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Short communication

Cold induced changes in the water balance affect immunocytolocalization pattern of one of the aquaporins in the vascular system in the leaves of maize (*Zea mays* L.)

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ABSTRACT

Chilling stress is known to affect the water balance in plants, which often manifests itself in the decrease of the water potential in different organs. Relationships between chilling, assimilate transport and water balance are far from being understood. Although aquaporins play a key role in regulating water balance in plants, especially under stress conditions, the role of individual aquaporins in stress response remains unclear. In this report we show the specific localization within plasma membranes of one of the aquaporins (PIP2;3) in the leaves of two maize inbred lines differing in their chilling-sensitivity. This form of aquaporin has been also observed in thick-walled sieve elements – an additional type of sieve tubes of unclear function found only in monocotyledons. Moderate chilling (about 15 °C) caused significant reduction of labelling in these cells accompanied by a steep decrease in the water potential in leaves of chilling-sensitive maize line. Our results suggest that both PIP2;3 and thick-walled sieve tubes may be an unknown element of the mechanism of the response of maize to cold stress.

1. Introduction

A rapid response of physiological processes to environmental changes is a key feature for survival. While this is true for any organism, it is especially important for plants, which are immobile and must cope with a plethora of stressors. Most stresses affect, directly or indirectly, their water balance, thus – compared to other organisms – plants possess a sophisticated system for regulating water content in their cells. Chilling stress, is no exception here and it is reported to decrease water potential in different organs of most plants, especially those susceptible to it, such as maize. It is supposed to reduce root hydraulic conductivity, root pressure and sap flow, which results in water deficit symptoms in shoots (Melkonian et al., 2004; Maurel, 2007). It seems that water balance and assimilate transport respond to chilling and this response may be interconnected (Melkonian et al., 2004; Sowiński 1995), but the precise relationship between these factors still needs to be elucidated. It is clear, that changes in water potential are steered by aquaporins, which may play central role in response of the plants to various stresses (Maurel, 2007). This fact is reflected in the genomes of many plant species, for instance in *Arabidopsis*, maize or rice there are over 30 genes coding aquaporins (Chaumont et al., 2001; Quigley et al., 2002; Sakurai et al., 2008). The largest subfamily of plant aquaporins – PIPs (Plasma membrane Intrinsic Proteins) are divided into two subgroups – PIP1 and PIP2 where PIP2 proteins are usually exhibit higher water conducting activity (Maurel, 2007).

Although, as noted earlier, water stress following chilling affects whole plant – and it seems to affect assimilate export from the leaves, changes in aquaporin activity, aquaporin gene expression or aquaporin immunocytolocalization were predominantly studied in the roots (Aroca et al., 2005; Hachez et al., 2006; Sakurai et al., 2008; Matsumoto et al., 2009). Aroca et al. (2005) found that relative amount of aquaporins has been increased in stressed maize roots while the expression of genes coding these proteins has been decreased after chilling-treatment. In addition, aquaporin expression in roots and in leaves may be significantly different

Abbreviations: CC, companion cell; CS, chilling-sensitive maize line; CT, chilling-tolerant maize line; PIP, plasma membrane intrinsic protein; SE, thin-walled sieve tube; SET, thick-walled sieve tube; VP, vascular parenchyma.

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(Matsumoto et al., 2009). It seems that the regulation of water balance dynamics in response to low temperature is a very complex process. Aquaporins seem to play a key role in this mechanism, however it is still not clear what is the function of the individual PIP proteins.

In this paper we report the results of changes in the water potential and immunogold localization of three forms of aquaporins (PIP1;3, PIP2;3 and PIP2;5) in cold-treated leaves of two maize inbred lines differing in chilling-sensitivity. A hypothesis that has been verified here states that low temperature can affect the water potential, which can be connected to changes in the localization (and/or the intensity of labelling) of aquaporins in cells of maize leaves. These proteins may play a crucial role in water balance dynamics in the leaf under chilling stress, by associating with specific cells of the vascular system of maize.

2. Material and methods

2.1. Plant material

Chilling-tolerant (CT) KW 1074 (*Zea mays* spp. *indurata*, flint) and chilling-sensitive (CS) CM 109 (*Z. mays* spp. *indentata*, dent) maize lines were used as an experimental material. The differences in the chilling sensitivity of these inbred lines have been described elsewhere (Sowiński, 1995). Kernels were germinated in wet sand in darkness at 25 °C. The seedlings were grown on Knop solution supplemented with Hoagland's micronutrients in a growth chamber (14 h/10 h light/darkness, irradiance 250 μmol quanta m^{-2} s^{-1} at 24/22 °C day/night temperature). At V3 stage (third leaf fully developed) half of the plants was transferred to a cold growth chamber (14/12 °C day/night temperature) for 4 and 28 h.

2.2. Water potential

Water potential in the leaves (Ψ_w) was measured with an HR 33T dewpoint microvoltometer (Vescor, USA) connected with C-52 Sample Chambers according to Rapacz (1998). The results were statistically analyzed using R (version 3.3.0).

2.3. Immunocytolocalization of aquaporins

For immunolocalization of aquaporins, leaf samples were fixed in 4% paraformaldehyde with 0.5% glutaraldehyde in 0.1 M PIPES (1,4-piperazinediethanesulfonic acid) buffer, pH 7.3 at 4 °C for 4 h. After washing (PIPES buffer) and dehydration (ethanol 10–100%) the material was embedded in LR White resin (London Resin Company, UK) and polymerized for 24 h at 37 °C. Ultrathin sections (80 nm) mounted on copper grids were obtained using a Leica Ultracut UTC ultramicrotome. In preliminary studies, three different antibodies: anti-PIP1;3, anti-PIP2;3 and anti-PIP2;5 antibodies (Agrisera, Sweden) for plasma membrane intrinsic proteins (PIPs) with confirmed reactivity for monocots (Sakurai et al., 2008) were tested. For this purpose, the unspecific epitopes were blocked in 4% bovine albumin (BSA) in phosphate buffered saline (PBS, 0.01 M, pH 7.3). After washing in the washing mixture (1% BSA/PBS), samples were incubated for 1.5 h in (in PBS) with primary antibodies: anti-PIP1;3, anti-PIP2;3 and anti-PIP2;5 in the dilution: 1:100 (selected from tested dilutions: 1:200, 1:100, 1:50 and 1:30). For the negative control, the incubation with primary antibodies was omitted. After series of washings – 1% BSA (in PBS), PBS and water – material was incubated with secondary antibodies conjugated to 10 nm gold particles (goat anti-rabbit, SIGMA) for 1.5 h. After washing (water) samples were contrasted with uranyl acetate (5%) for 20 min and lead citrate (0.04%) for 30 min. The observations were performed using transmission electron microscope (model JEM 1400; JEOL Co., Japan).

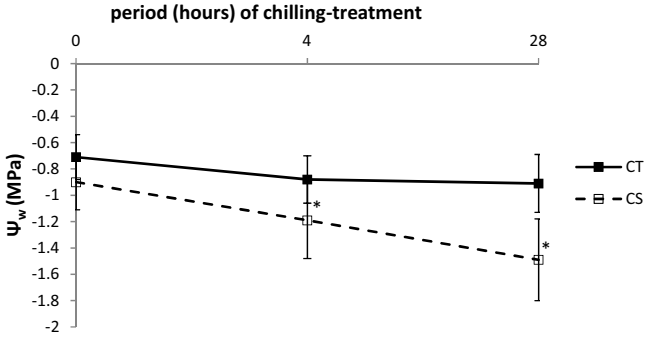

Fig. 1. Changes in the water potential (Ψ_w, in MPa) in leaves of two maize lines: chilling-tolerant (CT) and chilling-sensitive (CS) treated with low temperature for 4 and 28 h. Bars represent means ± SD values. *P < 0.01.

Table 1
Labeling density of aquaporin **PIP 2;3** in cells along the plasma membrane in control (not-chilled) and chilled for 28 h plants of chilling-tolerant (CT) and chilling-sensitive (CS) maize inbred lines. Abbreviations: 0 (0–5 gold particles); + (6–10 gold particles); ++ (11–20 gold particles); +++ (21> gold particles) along plasmalemma of the cell type. Data were collected in three independent experiments.

	CT line		CS line	
	control	cold	control	cold
Vascular parenchyma	+	0	0	0
Companion cell	0	+	0	0
Thin-walled sieve tube	0	+	+	+
Thick-walled sieve tube	+	+	+++	+

3. Results

Water potential (Ψ_w) in the leaves was found to decrease due to chilling in both lines, however major changes (almost 2-fold decrease) were observed in the leaves of CS maize (Fig. 1). Moreover, even in control plants, the CS line was characterized by lower (Ψ_w) values.

Based on the results from preliminary studies, the dilution of 1:100 for anti-PIPs antibodies was selected. Two of the antibodies – PIP1;3, (Fig. 2A–C) and PIP2;5 (Fig. 2D–F) turned out to lack any specificity in their localization in any cells of maize leaves. Gold particles for these two antibodies were found in different cell types and in almost all organelles, including cell wall (Fig. 2A–F), plastids and mitochondria (Fig. 2A–C, E) and endoplasmic reticulum (Fig. 2C, F). At the same time, the negative control, where primary antibodies were omitted, showed no labelling (Fig. 2G–I).

The PIP2;3 protein was found along plasma membrane in all studied types of cells of both maize lines: vascular parenchyma (VP), companion cells (CC), thin-walled sieve elements (SE) and thick-walled sieve elements (SET) (Fig. 3 and Table 1). In control (non-chilled) leaves of CT maize line PIP2;3 form was localized mainly in VP cells and SETs (Fig. 3A, C and Table 1). Low temperature caused slight increase of gold labelling in CC/SE complex (Fig. 3E and Table 1). At the same time, in control plants of CS maize line, SETs were hardly stained with gold particles of PIP2;3 form (Fig. 3H and Table 1). In chilled plants of this maize line a clear decrease of labeling in SETs was observed (Fig. 3J and Table 1).

4. Discussion

In this work we demonstrated that moderate chilling stress with temperature range: 12–14 °C caused clear changes in the water status of leaves of the chilling-sensitive maize line in comparison