypes. However, the value of L increased in the chilling tolerant genotype after 30 h at 5 °C; in the chilling sensitive Penjalinan genotype there was no such increase. Osmotic root hydraulic conductance was measured in excised root systems exuding under atmospheric pressure. We observed a progressive decline during the chilling treatment of the osmotic root hydraulic conductance in the chilling sensitive Penjalinan plants; however, after 54 h at 5 °C, the chilling tolerant Z7 plants had a significantly higher osmotic hydraulic conductance. Moreover, in the chilling tolerant plants we found an increase in the inhibition caused by HgCl₂ of the osmotic hydraulic conductance during the chilling treatment, indicating a possible increase in the contribution of aquaporins to root hydraulic conductance in the chilling tolerant Z7 plants during chilling treatment.

Chilling / Low temperature acclimation / Mercuric chloride / Root hydraulic conductance / Water channels / Zea mays

CRHC, Composite root hydraulic conductance / E, leaf transpiration rate / Jₑ, Exuded sap water flow / Lₑ, Hydrostatic root hydraulic conductance / PPFD, Photosynthetic photon flux density / RH, Relative humidity / RWC, Relative water content / U, Root water absorption / VPD, Vapour pressure deficit / ψₛ, Xylem osmotic potential / α, Osmotic reflection coefficient

1. INTRODUCTION

It is well documented that low temperature causes water deficit in thermophilic crops such as maize [3, 13, 24, 28]. This water deficit is caused by an inhibition of root water uptake without an accompanying decline of leaf transpiration during low temperature periods [13, 24]. In fact, the difference in chilling tolerance between maize genotypes is correlated with their capacity to close their stomates during chilling periods [13, 24, 28].

Little research has been done on maize root hydraulic properties as affected by exposure to low temperatures. Using an anatomical approach, Ciamperová and Dekánková [5] found that root tissues of a chilling-tolerant maize variety had greater conductive capacity than that of a chilling-sensitive variety after 7 d at 6 °C. In addition, Richner et al. [27] found that a chilling-sensitive maize variety decreased its root length and root surface area while a chilling-tolerant variety maintained it during different low temperature treatments. However, so far not much attention has been given to the comparison of root hydraulic properties of chilling-tolerant and sensitive genotypes of plants.

The study of plant water transport has undergone important changes in the past few years since the composite water transport model was proposed by Steudle and Peterson [31]. Thus, under hydrostatic force conditions (e.g. transpiring plants), water flow occurs mainly through the apoplastic path. However,
under an osmotic force, when transpiration is restricted by different conditions (e.g. drought, salinity, or high humidity), water movement takes place by the cell-to-cell path (for review see [31]). Moreover, the discovery of water channel proteins (aquaporins) in plants [15, 21] has caused us to reinterpret root hydraulic properties because the contribution of aquaporins to overall root water transport can dominate under certain conditions (see [32]). In fact, transgenic Arabidopsis thaliana plants that do not express the PIP1 family of plasma membrane aquaporins have a higher root:shoot ratio and lower protoplast water permeability as compared to control Arabidopsis plants [14].

The objective of the present research was to evaluate the effects of chilling on root hydraulic properties of two maize genotypes differing in chilling sensitivity: Z7, a chilling-tolerant genotype, and Penjalinan, a chilling-sensitive one [3, 5, 24, 27]. The measurements of root hydraulic conductance were performed under a hydrostatic force using a pressure chamber [19] and under an osmotic force using detached root systems exuding at atmospheric pressure [10]. We also investigated the involvement of aquaporins on maize root hydraulic properties during chilling by the application of HgCl2 to the detached root system exuding at atmospheric pressure [4, 26]. Furthermore, we compared the results obtained in excised roots with results obtained in intact plants using a potometer [22].

2. RESULTS

2.1. Water status

Figure 1 shows the effect of chilling on leaf relative water content (RWC) of the two maize genotypes under study: Z7 (tolerant) and Penjalinan (sensitive). During chilling treatment, Z7 plants maintained their RWC at control values; however, Penjalinan plants after 6 h of chilling already had a lower RWC. From 30 to 72 h of chilling, Z7 plants had a higher RWC than Penjalinan plants. Thus, after 72 h of chilling, the RWC values of Z7 and Penjalinan plants were 89 and 69 % respectively.

2.2. Hydrostatic and osmotic root hydraulic conductance

Hydrostatic root hydraulic conductance (Lh) of both genotypes grown under control conditions had the same values (Figure 2). In addition, after 6 h of chilling treatment both genotypes decreased their Lh practically to the same extent (23 % in Z7 and 17 % in Penjalinan with respect to the control values). However, after 30 and 54 h, the chilling-tolerant Z7 plants increased their Lh close to that of control values whereas Penjalinan plants maintained their low Lh values (Figure 2).

When control excised roots were allowed to exude at atmospheric pressure, Penjalinan showed higher water flux rate (Jw) and composite root hydraulic conductance (CRHC) than Z7 plants (Figure 2). However, during chilling treatment, the chilling-tolerant Z7 plants maintained their Jw value and even increased it after 6 h of chilling treatment (Figure 2). In contrast, the Jw of Penjalinan plants decreased progressively during chilling, reaching nearly 0 value after 54 h of chilling treatment (Figure 2). In Penjalinan, CRHC also changed in the same direction as Jw (Figure 2).
On the other hand, in Z7 CRHC increased significantly \((P < 0.05)\) after 54 h of chilling treatment, simultaneously with an increase in xylem osmotic potential \(\psi_s\) (Figure 3B).

### 2.3. HgCl₂ treatment

The effect of HgCl₂ on excised roots exuding at atmospheric pressure is also shown in Figure 3. Penjalinan plants showed the same percentage of inhibition by HgCl₂ on \(J_v\) and CRHC in control conditions and after 6 and 30 h of chilling treatment (around 70 and 90 % for \(J_v\) and CRHC respectively) (Figure 3A). However at 54 h of chilling, no inhibition of HgCl₂ of \(J_v\) and CRHC was found (Figure 3A, C). On the other hand, Z7 chilled plants increased the inhibition of HgCl₂ on \(J_v\) and CRHC with respect to control plants (Figure 3A, C). Thus, at control conditions the HgCl₂ inhibition on CRHC was 53 %, while after 54 h of chilling it was 92 %. The HgCl₂ treatment had no effect on \(\psi_s\), except after 30 and 54 h of chilling treatment in Penjalinan plants, when \(\psi_s\) decreased slightly after 30 and 54 h of chilling treatment (Figure 3B).

### 2.4. Potometer system

During chilling treatment, chilling-sensitive Penjalinan plants exhibited under light conditions a higher leaf transpiration rate (E) than Z7 plants except between 48 and 54 h of chilling treatment (Figure 4). Penjalinan decreased its E on the third day of chilling, whereas its root water absorption (U) decreased on the second day (Figure 4). During the chilling period between 54–60 h, Penjalinan E and U were 61 and 48 % with respect to the initial values respectively. Nevertheless, Penjalinan plants consistently showed higher U than Z7 plants. On the other hand, Z7 plants exhibited less change in E than did Penjalinan plants and their U values remained almost unchanged during the chilling treatment (Figure 4).

### 3. DISCUSSION

In the present research, we used two maize genotypes that clearly differ in the degree of water stress suffered under a low temperature regime (Penjalinan, a...
chilling-sensitive genotype and Z7, a chilling-tolerant one). Penjalinan plants showed greater dehydration than Z7 plants as indicated by their lower relative water content (RWC; Figure 1). In addition, others have previously found that the Penjalinan variety shows a higher necrotic leaf area and less plant survival than Z7 after a chilling period [3]. These results confirm that the Penjalinan genotype is more sensitive to chilling than Z7 [3, 24]. Having confirmed the differences in chilling sensitivity between these two maize genotypes, we studied the possible differences in root hydraulic properties between the two genotypes during chilling.

In the experiment with the pressure chamber using excised root systems, we observed that Z7 plants underwent an acclimation of their hydrostatic root hydraulic conductance ($L_h$) after 30 h of chilling treatment (Figure 2). Under a hydrostatic force (e.g. applied by a pressure chamber), water flows through the inner part of the root, mainly by the apoplastic path, although the cell-to-cell path also exists [18, 31]. Therefore, we can hypothesize that during chilling, Z7 plants increased their apoplastic water conductance. On the other hand, we found that Z7 plants had a higher $L_h$ than Penjalinan during chilling treatment, in agreement with the higher branching density, larger conducting area, and greater conductive capacity observed by Ciamporová and Dekánková [5] in Z7 roots after 7 d at 5 °C. Also, Fennell and Markhart [9] found a root hydraulic acclimation in spinach (a chilling-resistant species) roots similar to behavior we have found in Z7 excised roots. Fennell and Markhart [9] hypothesized that this root acclimation could be due to an increase in root membrane water permeability.

Membrane water permeability can increase due to a rise in unsaturated fatty acids as occurs in several species during periods of low temperature [11]. Other possibilities for increasing membrane water permeability is to increase the quantity or the activity of aquaporins (see [32]). Aquaporin involvement in chilling acclimation of maize roots has been studied with detached root systems exuding at atmospheric pressure. Under such conditions, no hydrostatic force is present, and water flows across the cell-to-cell path [31]. Therefore, we can develop an approach to study how water crosses root maize membranes as conducted previously by Gibbs et al. [10].

We expressed the root hydraulic conductance under an osmotic force as a composite root hydraulic conductance (CRHC) as proposed by Bigot and Boucaud [2] because we could not determine the osmotic reflection coefficient ($\sigma$) (see Methods section). Penjalinan plants decreased their CRHC to close to zero after 54 h of chilling treatment in parallel with a decrease in its $J_v$, although no changes in its $\psi_s$ were observed (Figure 3). Normally, osmotic hydraulic conductance is very low compared to hydrostatic conductance [31]. However, we found no differences between the values of $L_h$ and CRHC in Penjalinan control plants (Figures 2, 3C). Similar results have also been found in Phaseolus coccineus and Hordeum distichon using root and cell pressure probes due to higher membrane water conductivity [29, 30]. However, pressure probes should be used to verify this hypothesis in Penjalinan plants.

On the other hand, Z7 plants increased their CRHC after 54 h of chilling treatment due mainly to a decrease in their $\psi_s$, since water flux ($J_v$) barely exhibited a change (Figure 3). This fact can be explained by an increase in the proportion of water that flows independently of an osmotic gradient in Z7 chilled roots. All measurements were made at 25 °C because at 5 °C, $J_v$ was too low to be accurately measured by our methods. Thus, in several species a similar increase in osmotic hydraulic properties has been found at warm temperatures after a period of low temperatures [1, 6, 20].

A slight decrease in $\psi_s$ from HgCl$_2$ was observed (Figure 3B). Such a slight decrease could be explained as being a consequence of solute concentration, since $J_v$ decreases as a result of HgCl$_2$ treatment (Figure 3A).
Therefore, the inhibition observed on CRHC by HgCl₂ (figure 2C) might be caused by a direct effect on osmotic hydraulic conductance and not by effects on Ψᵣ.

The percentage of inhibition by HgCl₂ on CRHC was different in both genotypes during the chilling treatment. While Z7 plants increased their percentage of inhibition with respect to control values, inhibition decreased in Penjalinan plants (figure 3C). Therefore, we can conclude that chilled roots of Z7 plants result in an increase in the amount of water that flows across aquaporins, which also may explain the Lₜ acclimation observed in this genotype (figure 3). In fact, recently Li et al. [16] have found an increase in aquaporin mRNA after chilling stress in rice chilling-acclimated plants. However, the studies of aquaporin function using HgCl₂ should be interpreted with caution. First, because not all aquaporins are sensitive to HgCl₂ and second, because HgCl₂ may have metabolic secondary effects (see [18]). Therefore, the HgCl₂ results observed in this research should be taken as a preliminary approach towards the study of root aquaporin function during chilling in maize.

We tried to corroborate the above results with those from excised roots in intact plants using a potometer system. We found that Penjalinan plants had a higher leaf transpiration rate (E) and root water absorption (U) than Z7 plants during chilling treatment (figure 4). Therefore, we suggest that Z7 plants exhibit tolerance to chilling stress due to their capacity to close stomata during low temperature events as previously proposed [3, 24]. However, during chilling treatment, Z7 plants maintained their E and U values, whereas Penjalinan plants decreased their U more quickly than E (figure 4). Thus, the different behaviour between U and E in Penjalinan plants during chilling treatment could cause the observed water deficit (figure 1). The decline observed in U values of Penjalinan plants may also be caused by a decrease in their root hydraulic conductance (figures 2, 3C). Nevertheless, the root hydraulic conductance acclimation to low temperatures observed in the excised roots of Z7 plants (figures 2, 3) was not found in intact plants. This fact could be caused by the influence of leaf transpiration on root water uptake [31].

In summary, root hydraulic conductance of Z7 plants was able to acclimate to low temperatures while that of Penjalinan plants could not. This is the first documentation of root hydraulic acclimation in maize. In addition, we proposed a possible function of aquaporins in this acclimation process, although this statement should be examined more closely in subsequent research.

4. METHODS

4.1. Plant material and experimental design

Two maize genotypes (Zea mays L.) with different chilling tolerances were used: Z7, a chilling-tolerant genotype and Penjalinan, a chilling-sensitive one [3, 5, 14, 27]. Seeds of the two genotypes were germinated in wet expanded clay at 25 °C. After 5 d 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 (air temperatures 25 ± 1 °C, 60 % RH corresponding to 1.1 kPa of VPD, 12-h photoperiod, 150 µmol·m⁻²·s⁻¹ PPFD from fluorescent lamps). At 11 d, seedlings were transferred during 3 d to a chamber at 5.0 ± 0.5 °C, 60 % RH corresponding to 0.3 kPa of VPD in the same light conditions of the original growth chamber. The same nutrient solution was used during the chilling treatment and determinations of root hydraulic properties in the laboratory.

4.2. Leaf water status

Leaf water status was assayed by the measurement of relative water content (RWC) by Weatherley’s method [33] on three to five leaf samples of each variety grown at 25 °C (Control) or chilled (5 °C) during 6, 30, 54 or 72 h.

4.3. Hydrostatic root hydraulic conductance (Lₜ)

Hydrostatic root hydraulic conductance (Lₜ) was estimated by using a pressure chamber technique [19]. Three to six plants of each variety grown at 25 °C (Control) or chilled (5 °C) during 6, 30 or 54 h were cut below the first leaf and placed into a pressure chamber with the cut stump protruding through the lid. Their steady-state exudation rates were measured sequentially at 0.1, 0.3, 0.5 and 0.7 MPa. Measurements were made at 25 °C in control plants and at 5 °C in chilled plants. All Lₜ measurements were made at the middle of the photoperiod to avoid differences in the Lₜ values resulting from the time of day [23]. Lₜ was calculated by the slope between exudation flux rate and applied pressure [19]. Only the root systems that exhibited a significant (P > 0.95) regression coefficient between exudation flux rate and applied pressure were chosen to calculate Lₜ.
4.4. Osmotic root hydraulic conductance

Osmotic root hydraulic conductance was estimated using a detached root system exuding at atmospheric pressure. Three to six plants of each variety grown at 25 °C (Control) or chilled (5 °C) during 6, 30 or 54 h were cut below the first leaf and a pipette was attached to the root stump with a flexible silicone tube. Then, the roots were transferred to an aerated nutrient solution at 25 °C and the exuded sap was collected with a syringe after 3 h (the first 30 min of collected exudate was discarded). Exuded sap and nutrient solution osmolalities were determined using a cryoscopic osmometer (Osmomat 030, Gonotec GmbH, Berlin, Germany). Osmotic root hydraulic conductance was calculated by the following equation: 

\[ J_v = \tau \times L_o \times \Delta \psi \]

where \( J_v \) is the exuded sap flow rate, \( \tau \) the osmotic reflection coefficient, \( L_o \) the root osmotic conductance and \( \Delta \psi \) the osmotic potential difference between the exuded sap and nutrient solution. The osmotic potential of the nutrient solution was –0.10 MPa. Under our experimental conditions, we could not determine \( \tau \); therefore we expressed root osmotic conductance as \( \sigma \times L_o \). We called this parameter the composite root hydraulic conductance (CRHC) as was recently proposed by Bigot and Boucaud.

4.5. HgCl₂ treatment

Certain aquaporins are sensitive to mercurial reagents. In fact, HgCl₂ is commonly used to study aquaporin function. In the present research, we used the same HgCl₂ concentration that has been used by other authors. HgCl₂ experiments were conducted on three to six plants of each variety grown at 25 °C (Control) or chilled (5 °C) during 6, 30 or 54 h. Roots of intact plants were exposed to a nutrient solution with a concentration of 50 µM of HgCl₂ for 15 min. After this HgCl₂ exposure period, excised roots were washed and placed in a free HgCl₂ nutrient solution, and then the same procedure described in section 4.4 was followed.

4.6. Leaf transpiration (E) and root water absorption (U)

At different time intervals of the chilling treatment, the rates of leaf transpiration (E) and root water absorption (U) were measured by a potometer system as described in Pardossi et al. Intact, bare-root plants were sealed in 50-mL vessels (one plant per vessel) through a rubber bung with a graduated pipette attached. E was determined by the rate of weight loss of the potometer-plant apparatus, and U was estimated by weighting the nutrient solution used to refill the graduate pipette to the reference point.

4.7. Statistical analysis

Means of all treatments of each parameter were compared using ANOVA and Fisher LSD tests.

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