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Cold Acclimation Can Induce Microtubular Cold Stability in a Manner Distinct from Abscisic Acid

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The response of cortical microtubules to low temperature was investigated for the Chinese winter wheat (*Triticum aestivum* L.) cultivar Jing Nong 934. Microtubules in the cortex of the root elongation zone disassembled rapidly in response to a cold shock of –7°C and reassembled upon rewarming to 25°C. The microtubules acquired resistance against this cold shock in response to cold acclimation in chilling, but non-freezing, temperature or after a treatment with abscisic acid (ABA). Cold acclimation and ABA differed with respect to the appearance of microtubules: fine, transverse strands were observed after cold acclimation, whereas ABA produced steeply oblique microtubule bundles. The findings are discussed in terms of an ABA-independent pathway for acquired cold stability of microtubules.

Key words: Abscisic acid — Cold acclimation — Immunofluorescence — Microtubules — Winter wheat (*Triticum aestivum* L.)

Abbreviations: ABA, abscisic acid; EGTA, ethylene glycol bis(2-aminoethyl)-tetraacetic acid; MSB, microtubule-stabilizing buffer; PIPES, 1,4-piperazine-diethanesulfonic acid; TBS, Tris-buffered saline.

Introduction

Low temperatures pose severe constraints upon the productivity of cultivated plants. The sensitive step seems to be cell growth delaying the unfolding of the leaves, because it is leaf area rather than photosynthetic capacity that limits the accumulation of biomass (for a review see Nick 2000). One approach to overcome this problem was the creation of wheat cultivars that can be planted in autumn and are able to survive the winter such that leaf development is more advanced with the arrival of the spring season. These cultivars are characterized by a high resistance to low temperature that develops in autumn, as the temperature gradually drops.

Plant hormones such as abscisic acid (ABA) can induce cold hardiness (Lee and Chen 1993, Sakiyama and Shibaoka 1990, Tanino et al. 1990). In parallel, many plants are able to acquire cold hardiness through cold acclimation, which is defined as a prolonged exposure to chilling, but non-freezing temperatures. This parallel between cold acclimation and ABA gave rise to the idea that cold acclimation is mediated by elevated levels of ABA. In fact, the cytological and metabolic changes in response to exogenous ABA and to cold acclimation were observed to be very similar (Mohapatra et al. 1988a, Mohapatra et al. 1988b, Tanino et al. 1990). The similarity includes the induction of specific proteins (Robertson et al. 1987), the ultrastructure of the cell (Tanino et al. 1991), and the pattern of translatable RNA species (Lee et al. 1991, Lee et al. 1992) supporting the idea that cold acclimation is brought about by elevated levels of ABA leading to the induction of specific proteins that are responsible for the increased cold hardiness (Chen et al. 1983). However, ABA can only induce a partial cold hardiness. For the development of maximum tolerance, cold acclimation is essential (Mohapatra et al. 1988a, Mohapatra et al. 1988b).

Microtubules are key candidates for the pronounced cold sensitivity of cell growth, as they support cell elongation (for review see Nick 1998) and depolymerize in response to low temperatures (Bartolo and Carter 1991a, Bartolo and Carter 1991b, Chu et al. 1992, Mizuta et al. 1994, Mizuta et al. 1995). Moreover, the cold stability of microtubules varies between plants (Burch and Marchant 1983, Hogetsu 1986) in correlation with the general cold-hardiness of the respective species (Jian et al. 1989). Therefore, microtubules have been discussed for a long time as potential targets for cold sensitivity. In fact, factors that are known to increase the general cold hardiness of a plant were found to induce an elevated cold-stability of microtubules (Sakiyama and Shibaoka 1990 for ABA; Kerr and Carter 1990a, Pihakaski-Maunsbach and Puhakainen 1995 for cold acclimation). Conversely, antimicrotubular drugs significantly accelerated and enhanced chilling injury in cotton, whereas a pretreatment with ABA could prevent chilling injury and counteracted the sensitizing effect of antimicrotubular drugs. Chilling injury was accompanied by a destruction of the microtubular network, and ABA prevented this microtubular destruction (Rikin et al. 1980, Rikin et al. 1983).

Although ABA has been extensively studied for its role in the response to environmental stresses, its role in cold acclimation is far from being understood. The present work demonstrates that cold acclimation and ABA are able to increase the cold stability of cortical microtubules in winter wheat but that they differ with respect to microtubular organization. This suggests that cold acclimation utilizes a pathway that is at least partially independent of ABA.

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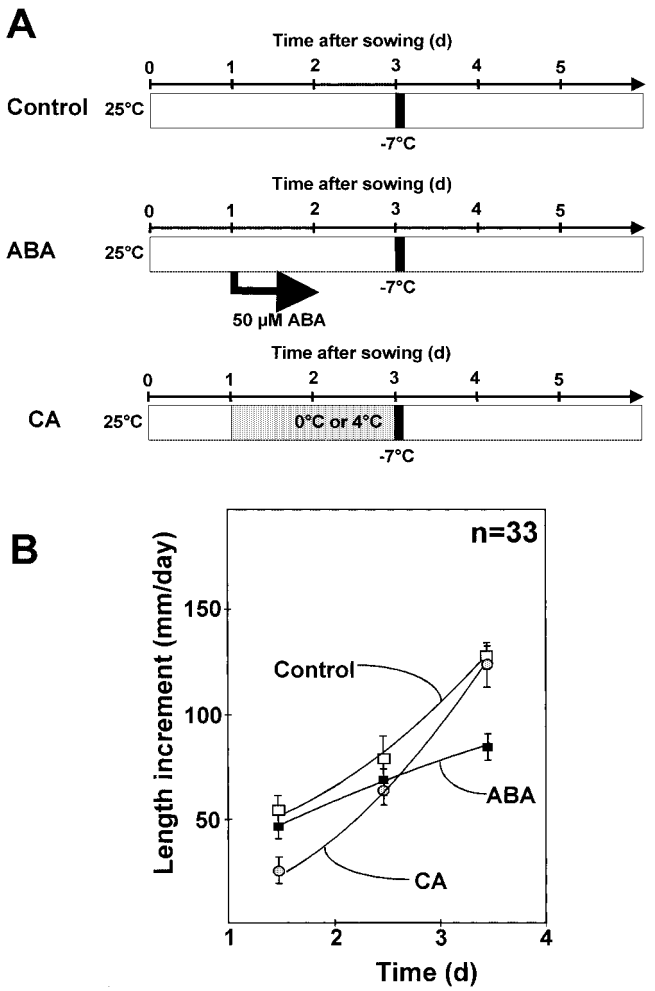

Fig. 1 Experimental protocols used in the present studies (A) and their effect on root elongation (B). ABA, treatment with 50 µM of ABA; CA, cold acclimation. The ABA-treatment and the cold-acclimation (at 0°C) treatment in B was administered for 3.5 h at day 1 after sowing.

Results

ABA and cold acclimation affect root elongation in a different manner

The effect of ABA and cold acclimation on root elongation was followed for a pulse-treatment of 3.5 h duration (Fig. 1B). In contrast to the immunofluorescence studies, where the treatment lasted for 1 d (Fig. 1A), this experiment was designed as a pulse experiment to minimize differences in overall length, since for longer treatments the control group would mature too far during the time when the cold-acclimated group would be halted during the cold treatment. ABA was observed to decrease growth rate by about a third as compared to the untreated controls. Interestingly, the onset of root elongation (taking place between days 1 and 2 after sowing) was not affected by this treatment. In contrast, a short cold acclimation at 0°C lasting for only 3.5 h delayed root elongation considerably by around half a day. Once the roots had initiated elongation, they rapidly recovered the same elongation rates as the controls.

Cortical microtubules depolymerize in response to cold shock and repolymerize upon rewarming

Prior to the cold shock, the control group (Fig. 1A, upper diagram), predominantly had slightly oblique, dense microtubules in subepidermal cells throughout the elongation zone that had been determined in preliminary experiments to be situated between 3 and 11 mm from the tip (Fig. 2A–C). A cold shock (–7°C) caused progressive microtubule disassembly in the majority of subepidermal cells (Fig. 2G), ranging from microtubular fragments (Fig. 2D, E) to the complete elimination of microtubules (Fig. 2F, G). This disassembly is reversible. When the plants are returned to 25°C, microtubules rapidly repolymerized within 90 min (Fig. 2I, J) restoring the same orientation as prior to the cold shock (Fig. 2K). Microtubule frequency, defined as number of microtubules intersected by a line of 100 µm, decreases dramatically in response to the cold shock, but recovers almost to the control level after 90 min of rewarming (Fig. 2H).

ABA can induce cold stability and longitudinal reorientation of microtubules

ABA causes a reorientation of microtubules towards steeper arrays (Fig. 3A, B, F). In addition, the frequency of microtubules is somewhat reduced as compared to the control (Fig. 3G). The pretreatment with ABA produced a conspicuous stabilization against a cold shock, since even after 2 h at 7°C the majority of cells maintained intact microtubule arrays (Fig. 3C–E). In contrast to the sharp decrease of observed microtubules in control roots after the cold shock, microtubule frequency was maintained in the roots pretreated with ABA (Fig. 3G).

Cold acclimation can induce cold stability and transverse reorientation of microtubules

Cold acclimation can induce a higher resistance of microtubules against a cold shock (Fig. 4A–E, gray bars). The stabilizing effect is more pronounced when the acclimation takes place at 4°C (Fig. 4E), whereas acclimation at 0°C was less effective (Fig. 4E, black bars). In both cases, however, the microtubule frequency prior to the cold shock is higher as compared to the control and much higher as compared to cells pretreated with ABA (Fig. 3G). In addition, the microtubule arrays that were found in response to cold acclimation were oriented more transversely as compared to the controls (Fig. 4F). In contrast, the microtubule arrays observed after treatment with ABA were oriented more longitudinally than the controls (Fig. 4F).

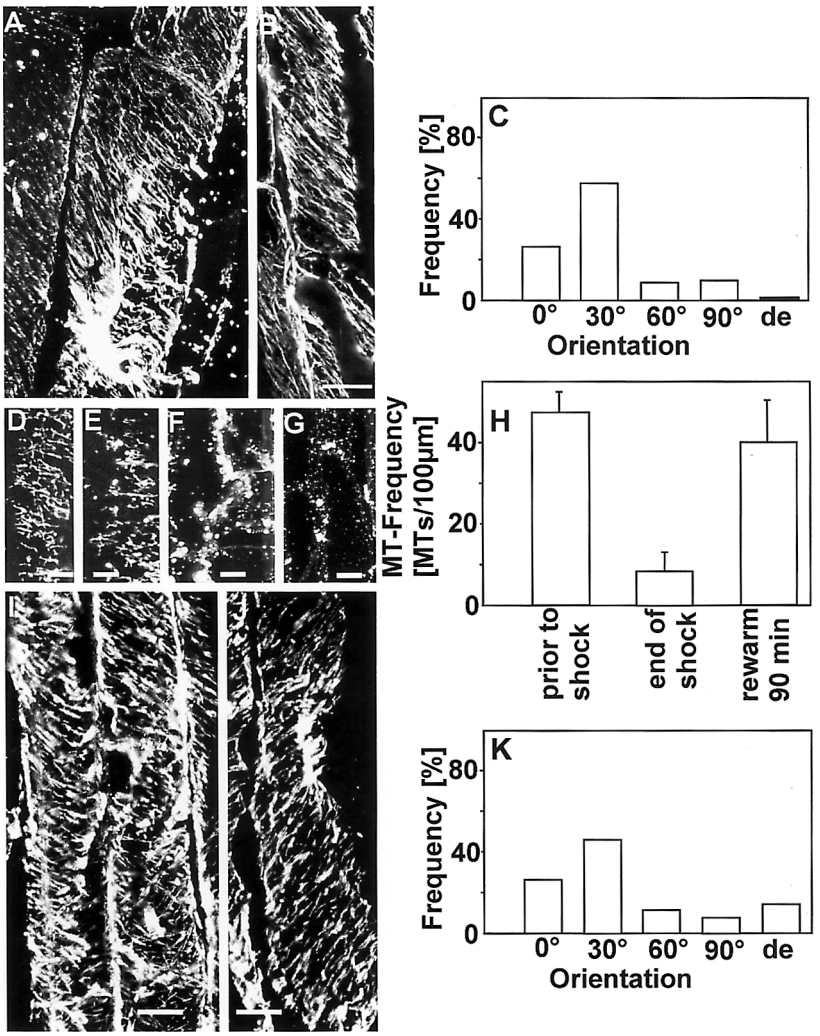

Fig. 2 Reversible depolymerization of microtubules in response to cold shock in control seedlings. (A–C) Microtubule organization at 25°C prior to the cold shock. (D–H) Effect of cold shock (–7°C, 2 h). (I–K) Recovery from the cold shock after returning the plants for 90 min to 25°C. Immunofluorescence images of cortical cells in the root-elongation zone are shown in (A–B), (D–G), and (I–J), respectively. Frequency distributions of microtubule orientation are shown in C and K, the effect of cold shock and recovery on microtubule frequency in H. The plots typically are constructed from 180–250 individual cells from 20–30 individual roots. De cells with disassembled microtubules. The scale corresponds to 15 µm.

Discussion

The present study was motivated by the question: is the cold resistance of microtubules increased by treatments that are known to increase general cold hardiness i.e. ABA and cold acclimation at chilling, but non-freezing temperatures? Winter wheat was chosen for its pronounced capacity for cold acclimation. The severity of the cold shock (–7°C for 2 h) was adjusted such that it caused depolymerization of microtubules in the majority of cells (Fig. 2H), but did not cause irreversible damage. When the plants were returned to 25°C after this cold shock, microtubules reassembled rapidly (Fig. 2I–K) demonstrating that the viability of the cells was not affected by the cold shock.

Preliminary studies, in various winter wheat cultivars, on the growth pattern of different root zones in response to cold revealed that the zone 3–11 mm from the root tip was the center of growth activity and most sensitive to low temperature with respect to growth and microtubule disassembly. The longitudinal microtubules in the cells proximal of this zone were found to be essentially unaffected by a cold shock of 2 h duration as well as the mitotic spindles in meristem. This is consistent with published results on maize roots, where cold-induced microtubule disassembly was observed to depend on position and cell type with the elongation zone exhibiting the most pronounced effect for short-term exposure (Baluška et al. 1993).

Although different tissue layers were observed during this study, the attention was focussed on the subepidermal cells of

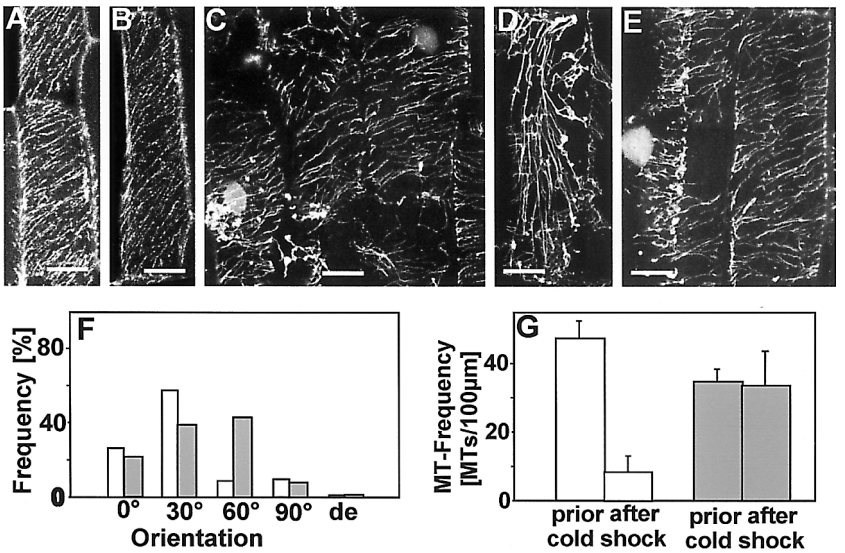

Fig. 3 Stabilization of microtubules against cold shock by pretreatment with ABA. (A, B, F) Microtubule organization at 25°C prior to the cold shock. (C–E, G) and at the end of the cold shock. Representative immunofluorescence images are shown in (A–E). The frequency distribution of microtubule orientation prior to the cold shock is shown in F for the control treatment (white bars) and the ABA treatment (bars in dark gray), microtubule frequency prior and after the cold shock in G. For further details refer to the legend of Fig. 2. The scale corresponds to 15 µm.

the outer cortex, because these cells responded most sensitively to the cold shock. Moreover, this cell layer is most susceptible to stimuli that regulate root growth, such as gravity (Blancaflor and Hasenstein 1993) or auxin (Baluška et al. 1996). It seems that it is the response of this tissue layer that limits root growth in low temperature.

The cold resistance of microtubules was conspicuously increased by a pretreatment with ABA (Fig. 3), a well-known inducer of cold hardiness (Lee and Chen 1993, Sakiyama and Shibaoka 1990, Tanino et al. 1990). For these cold-stable microtubules, longitudinal and steeply oblique arrays were predominant as compared to a more transverse orientation in control plants (Fig. 3F). This microtubule reorientation is presumably responsible for the reduced rate of root elongation observed after treatment with ABA (Fig. 1B). A similar change towards more longitudinal orientations along with an increased cold resistance has been reported for dwarf pea epicotyls in response to a treatment with ABA (Sakiyama and Shibaoka 1990, Sakiyama-Sogo and Shibaoka 1993, Shibaoka 1994). The reduced microtubule frequency observed in the ABA-treated roots might be connected to the downregulation of tubulin genes that has been observed in other Graminea (Gianí et al. 1998).

In an attempt to mimic the natural situation during late autumn, the plants were subjected to a cold-acclimation protocol (Fig. 1A), and cold acclimation was found to be effective in increasing the cold stability of microtubules (Fig. 4) with 4°C being more effective than 0°C (Fig. 4E). In this case, microtubules were oriented into transverse arrays (Fig. 4A–D) that were able to support root elongation. Consistently, although cold acclimation causes a delay of root elongation, a high rate of growth is recovered as soon as the temperature returns to 25°C (Fig. 1B). These findings concur with previous studies, in which cold acclimation of rye roots rendered microtubules resistant to a cold shock of –10°C that completely eliminated microtubules in non-acclimated control roots (Pihakaski-Maunsbach and Puhakainen 1995).

The ability of ABA and cold acclimation to induce microtubular cold stability is consistent with the idea that cold acclimation is brought about by elevated levels of ABA (Chen et al. 1983). However, the response of microtubules to these two factors differs. Upon pretreatment with ABA, the frequency of microtubules is lower than in the controls (Fig. 3G), and they are organized into steeply oblique or even longitudinal arrays (Fig. 3A, B, F). In contrast, cold acclimation produces dense, transverse arrays of microtubules (Fig. 4A–D, F). Therefore at least some events of the microtubular response to cold acclimation cannot be mimicked by ABA. This finding supports previous reports on the regulation of cold-induced genes by ABA-independent pathways (Mäntylä et al. 1995, Medina et al. 1999).

The molecular basis of microtubular cold resistance has remained enigmatic so far but seems to involve the differential induction of tubulin isotypes during cold acclimation (rye roots: Kerr and Carter 1990b; Arabidopsis: Chu et al. 1993). It seems that the cold-induced disassembly of microtubules is related to the carboxy terminus of tubulin, since TUB9 that is upregulated during cold acclimation in Arabidopsis, is characterized by a short carboxy terminus (Chu et al. 1993). Consistently, the highly cold-sensitive microtubules of the maize Black Mexican Sweet cell culture can be rendered cold stable by pro-

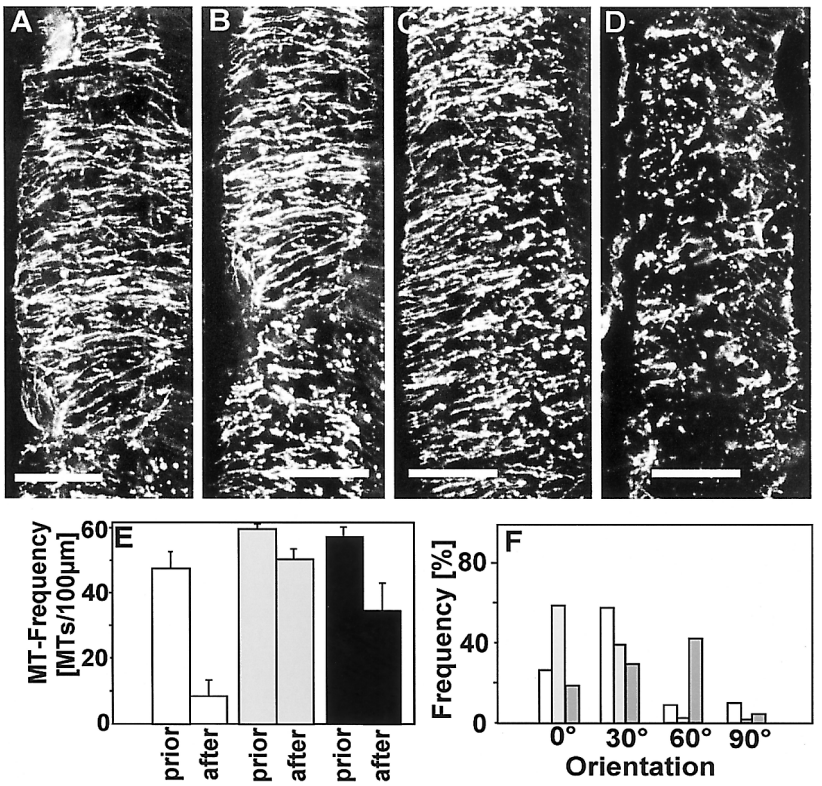

Fig. 4 Stabilization of microtubules against cold shock by cold acclimation. Representative immunofluorescence images are shown in (A–D) for an acclimation temperature of 0°C followed by a cold shock (–7°C, 2 h). Microtubule frequenies prior and after the cold shock are shown in E for the control treatment (white bars), cold acclimation at 4°C (bars in light gray) and cold acclimation at 0°C (black bars). The frequency distribution of microtubule orientation prior to the cold shock is shown in F for the control treatment (white bars), a cold acclimation at 4°C (bars in light gray), and ABA treatment (bars in dark gray). For further details refer to the legend of Fig. 2. Scale 15 µm.

teolytic cleavage of the carboxy terminus (Bokros et al. 1996). This indicates that the carboxy terminus of certain tubulin isotypes might be the target for cold-induced signaling. Therefore future work will be dedicated to how the pattern of tubulin isotypes responds to ABA and cold acclimation.

Material and Methods

Plant material

Caryopses of winter wheat (*Triticum aestivum* L. cv. Jing Nong 934; Chinese Academy of Agriculture, Beijing, China) were soaked in water for 2 h at room temperature, and then sown equidistantly, embryo up, on moistened double-layered filter paper in plexiglass boxes (95×95×60 mm, 20 seeds for each box). In order to obtain well-grown roots, cultivation took place in complete darkness. The plexiglass boxes were placed in light-tight black boxes covered with black cloth and kept in a dark room at 25°C for 1–6 d until the seedlings were used for the experiments.

ABA and cold treatments

All treatments took place in the dark. The seedlings were subjected to three treatments. The control group was cultivated at 25°C throughout the experiment (Fig. 1A, upper diagram) and then challenged at day 3 by a cold shock at –7°C (±1°C) lasting for 2 h. In preliminary experiments, where a range of winter wheat cultivars, temperatures and time intervals had been tested, this treatment had been found to disassemble microtubules in the majority of root cells without causing cell death. For the ABA-treatment, the seedlings were cultivated from day 1 after sowing on filter paper soaked with 50 µM ABA (Fluka, Neu-Ulm; Germany). To avoid the induction of the plant photoreceptor phytochrome, the hormone was added under dim green safelight (λ_{max} 550 nm, 20 mW m^{-2}). Again, this group was challenged by the same cold shock as in the controls (Fig. 1A, central diagram). For cold acclimation, the seedlings (again at day 1 after sowing) were cultivated in complete darkness at 4°C (±1°C) and again challenged by the same cold shock (Fig. 1A, lower diagram), in some experiments the cold acclimation was performed at 0°C (±1°C). For cold acclimation and the cold shock, a temperature-controlled incubation chamber (Fryka Kältetechnik, Esslingen, Germany) was utilized. The immunofluorescence data were obtained for a pretreatment (cold shock or ABA) lasting for 1 d (Fig. 1A), for the growth data, the pretreatment was shorter (3.5 h) to minimize differences in the starting point of root length (since the roots will not elongate in the acclimation experiment, in contrast to the ABA-treated roots).

Visualization of cortical microtubules by immunofluorescence

The analysis focussed on segments of 8 mm length (from 3 to 11 mm from the root tip) comprising the elongation zone. Preliminary measurements, where ink marks had been placed at different positions along the root, had shown that this zone was the center of growth activity and most sensitive to the effect of low temperature, consistent

with published results on maize roots (Baluška et al. 1993). Moreover, microtubules were found to progressively deviate towards more longitudinal orientations if the distance was more than 11 mm from the tip. For distances less than 3 mm from the tip, many cells had not yet developed the typical cortical array, but displayed other microtubule structures such as endoplasmic or perinuclear microtubules or mitotic spindles. The zone from 3 to 11 mm from the tip was therefore considered to represent the elongation zone. Although several tissue layers were examined, the study focussed on the subepidermal cells of the outer cortex layer, where the microtubule response to signals that control root growth is most sensitive (see for instance Blancaflor and Hasenstein 1993 for gravity; Baluška et al. 1996 for auxin) and where in our hands the response to cold was observed to be most sensitive.

The segments were fixed by 3.7% w/v paraformaldehyde in microtubule stabilizing buffer (MSB: 50 mM PIPES, 5 mM EGTA, 1 mM $MgSO_4$, 1% v/v glycerol, 0.25% v/v Triton-X100, pH 6.9) for 1 h at the respective temperature depending on the treatment prior to fixation.

After fixation, tangential sections were obtained using a vibratome as described in Waller and Nick (1997). The sections were collected and placed on a glass slide in a drop of 1.2% w/v agar dissolved in MSB and then incubated with 5% v/v normal goat serum (Sigma, Neu-Ulm, Germany) in Tris-buffered saline (TBS: 150 mM NaCl, 20 mM Tris-HCl, 0.25% v/v Triton-X100, pH 7.4) for 20 min to block non-specific interactions. After incubation for 1 h at 37°C or overnight at 4°C with mouse monoclonal antibodies directed against α- and β-tubulin (Amersham, Braunschweig, Germany, dilution 1 : 100 in TBS), the sections were washed 3 times for 5 min with TBS, and reincubated for 1 h at 37°C or overnight at 4°C with a fluorescein-isothiocyanate-conjugated secondary antibody (anti-mouse immunglobulin G from goat; Sigma, Deisenhofen, Germany) diluted 1 : 20 in TBS. Sections were thoroughly washed five times for 5 min in TBS and then mounted in anti-fade medium (Moviol containing 0.1% *p*-phenylenediamine). Slides were analyzed by confocal microscopy.

Confocal microscopy and quantification of microtubular responses

Microtubule images were obtained by confocal laser scanning microscopy (DM RBE; Leica, Bensheim, Germany) using the 488-nm line of an argon-krypton laser for excitation, a beam splitter at 510 nm and a bandpass filter at 515 nm, and a line algorithm averaging eight individual scans. Each cell layer was optically sectioned into eight slices and these individual sections were then projected into one stack. The microtubules adjacent to the outer cell wall were classified into five classes according to their deviation from a transverse orientation: strictly transverse (0°), slightly oblique (30°), steeply oblique (60°), longitudinal (90°), and partially or completely destroyed (de). Frequency distributions were constructed representing the data of around 100–200 cells corresponding to 30–50 individual roots from at least three independent experimental series. To quantify microtubule frequency, images were processed using the Scion Image software (Scion Corporation, Frederick, MD, U.S.A.). After transformation into binary images to eliminate differences in overall intensity, the images were filtered using the Find Edge algorithm. The result was an image, where a profile across each microtubule yielded the same integrated density irrespective of the thickness of the microtubule or its original fluorescence intensity. Under these conditions, it was possible to obtain a linear function between integrated density along a line intersecting the microtubule array perpendicular to the orientation of individual microtubules and the number of microtubules intersected by this line. This function was used for calibration of the sample data. To obtain the sample data, a lattice of five equally spaced, parallel lines of eight pixels thickness was laid over each individual cell such that the lines were oriented perpendicular to the microtubule array and did not touch the cell wall. The integrated density along each line was then determined with the Analyze algorithm, averaged for each cell and corrected for background measurements obtained from the same image. Microtubule frequency (defined as number of microtubules that are intersected by a line of 100 μm length) was calculated from these measured values for integrated density by means of the calibration function. Each value for microtubule frequency represents the average from 180–250 individual cells from 20–30 individual roots.

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References

Baluška, F., Barlow, P.W. and Volkmann, D. (1996) Complete disintegration of the microtubular cytoskeleton precedes its auxin-mediated reconstruction in postmitotic maize root cells. *Plant Cell Physiol.* 37: 1013–1021.

Baluška, F., Parker, J.S. and Barlow, P.W. (1993) The microtubular cytoskeleton in cells of cold-treated roots of maize (*Zea mays* L.) shows tissue-specific responses. *Protoplasma* 172: 84–96.

Bartolo, M.E. and Carter, J.V. (1991a) Microtubules in mesophyll cell of nonacclimated and cold-acclimated spinach. *Plant Physiol.* 97: 175–181.

Bartolo, M.E. and Carter, J.V. (1991b) Effect of microtubule stabilization on the freezing tolerance of mesophyll cells of spinach. *Plant Physiol.* 97: 182–187.

Blancaflor, E.B. and Hasenstein, K.H. (1993) Organization of cortical microtubules in gravi-responding maize roots. *Planta* 191: 231–237.

Bokros, C.L., Hugdahl, J.D., Blumenthal, S.S.D. and Morejohn, L.C. (1996) Proteolytic analysis of polymerized maize tubulin: regulation of microtubule stability to low temperature and Ca^{2+} by the carboxyl terminus of β-tubulin. *Plant Cell Environ.* 19: 539–548.

Burch, M.D. and Marchant, J.H. (1983) Motility and microtubule stability of antarctic algae at sub-zero temperatures. *Protoplasma* 115: 240–242.

Chen, H.H., Li, P.H. and Brenner, M.L. (1983) Involvement of abscisic acid in potato cold acclimation. *Plant Physiol.* 71: 362–365.

Chu, B., Snustad, D.P. and Carter, J.V. (1993) Alteration of beta-tubulin gene expression during low-temperature exposure in leaves of *Arabidopsis thaliana*. *Plant Physiol.* 103: 371–377.

Chu, B., Xin, Z., Li, H. and Carter, J.V. (1992) Depolymerization of cortical microtubules is not a primary cause of chilling injury in corn (*Zea mays* L. cv Black Mexican Sweet) suspension culture cells. *Plant Cell Environ.* 15: 307–312.

Gianí, S., Qin, X., Faoro, F. and Breviario, D. (1998) In rice, oryzalin and abscisic acid differentially affect tubulin mRNA and protein levels. *Planta* 205: 334–341.

Hogetsu, T. (1986) Re-formation of microtubules in Closterium ehrenbergii after cold-induced depolymerization. *Planta* 167: 437–443.

Jian, L.C., Sun, L.H. and Liu, Z.P. (1989) Studies on microtubule cold stability in relation to plant cold hardiness. *Acta Bot. Sinica* 31: 737–741.

Kerr, G.P. and Carter, J.V. (1990a) Relationship between freezing tolerance of root-tip cells and cold stability of microtubules in rye (*Secale cereale* L. cv Puma). *Plant Physiol.* 93: 77–82.

Kerr, G.P. and Carter, J.V. (1990b) Tubulin isotypes in rye roots are altered during cold acclimation. *Plant Physiol.* 93: 83–88.

Lee, S.P. and Chen, T.H.H. (1993) Molecular cloning of abscisic acid-responsive mRNAs expressed during the induction of freezing tolerance in bromegrass (*Bromus inermis* Leyss) suspension culture. *Plant Physiol.* 101: 1089–1096.

Lee, S.P., Chen, T.H.H., and Fuchigami, L.H. (1991) Changes in the translateable RNA population during abscisic acid induced freezing tolerance in bromegrass suspension culture. *Plant Cell Physiol.* 32: 45–56.

Lee, S.P., Zhu, B., Chen, T.H.H. and Li, P.H. (1992) Induction of freezing tolerance in potato (*Solanum commersonii*) suspension cultured cells. *Physiol. Plant.* 84: 41–48.

Mäntylä, E., Lång, V. and Palva, E.T. (1995) Role of abscisic acid in drought-induced freezing tolerance, cold acclimation, and accumulation of LTI78 and

RAB18 proteins in *Arabidopsis thaliana*. *Plant Physiol.* 107: 141–148.

Medina, J., Bargues, M., Terol, J., Pérez-Alonso, M. and Salinas, J. (1999) The Arabidopsis *CBF* gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but not by abscisic acid or dehydration. *Plant Physiol.* 119: 463–469.

Mizuta, S., Kaneko, M., Kimura, S. and Okuda, K. (1994) Experimental studies on the stability of the cortical microtubule cytoskeleton in relation to polarity and cell elongation in the coenocytic green alga, *Chaetomorpha moniligera. Ann. Bot.* 73: 273–280.

Mizuta, S., Kaneko, M. and Tsurumi, S. (1995) Assembly of cortical microtubules during cold treatment of the coenocytic green alga, *Chaetomorpha moniligera*. *Planta* 196: 190–192.

Mohapatra, S.S., Poole, R.J. and Dhindsa, R.S. (1988a) Detection of two membrane polypeptides induced by abscisic acid and cold acclimation: possible role in freezing tolerance. *Plant Cell Physiol.* 29: 727–730.

Mohapatra, S.S., Poole, R.J. and Dhindsa, R.S. (1988b) Abscisic acid-regulated gene expression in relation to freezing tolerance in alfalfa. *Plant Physiol.* 87: 468–473.

Nick, P. (1998) Signaling to the microtubular cytoskeleton in plants. *Int. Rev. Cytol.* 184: 33–71.

Nick, P. (2000) Control of the response to low temperatures. *In* Plant Microtubules. Edited by Nick, P. pp. 121–135. Springer, Berlin, Heidelberg.

Pihakaski-Maunsbach, K. and Puhakainen, T. (1995) Effect of cold exposure on cortical microtubules of rye (*Secale cereale*) as observed by immunocytochemistry. *Physiol. Plant.* 93: 563–571.

Rikin, A., Atsmon, D. and Gitler, C. (1980) Chilling injury in cotton (*Gossypium hirsutum* L.): Effect of antimicrotubular drugs. *Plant Cell Physiol.* 21: 829–837.

Rikin, A., Atsmon, D. and Gitler, C. (1983) Quantitation of chill-induced release of a tubulin-like factor and its prevention by abscisic acid in *Gossypium hirsutum* L. *Plant Physiol.* 71: 747–748.

Robertson, A.J., Gusta, L.V., Reaney, M.J.T. and Ishikawa, M. (1987) Protein synthesis in bromegrass (*Bromus inermis* Leyss.) cultured cells during the induction of frost tolerance by abscisic acid or low temperature. *Plant Physiol.* 84: 1331–1336.

Sakiyama, M. and Shibaoka, H. (1990) Effects of abscisic acid on the orientation and cold stability of cortical microtubules in epicotyl cells of the dwarf pea. *Protoplasma* 157: 165–171.

Sakiyama-Sogo, M. and Shibaoka, H. (1993) Gibberellin A_3 and abscisic acid cause the reorientation of cortical microtubules in epicotyl cells of decapitated dwarf pea. *Plant Cell Physiol.* 34: 431–437.

Shibaoka, H. (1994) Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 45: 527–544.

Tanino, K.K., Chen, T.H.H., Fuchigami, L.H. and Weiser, C.J. (1990) Metabolic alterations associated with abscisic acid-induced frost hardiness in bromegrass suspension culture cells. *Plant Cell Physiol.* 31: 505–511.

Tanino, K.K., Chen, T.H.H., Fuchigami, L.H. and Weiser, C.J. (1991) Abscisic acid-induced cellular alterations during the induction of freezing tolerance in bromegrass cells. *J. Plant Physiol.* 137: 619–624.

Waller, F. and Nick, P. (1997) Response of actin microfilaments during phytochrome-controlled growth of maize seedlings. *Protoplasma* 200: 154–162.

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