

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

Ricardo Aroca^a, Franco Tognoni^b, Juan José Irigoyen^a, Manuel Sánchez-Díaz^{a*}, Alberto Pardossi^c

^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, Viale delle Piagge 23, 56100 Pisa, Italy

^c Dipartimento di Produzione Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

Received 4 January 2001; accepted 23 July 2001

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.
© 2001 Éditions scientifiques et médicales Elsevier SAS

chilling / low temperature acclimation / mercuric chloride / root hydraulic conductance / water channels / *Zea mays*

CRHC, composite root hydraulic conductance / E, leaf transpiration rate / J_v, exuded sap water flow / L_h, hydrostatic root hydraulic conductance / PPF, photosynthetic photon flux density / RH, relative humidity / RWC, relative water content / U, root water absorption / VPD, vapour pressure deficit / ψ_s , xylem osmotic potential / σ , 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 [3, 24, 28].

Little research has been done on maize root hydraulic properties as affected by exposure to low temperatures. Using an anatomical approach, Ciamporová 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,

*Correspondence and reprints: fax +34 948 425649.

E-mail address: msanchez@unav.es (M. Sánchez-Díaz).

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 HgCl_2 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 (L_h) 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 L_h practically to the same extent (23 % in Z7 and 17 % in

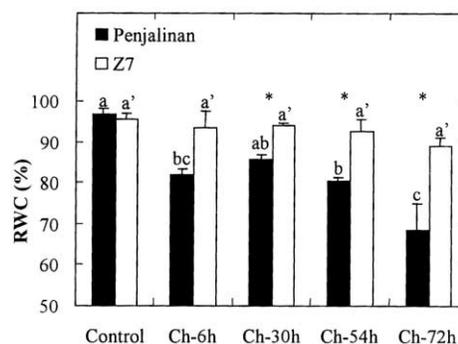


Figure 1. Leaf relative water content (RWC) of Penjalinan (in black) and Z7 (in white) plants at 25 °C (Control) or at 5 °C during 6 (Ch-6h), 30 (Ch-30), 54 (Ch-54) or 72 h (Ch-72). Different letters indicate significant differences ($P < 0.05$) among treatments of each variety (e.g. a, b or c for Penjalinan and a', or b', c' for Z7). * Significant difference ($P < 0.05$) between genotypes of each treatment. Means \pm S.E. are represented ($n = 3-6$).

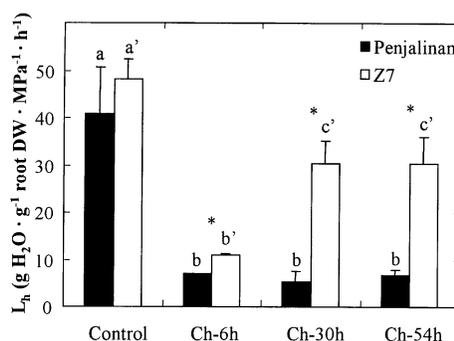


Figure 2. Hydrostatic root conductance (L_h) calculated by pressure chamber in excised roots. Measurement were made at 25 °C in control root system (Control) or at 5 °C on chilled ones (Ch). Otherwise as for figure 1.

Penjalinan with respect to the control values). However, after 30 and 54 h, the chilling-tolerant Z7 plants increased their L_h close to that of control values whereas Penjalinan plants maintained their low L_h values (figure 2).

When control excised roots were allowed to exude at atmospheric pressure, Penjalinan showed higher water flux rate (J_v) and composite root hydraulic conductance (CRHC) than Z7 plants (figure 3A, C). However, during chilling treatment, the chilling-tolerant Z7 plants maintained their J_v value and even increased it after 6 h of chilling treatment (figure 3A). In contrast, the J_v of Penjalinan plants decreased progressively during chilling, reaching nearly 0 value after 54 h of chilling treatment (figure 3A). In Penjalinan, CRHC also changed in the same direction as J_v (figure 3A, C).

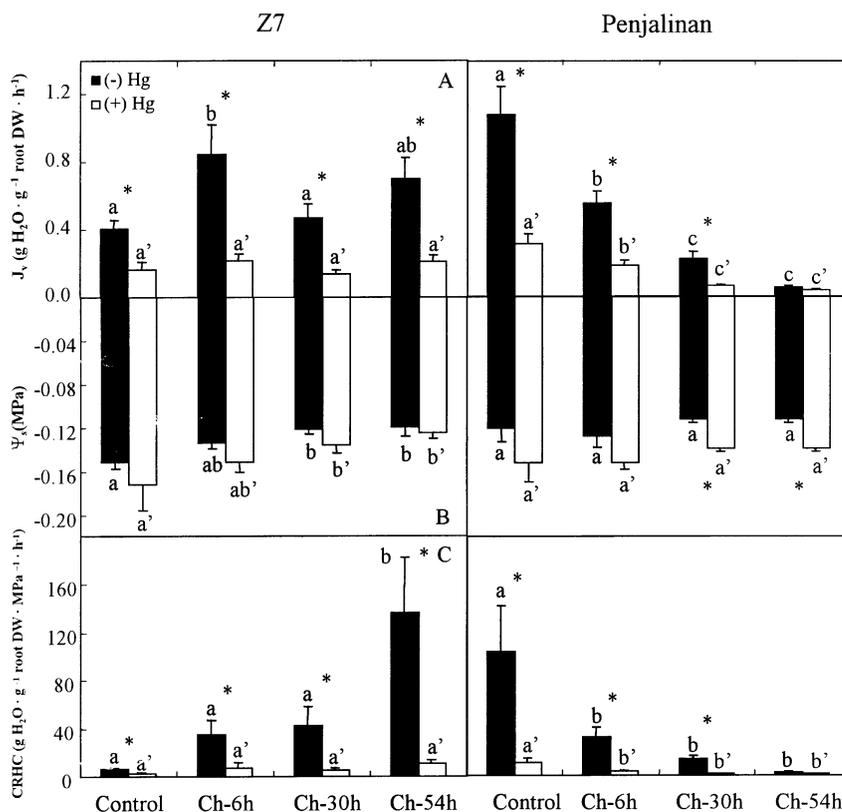


Figure 3. Osmotic water flow rate (J_v) (A), osmotic xylem pressure (ψ_s) (B) and composite root hydraulic conductance (CRHC) (C) of Z7 (left panels) and Penjalinan (right panels) from excised roots treated with 50 μ M HgCl_2 (in white) or not (in black). All measurements were made at 25 °C. Different letters indicate significant differences ($P < 0.05$) among control (Control) and chilled roots (Ch) of each HgCl_2 treatment (e.g. a, b or c for -Hg and a', b' or c' for +Hg). * Significant difference ($P < 0.05$) between -Hg and +Hg treatments at each temperature treatment. Otherwise as for figure 1.

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 (ψ_s) (figure 3B).

2.3. HgCl_2 treatment

The effect of HgCl_2 on excised roots exuding at atmospheric pressure is also shown in figure 3. Penjalinan plants showed the same percentage of inhibition by HgCl_2 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, C). However at 54 h of chilling, no inhibition of HgCl_2 of J_v and CRHC was found (figure 3A, C). On the other hand, Z7 chilled plants increased the inhibition of HgCl_2 on J_v and CRHC with respect to control plants (figure 3A, C). Thus, at control conditions the HgCl_2 inhibition on CRHC was 53 %, while after 54 h of chilling it was 92 %. The HgCl_2 treatment had no effect on ψ_s , except after 30 and 54 h of chilling treatment in Penjalinan plants, when ψ_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

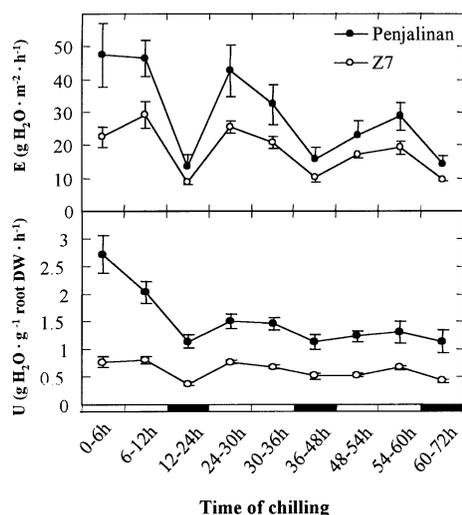


Figure 4. Potometer leaf transpiration (E) and root water absorption rates (U) of Penjalinan (in black) and Z7 (in white) intact plants at different intervals of chilling treatment. Horizontal black bars indicate night period. Otherwise as for *figure 1*.

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 in part 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 (σ) (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 ψ_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 ψ_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 ψ_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 HgCl_2 (figure 3C) might be caused by a direct effect on osmotic hydraulic conductance and not by effects on ψ_s .

The percentage of inhibition by HgCl_2 on CRHC was different in both genotypes along 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 L_h acclimation observed in this genotype (figure 2) [18]. 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 HgCl_2 should be interpreted with caution. First, because not all aquaporins are sensitive to HgCl_2 and second, because HgCl_2 may have metabolic secondary effects (see [8]). Therefore, the HgCl_2 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 aqua-

porins 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, 24, 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, $150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 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 (L_h)

Hydrostatic root hydraulic conductance (L_h) 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 L_h measurements were made at the middle of the photoperiod to avoid differences in the L_h values resulting from the time of day [23]. L_h 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 L_h .

4.4. Osmotic root hydraulic conductance

Osmotic root hydraulic conductance was estimated using a detached root system exuding at atmospheric pressure [10]. 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: $J_v = \sigma \times L_o \times \Delta\psi_s$, where J_v is the exuded sap flow rate, σ the osmotic reflection coefficient, L_o the root osmotic conductance and $\Delta\psi_s$ 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 σ ; 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 [2].

4.5. HgCl₂ treatment

Certain aquaporins are sensitive to mercurial reagents [7, 25]. In fact, HgCl₂ is commonly used to study aquaporin function [4, 12, 17, 18, 26]. In the present research, we used the same HgCl₂ concentration that has been used by other authors [4, 12]. 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. [22]. 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.

Acknowledgments. The work was supported in part by MURST (Italy) and MCYT (Spain) – Concerted Action Italy-Spain (HI2000-124). Ricardo Aroca was the recipient of a grant from Asociación de Amigos de la Universidad de Navarra. The authors wish to thank Dr Y. Fracheboud from ETH (Zürich) for providing maize seeds. Technical support and comments during the experiments by Dr L. Incrocci have been most valuable. We thank Prof. M.J. Chrispeels for his careful editing of the manuscript.

REFERENCES

- [1] Bigot J., Boucaud J., 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.
- [2] Bigot J., Boucaud J., 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.
- [3] Capell B., Dörffling K., 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.
- [4] Carvajal M., Cooke D.T., Clarkson D.T., Responses of wheat plants to nutrient deprivation may involve the regulation of water-channel function, *Planta* 199 (1996) 372–381.
- [5] Ciamporová M., Dekánková K., Root system morphology and anatomy of cold-sensitive Penjalinan and cold-tolerant Z7 genotypes of maize, *Biol. Bratislava* 53 (1998) 133–139.
- [6] Clarkson D.T., The influence of temperature on the exudation of xylem sap from detached root systems of rye (*Secale cereale*) and barley (*Hordeum vulgare*), *Planta* 132 (1976) 297–304.
- [7] Daniels M.J., Chaumont F., Mirkov T.E., Chrispeels M.J., Characterization of a new vacuolar membrane aquaporin sensitive to mercury at unique site, *Plant Cell* 8 (1996) 587–599.
- [8] Eckert M., Biela A., Siefritz F., Kaldenhoff R., New aspects of plant aquaporin regulation and specificity, *J. Exp. Bot.* 150 (1999) 1541–1545.
- [9] Fennell A., Markhart III A.H., Rapid acclimation of root hydraulic conductivity to low temperature, *J. Exp. Bot.* 49 (1998) 879–884.

- [10] Gibbs J., Turner D.W., Armstrong W., Sivasithamparam K., Greenway H., 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.
- [11] Harwood J.L., Jones A.L., Perry H.J., Rutter A.J., Smith K.L., Williams M., Changes in plant lipids during temperature adaptation, in: Cossins A.R. (Ed.), *Temperature Adaptation of Biological Membranes*, Portland Press, London, 1994, pp. 107–118.
- [12] Hejnowicz Z., Sievers A., Reversible closure of water channels in parenchymatic cells of sunflower hypocotyls depends on turgor status of the cells, *J. Plant Physiol.* 147 (1996) 516–520.
- [13] Irigoyen J.J., Pérez de Juan J., Sánchez-Díaz M., Drought enhances chilling tolerance in a chilling-sensitive maize (*Zea mays*) variety, *New Phytol.* 134 (1996) 53–59.
- [14] Kaldenhoff R., Grote K., Zhu J.J., Zimmermann U., Significance of plasmalemma aquaporins for water transport in *Arabidopsis thaliana*, *Plant J.* 14 (1998) 121–128.
- [15] Kammerloher W., Fischer U., Pietchotka G.P., Schäffner A.R., Water channel in the plant plasma membrane cloned by immunoselection from a mammalian expression system, *Plant J.* 6 (1994) 187–199.
- [16] Li L.G., Li S.F., Tao Y., Kitagawa Y., Molecular cloning of a novel water channel from rice: its products expression in *Xenopus* oocytes and involvement in chilling tolerance, *Plant Sci.* 154 (2000) 43–51.
- [17] Lu Z., Newmann P.M., Water stress inhibits hydraulic conductance and leaf growth in rice seedlings but not the transport of water via mercury-sensitive water channels in the root, *Plant Physiol.* 120 (1999) 143–151.
- [18] Maggio A., Joly R.J., Effects of mercuric chloride on the hydraulic conductivity of tomato root systems: evidence for a channel-mediated pathway, *Plant Physiol.* 109 (1995) 331–335.
- [19] Markhart III A.H., Smit B., Measurement of root hydraulic conductance, *HortScience* 25 (1990) 282–287.
- [20] Markhart III A.H., Fiscus E.L., Naylor A.W., Kramer P.J., Effect of temperature on water and ion transport in soybean and Broccoli systems, *Plant Physiol.* 64 (1979) 83–87.
- [21] Maurel C., Reizer J., Schroeder J.I., Chrispeels M.J., The vacuolar membrane protein γ TIP creates water specific channels in *Xenopus* oocytes, *EMBO J.* 12 (1993) 2241–2247.
- [22] Pardossi A., Vernieri P., Tognoni F., Involvement of abscisic acid in regulating water status in *Phaseolus vulgaris* L. during chilling, *Plant Physiol.* 100 (1992) 1243–1250.
- [23] Parsons L.R., Kramer P.J., Diurnal cycling in the root resistance to water movement, *Physiol. Plant.* 30 (1974) 19–23.
- [24] Pérez de Juan J., Irigoyen J.J., Sánchez-Díaz M., 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.
- [25] Preston G.M., Jung J.S., Guggino W.B., Agre P., The mercury-sensitive residue at cysteine 189 in the CHIP28 water channel, *J. Biol. Chem.* 268 (1993) 17–20.
- [26] Quintero J.M., Fournier J.M., Benlloch M., Water transport in sunflower root systems: effects of ABA, Ca^{2+} status and $HgCl_2$, *J. Exp. Bot.* 50 (1999) 1607–1612.
- [27] Richner W., Soldati A., Stamp P., Shoot-to-root relations in field-grown maize seedlings, *Agron. J.* 88 (1996) 56–61.
- [28] Ristic Z., Yang G., Sterzinger A., Zhang L., 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.
- [29] Steudle E., Brinckmann E., The osmometer model of the root: water and solute relations of *Phaseolus coccineus*, *Bot. Acta* 102 (1989) 85–95.
- [30] Steudle E., Jeschke W.D., Water transport in barley roots, *Planta* 158 (1983) 237–248.
- [31] Steudle E., Peterson C.A., How does water get through roots?, *J. Exp. Bot.* 49 (1998) 755–788.
- [32] Tyerman S.D., Bohnert H.J., Maurel C., Steudle E., Smith J.A.C., Plant aquaporins: their molecular biology, biophysics and significance for plant water relations, *J. Exp. Bot.* 50 (1999) 1055–1071.
- [33] Weatherley P.E., Studies in the water relations of the cotton plant. I. The field measurements of water deficits in leaves, *New Phytol.* 49 (1950) 81–87.