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Water Deficit in Pea Root Tips: Effects on the Cell Cycle and on the Production of Dehydrin-like Proteins

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Dehydrin-like proteins have been detected in nuclei and cytoplasm of meristematic root tip cells from pea seedlings subjected to slow dehydration at 90% relative humidity for 48 h or more. Evidence was gained from Western blotting and immunocytochemical experiments using an antibody raised against the conserved domain of dehydrin proteins. Flow cytometer analysis has shown that cycling cells of root tip meristems from dehydrated seedlings are mostly arrested in G2 phase. Other stress treatments thought to involve water depletion (osmotic stress, cold treatment) or to modulate cell response to water deficit (abscisic acid) gave less clear-cut results with all treatments lowering the proportion of cells entering the S phase, but without a definite and persistent arrest in any preferential phase of the cycle. Possible interrelationships between G2 arrest and dehydrin production are discussed.

Key words: Cell cycle, dehydrins, flow cytometry, nuclei, pea, *Pisum sativum* L., water stress.

INTRODUCTION

Water deficit and related stresses (increased solutes, low temperature) which are first perceived as plasmalemma perturbations caused by loss in turgor pressure, start a signal transduction pathway that induces the expression of specific genes and leads, eventually, to changes at the cellular, physiological and developmental levels. Abscisic acid (ABA) has been reported to be involved in this stress-perception-response pathway in most cases and its administration induces reactions which mimic, at least in part, those induced by stresses involving water depletion. While a general decrease in bulk protein synthesis usually occurs under conditions of water deficiency, some gene products that are present at a basal level in vegetative tissues increase abruptly. Among these water stress-induced proteins, the family of dehydrins (Close, Kortt and Chandler, 1989), also named the Lea D-11 family (Dure, 1993) and Rab genes (Vilardell *et al*., 1990; Pla, Gomez and Pagès, 1991) have been described in a variety of monocot and dicot species; they are characterized by a highly conserved consensus sequence, probably essential to their function, near the carboxy terminus of their molecule, whose core KIKEKLPG is not present in any other proteins. All dehydrins are highly hydrophilic, randomly coiled throughout most of their length and heat stable, i.e. they remain soluble after boiling. This makes their separation from bulk proteins of a tissue easier. Dehydrin genes are usually expressed during late embryogenesis and peak towards the end of seed maturation; from this material several dehydrin genes have been isolated (Close *et al*., 1989; Galau and Close, 1992; Robertson and Chandler, 1992). However, dehydrins can be induced by water deficit or, in many cases, by ABA treatment in vegetative tissues of seedlings and plants (Hong, Barg and Ho, 1992; Close, Fenton and Moonan, 1993*a*; Moons *et al*., 1995). The high conservation of dehydrin sequences across distantly related species, and their induction by dehydration suggest that they play a key role in preventing cell damage during water stress. Their role is probably a structural one: it has been proposed that, like sucrose and other sugars which also increase in tissues subjected to water stress, they can hydrogen bond through their polar groups to macromolecules and to polar head groups of membranes replacing the hydration shell and providing the hydrophilic interactions necessary for stability in the dry state (Blackman *et al*., 1991). As for their subcellular localization, dehydrins were first described as being mainly present in the cytosol (Mundy and Chua 1988), in the cytoplasm and chloroplasts (Schneider *et al*., 1993) or in soluble fractions (Neven *et al*., 1993). More recently dehydrin related proteins have also been found in nuclei. In maize caryopses imbibed in ABA, nuclear dehydrin-like proteins have been described in the scutellar parenchyma and in the inner embryonic leaves by Asghar *et al*. (1994) and Goday *et al*. (1994). The latter report referred in particular to Rab 17 protein, which is the main dehydrin in maize. By means of immunogold electron microscopy, the dehydrin TAS 14, expressed in salt-stressed tomato plants has been localized in nuclei and cytoplasm of provascular and vascular tissues of stems and leaves (Godoy

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et al., 1994). Moreover, a 65 kDa protein which accumulates gradually during water stress has been found in nuclei of stressed tomato leaves (Tabaeizadeh *et al.*, 1995). A defined role for dehydrin-like proteins in the nuclear compartment can only be hypothesized, but a protective role for nuclear proteins against denaturation and for nuclear membranes, similar to the one possibly exerted in the cytoplasm, is easily predictable. In addition, depletion of water and increase in ionic strength can be deleterious to DNA topological organization and stability, thus stabilization and protection of this macromolecule is more likely to occur. The Rab 17 protein has also been proposed to play a role in nuclear protein transport (Goday *et al.*, 1994).

To our knowledge there have been no reports of nuclear dehydrin-like proteins, re-induced by dehydration, in meristematic tissues of root tips of germinated seedlings. Roots represent the part of the plant most vulnerable to water stress and the radicle tip is the first tissue killed by desiccation. The purpose of the present work was to verify, by means of an antibody raised against the conserved dehydrin core, whether, in conditions of slow drying, even meristematic root cells can be induced to express dehydrin-like proteins and if this production is linked to their survival. We have also investigated whether cycling cells of root tips are arrested in a particular phase of the cell cycle by water deficit. There are few reports on the response of the cell cycle to stress. Stress or ABA treatment have different effects in different materials. A general slowdown of the cycle in stress conditions has usually been reported (Robertson *et al.*, 1990), the most sensitive phases being, according to different papers, the S phase (Rost, 1977; Barlow and Pilet, 1984; Bouvier-Durand, Real and Côme, 1989), or the M phase (Myers *et al.*, 1990; Artlip, Madison and Setter, 1995).

MATERIALS AND METHODS

Plant material and seedling treatment

Pea (*Pisum sativum* L. cv. Lincoln) seeds were surface sterilized in 10% NaClO and germinated in moistened agriperlite at 25 °C in the dark. All treatments started when seedlings were 72 h old. Seedlings were either (*a*) transferred to glass desiccators above a solution of 25% glycerol (approx. 90% relative humidity); or, alternatively (*b*) transferred to moistened filter paper and left at 2 °C (cold treatment); transferred to filter paper saturated with (*c*) 100 μM abscisic acid or (*d*) 0·5 M mannitol. For these treatments, concentrations and temperature were chosen to give consistently reduced root elongation and to ensure full recovery of normal growth on removal of the stress condition. Solutions of ABA and mannitol were renewed daily. All treatments lasted 48 h; in some cases seedlings were withdrawn after 24 or 72 h of treatment. All inductions and subsequent analyses were performed at least three times. Control seedlings were grown in agriperlite for 72 or 120 h. No differences were noticed between seedlings of these two age classes either in dehydrin content or cell cycle course, therefore 72 h seedlings were generally used as controls.

Determination of relative water content

Twenty seedlings grown in agriperlite for 72 h were weighed at the beginning of the dehydration experiment and again after various periods of dehydration, either on the laboratory bench at room temperature and humidity (about 50% RH), or at 90% RH. To determine water loss by the apical part of the seedlings, 100 apices (2 mm in length) were excised from 72-h-old seedlings and weighed immediately. This was repeated several times to determine the mean fresh weight of hydrated apices. After different periods of dehydration of whole seedlings, apices were cut and immediately weighed, and the relative water content was calculated as the difference between the initial weight of hydrated apices and the actual weight of the same number of apices after dehydration.

Isolation of nuclei

Root tips (2 mm in length) were collected from control and treated seedlings, fixed for 20 min in 4% formaldehyde in Tris Buffer (10 mM Tris-HCl, pH 7·5; 10 mM EDTA; 100 mM NaCl) and washed in the same buffer for an additional 20 min. Root tips were then chopped with a glass rod for the extraction of nuclei (Levi *et al.*, 1986), filtered through 60 and 15 μm pore size nylon filters and either stained with DAPI (4′,6-diamidino-2-phenylindole), 2 μg ml^{-1}, and analysed by flow cytometry (see below) or spread on slides for immunocytochemical treatments.

For SDS-PAGE and immunoblotting analysis, the suspension with nuclei extracted as described above was then loaded on a discontinuous Percoll gradient as previously described (Dicorato *et al.*, 1995). After checking for nuclei purity by UV fluorescence microscopy after staining nuclei with the DNA specific fluorochrome DAPI, resuspended nuclei were sonicated and boiled for 10 min, left on ice for 15 min, and centrifuged at 8000 *g*. Four volumes of acetone were added overnight to the supernatant, containing the heat-stable proteins. After centrifugation the pellet was dissolved in sample buffer: 60 mM TRIS-HCl, pH 6·8, 4% (w/v) SDS, 5% (w/v) DTT (dithiothreitol); 10% (w/v) sucrose, 0.002% bromophenol blue, boiled for 3 min and run in SDS gels.

Preparation of the antiserum and IgG purification

A 16-amino-acid synthetic peptide EKKGIMD-KIKEKLPGQ containing the conserved consensus sequence (underlined) and flanked by amino acids characteristic of pea dehydrins 1 and 2 (Close *et al.*, 1993*b*) was constructed, coupled to BSA and used to immunize New Zealand rabbits by means of three identical boosters of about 400 μg of the synthetic peptide suspended in 2 ml of 67% Freund's complete adjuvant at 3-week intervals. Preimmune serum was collected from the rabbits before immunization. IgGs were then affinity purified using a Sepharose CNBr column (Pharmacia, Milan, Italy), according to the manufacturer's instructions.

Gel electrophoresis and immunoblot analysis

Equal quantities of protein were loaded and fractionated by discontinuous SDS-PAGE (Harlow and Lane, 1988) performed in separating gels containing 12% acrylamide (w/v) using Mini-Protean II electrophoresis cells (BioRad Laboratories). Gels were then stained with 0·1% Coomassie Brilliant Blue R 250. For immunoblot analysis, proteins were transferred electrophoretically from gels to nitrocellulose in 25 mM Tris-HCl, 192 mM glycine, 20% v/v methanol, using Mini-Transblot cells (BioRad Laboratories). Nitrocellulose filters were incubated with rabbit primary antibody diluted 1:2000, followed by goat anti-rabbit alkaline phosphatase conjugated IgG 1:9000. The secondary antibody was detected using 4-nitroblue tetrazolium chloride. Negative controls were nitrocellulose membranes reacted with preimmune serum 1:2000 and treated as above. The addition of the synthetic peptide to the primary antibody strongly reduced the signal.

Preparation of tissue for light microscopy

Root tips of control and dehydrated seedlings were fixed in 3% formaldehyde in 0·1 M phosphate buffer, pH 7, for 2 h, washed, dehydrated and embedded in LR White resin (Polysciences, Warrington, PA, U.S.A.). One μm thick sections were cut with a Reichert Jung ultramicrotome and mounted on glass slides.

Immunocytochemical analysis

Nuclei from root tips obtained as previously described were spread on glass slides and left to dry in the air. Slides with spread nuclei or with sectioned root tips were then treated with primary anti-dehydrin antibody, diluted 1:100 for 2 h at room temperature, followed by goat anti-rabbit biotinylated antibody (Boehringer, Mannheim) 1:200 for 1 h and finally by Texas Red conjugated streptavidin 1:400 for 15 min. Slides were finally counterstained with DAPI and examined and photographed with a Zeiss Axioplan fluorescence microscope using excitation filter LP 400 for DAPI, and excitation filter BP 530–585, FT 600 and LP 610 for Texas Red. Negative controls were nuclei and sections treated with 1:100 preimmune serum.

Flow cytometry analysis

Root tip nuclei from controls and differently treated seedlings were obtained as previously described and diluted to about 200000 nuclei ml^{-1}. DAPI was added to a final concentration of 2 μg ml^{-1} and nuclei were analysed for fluorescence with a Partec PAS II flow cytometer employing a 100 W mercury lamp, excitation filter UG1 2 mm, dichroic mirror TK 420 and barrier filter GG 435. About 20000 nuclei were scored for each sample.

Determination of mitotic index

Root tips collected after 0, 4, 9, 15, 30, 48, 72 h from the onset of the various treatments were fixed in ethanol-acetic acid (3:1), stained by the Feulgen reaction, squashed and scored for mitoses. Ten squashed tips and 3000 nuclei for each were counted. Root tips of seedlings transferred to water for the same period were scored as the control.

RESULTS

Immunodetection of dehydrin-like proteins in nuclei from slowly dehydrated root seedlings

When 72-h-old seedlings were exposed to air at about 50% relative humidity (RH) and subjected to rapid dehydration, root tips lost 90% of their fresh weight and reached their constant dry weight in only 2 h, when the whole seedlings had lost only 15% (Fig. 1). In these conditions primary root tips always survived and resumed growth on rewatering in seedlings dehydrated for 15 min, when root tips still retained 88% of their water content. After longer periods of rapid dehydration, and up to about 50 h, 100% of the seedlings survived desiccation but, on rewatering growth was resumed only by the aerial parts of the seedlings and by the proximal parts of their roots, which gave rise to lateral radicles. Under these conditions of rapid dehydration formation of dehydrins was never found in root tip nuclei (data not shown). When seedlings were dried slowly at 90% RH for 48 h, the relative water content of the whole seedlings dropped to 82% of its initial amount and

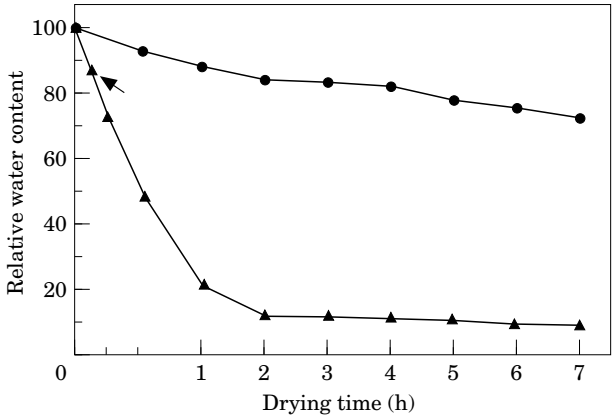

FIG. 1. Loss of water from whole 72-h-old seedlings (●——●) and from their root tips (▲——▲) when seedlings are dehydrated in the air. Arrow indicates the relative water content which allows 100% recovery of primary root growth.

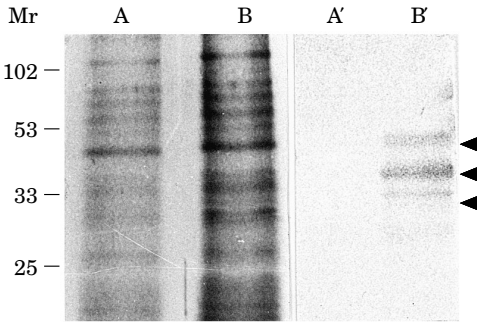

FIG. 2. A and B: SDS-PAGE of heat stable proteins from root tip nuclei of control (not dehydrated) seedlings (A) and of seedlings dehydrated for 72 h at 90% RH (B). A', B': Immunoblot analysis of the same samples, using the dehydrin oligopeptide antibody.

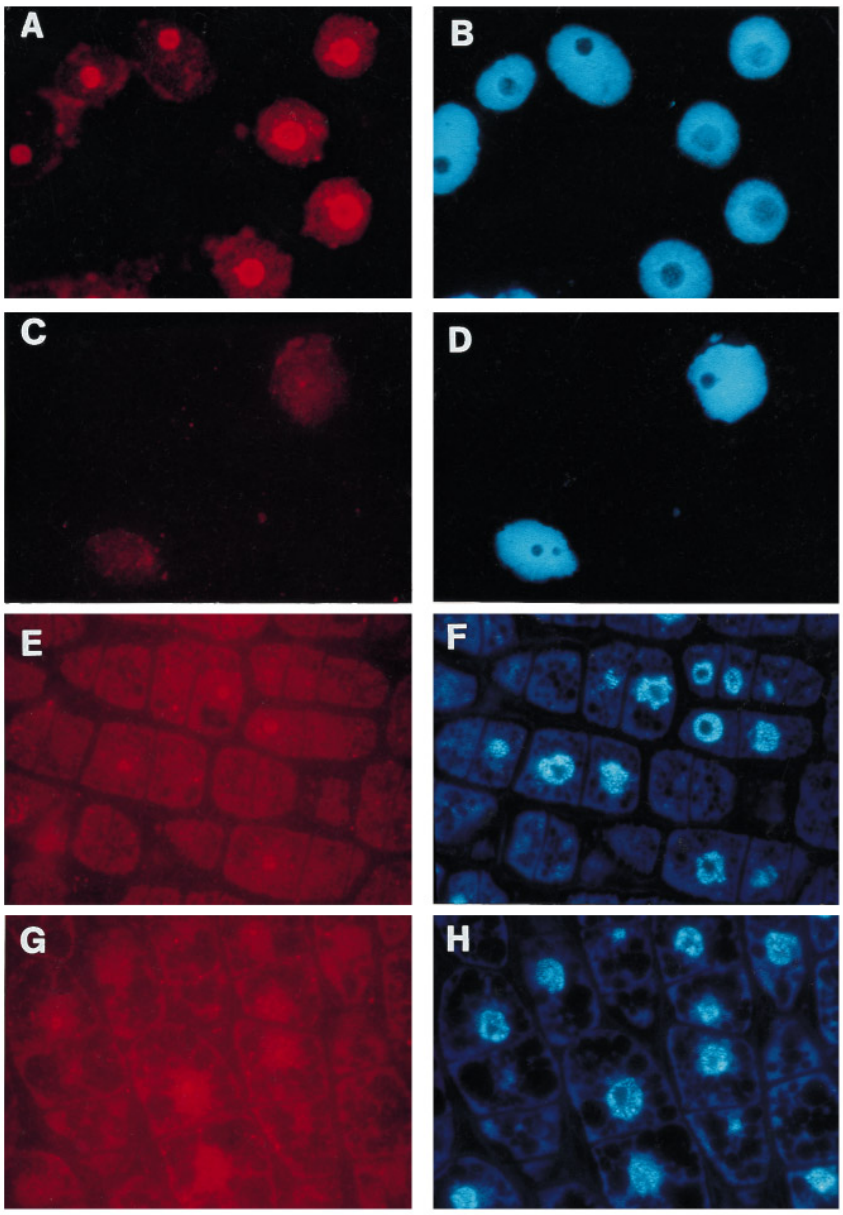

FIG. 3. Dehydrin-like proteins in isolated nuclei and in sectioned root tips from seedlings dehydrated for 48 h at 90% RH. A and C (×1000), Isolated nuclei from dehydrated (A) and control (C) root tips treated with antidehydrin antibody, antirabbit bitinylated secondary antibody and Texas Red-conjugated streptavidin; E and G (×1000), Pea root tips sectioned through meristematic (E) and enlarging (G) areas. B, D, F and H (×1000), The same nuclei as in A, C, E and G, respectively stained with DAPI.

that of root tips to 55%. Yet primary root tips were able to resume growth quickly after being retransferred to water. In these conditions root tip nuclei contained many heat stable polypeptides and three of these, of about 40, 36 and 30 kDa were recognized in Western blotting experiments using the dehydrin oligopeptide antibody (Fig. 2). The same polypeptides were detected when the immunoblot analysis was performed using total nuclear proteins, instead of heat stable ones (data not shown). It is possible that two of these polypeptides correspond to two dehydrins (of 40 and 29 kDa) found in maturing pea cotyledons (Robertson and Chandler, 1992), and whose subcellular localization has not been reported.

Immunocytochemical detection of nuclear dehydrin-like proteins in root tips from slowly dehydrated seedlings

Results from Western blotting were confirmed by immunocytochemical experiments both on whole nuclei, and on sectioned root tips from slowly dehydrated seedlings. In whole nuclei the labelling after treatment with anti-dehydrin antibodies was found throughout the nuclei, but particularly on the nucleolus (Fig. 3A and B). Nuclei from control samples were essentially free of labelling (Fig. 3C and D). The same was true for nuclei from dehydrated root tips treated with preimmune serum (data not shown). In sectioned root tips both nuclei and cytoplasm showed the

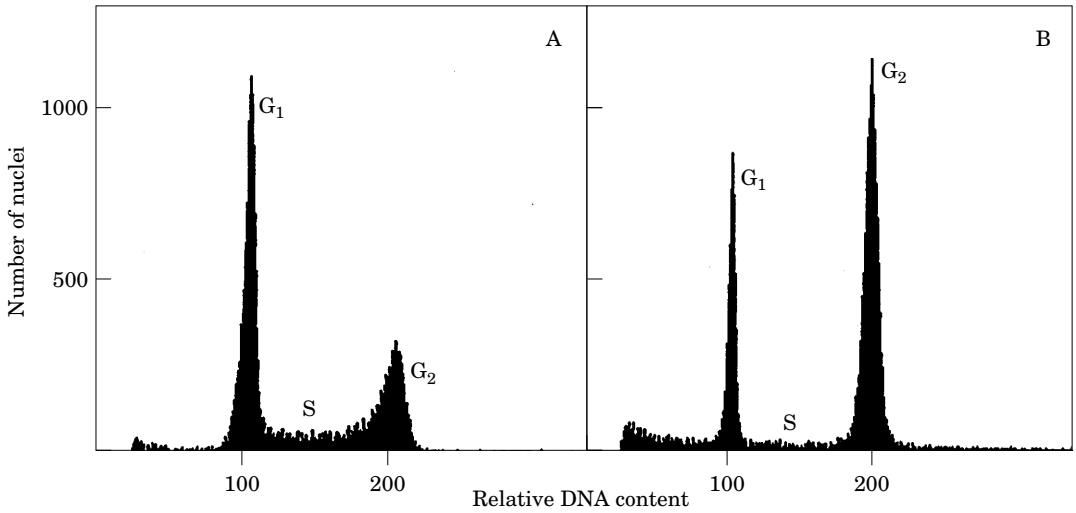

FIG. 4. Flow cytometry analysis of nuclei from root tips of control seedlings (A) and of 3-d-old seedlings treated for two additional days at 90% RH (B). G1, S and G2 show nuclei in the different phases of the cell cycle.

presence of dehydrin-like proteins. Once again, the nucleolus was strongly labelled. Dehydrin like proteins were present both in strictly meristematic tissue (Fig. 3E and F) and in cells starting to enlarge (Fig. 3G and H).

Flow cytometry analysis of the cell cycle course in nuclei from root tips of slowly dehydrated seedlings

When transferred to 90% RH the growth of seedling radicles was completely blocked, showing that cell elongation and meristematic activity were arrested; however, both activities soon recovered upon retransfer to water (see above). To test whether nuclei of cycling cells of the root tip were blocked in a specific stage of the cell cycle, nuclei isolated from control and slowly dehydrated seedlings were analyzed by flow cytometry. Frequency distributions, illustrating the number of nuclei in each DNA-content class are shown in Fig. 4. After 48 h only a negligible fraction of the nuclei of treated seedlings were still in S phase, while many were in the postsynthetic G2 phase; this result was maintained when nuclei were examined after longer periods of treatment (data not shown). The percentage of nuclei still having a 2C DNA content in these conditions was about 30%. However, it must be noted that in control root tips of 72-h-old pea seedlings, about 21% of cells are non cycling or very slowly proliferating cells in G0–G1 transition, both of which have a 2C DNA content (Sgorbati *et al.*, 1991; Citterio *et al.*, 1994). Thus the percentage of cycling cells blocked in G2 in dehydrated seedlings was estimated to be comparatively higher than it appeared from the cytometry data. This finding seemed of interest, since a possible causal relationship between the block in G2 phase and the presence of nuclear dehydrin could be inferred. For example, if dehydrins can stabilize the nuclear envelope membranes, as has been proposed in other cell membranes, the nuclear envelope could be prevented from disassembling prior to mitosis, thus holding cycling cells in G2 phase. To test whether the block in G2 phase and the presence of nuclear dehydrin-like proteins are always coupled, pea seedlings were subjected to other kinds of stresses, all related to water stress and presumably involving the possible production of dehydrins.

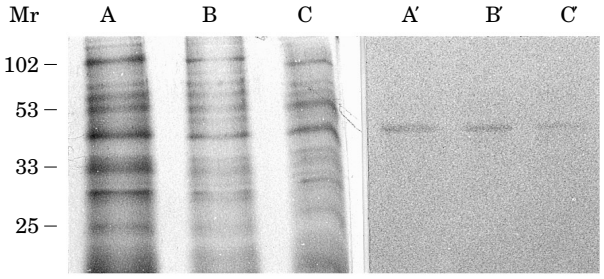

FIG. 5. SDS-PAGE (left) and immunoblot analysis (right) of heat stable proteins from root tip nuclei of 3-d-old seedlings transferred for 48 h to: 0·5 M mannitol (A,A'); 100 μM ABA (B,B') and chilled at 2 °C (C,C').

Effects of ABA, mannitol and cold temperature treatments on the production of dehydrin-like proteins in root meristems and on the cell cycle course

Three-day-old seedlings were transferred to 100 μM ABA, 0·5 M mannitol and to cold temperature (2 °C) for 48 h. Osmotic stress imposed by mannitol and cold temperature are known to cause cellular water loss to some extent (Asghar *et al.*, 1994; Bartels and Nelson, 1994). ABA is known to modulate the response of plants to water stress and has been reported to promote the expression of dehydrin genes. Saline stress was avoided so as not to superimpose possible effects induced by ion toxicity on osmotic effects. A very slight elongation of roots was seen in seedlings grown at 2 °C and in 0·5 M mannitol, while more consistent growth, though much lower than in controls, was observed in ABA treated seedlings (data not shown). As before, all the treatments were fully reversible and radicles recovered normal growth when transferred back to water. Nuclei were extracted from root tips of differently treated seedlings and checked for the presence of dehydrin-like proteins in Western blotting experiments. Results are reported in Fig. 5. All

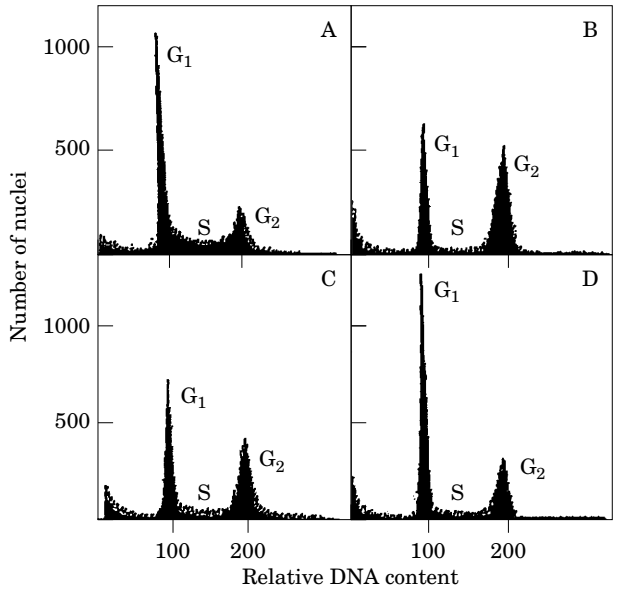

FIG. 6. Flow cytometry analysis of nuclei from root tips of 5-d-old seedlings (A) and of 3-d-old seedlings transferred for two additional days to: water (A, control); 0·5 M mannitol (B); cold temperature, 2 °C (C) and 100 μM ABA (D).

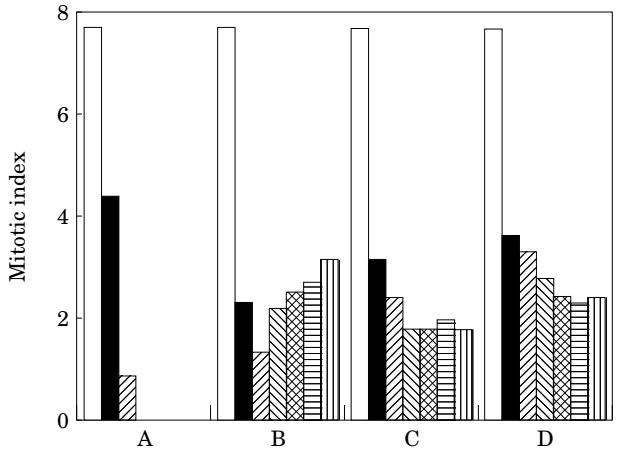

FIG. 7. Lowering of the mitotic index after transferring 3-d-old seedlings for up to 3 d to: 90% RH (A); 0·5 M mannitol (B); cold temperature, 2 °C (C) and 100 μM ABA (D). Bars show mitotic indices after 0 (□), 4 (■), 9 (▨), 15 (▧), 30 (▩), 48 (▤) and 72 h (▥).

treatments elicited the formation of a 40 kDa polypeptide, which is absent in control nuclei. Two polypeptides of higher mobility (36 and 30 kDa), always present in slowly dehydrated seedlings (see Fig. 2, lane B), were absent or extremely faint in ABA, mannitol and cold treated nuclei.

The course of the cell cycle after 48 h of each treatment was examined by means of flow cytometry analysis. In ABA and cold treatments (Fig. 6C and D), a lower number of nuclei in the S phase was observed, compared to controls, but the strong and persistent accumulation of nuclei in G2 phase found in nuclei subjected to water deficit was not present. A consistent percentage of nuclei in G2 phase was noticed only in mannitol treated samples (Fig. 6B). However, seedlings treated with mannitol had a tendency to regain a normal cell course after longer periods of treatment, as though meristematic tissue could develop some adaptation to stress conditions (data not shown). The mitotic index in root tip cells after different periods of the various treatments was also examined. Only in dehydrated root tips did it drop to zero, while in the other conditions the mitotic index settled at very low values (Fig. 7). A slight increase in mitotic index was only observed in mannitol treated samples after prolonged treatment (Fig. 7B), consistent with their partial adaptation to the osmotic stress, as mentioned above. Under control conditions, the mitotic index remained practically unchanged for up to 3 d, at about 8%.

DISCUSSION

Dehydrins and related drought induced proteins, formerly thought to be synthesized only in the cytoplasm in response to water deficit, have been found recently in nuclei of different tissues of mature seeds (Asghar *et al.*, 1994; Goday *et al.*, 1994) and in vegetative parts of seedlings and plants subjected to water stress (Godoy *et al.*, 1994; Tabaeizadeh *et al.*, 1994). In the present work it has been shown, by immunochemical and cytological evidence, that dehydrin-like proteins can be induced even in nuclei of meristematic cells of root tips, provided a sufficiently slow dehydration treatment is imposed. In fact, Western blot analysis using a polyclonal antibody raised against the conserved domain of dehydrins, demonstrated the appearance of three newly synthesized, boiling-stable polypeptides in a fraction of purified nuclei from root tips of slowly dehydrated pea seedlings. Moreover, immunomicroscopy experiments using the same antibody showed the presence of dehydrin-like proteins both in the cytoplasm and in nuclear compartments, with higher labelling apparent in the nucleolar regions. Nevertheless, other authors failed to find dehydrin-like proteins in pea roots of seedlings undergoing slow dehydration (Robertson and Chandler, 1992; Baker *et al.*, 1995), even though, in the former case, the respective mRNAs were present. However, the authors used a maize dehydrin antiserum which, as shown later (Robertson and Chandler, 1994), behaved differently from the one directed to the conserved consensus sequence. Our differing results can be ascribed to the different cultivar of pea used (Lincoln *vs.* Greenfast and Alaska): it is known that, for instance, different cultivars of rice differ in their ability to produce dehydrin proteins (Moons *et al.*, 1995). Finally, it has to be added that the periods of dehydration imposed upon seedlings were shorter in our case than those used by other authors. It is possible that dehydrin production in roots is a transient event and that it might not be present after longer periods.

With regard to nuclear labelling, our material showed higher fluorescence in the nucleolar regions. This is in line with the results of Godoy *et al.* (1994) concerning TAS 14 protein, which is induced in tomato by osmotic stress or abscisic acid; immunogold labelling was associated mainly with nuclear and nucleolar transcribing chromatin. Accumulation at the nucleolar level could actually be required to preserve nucleolar structures under conditions of water deficit, or could simply be a consequence of a reduced and

altered nuclear metabolism in these conditions. Anyway, at a molecular level, three-dimensional structures of membranes, proteins and nucleic acids (all of which are present in nuclear compartments) are dependent on the physico-chemical characteristic of water. These structures must be maintained during water loss or arranged in such a way as to recover, spontaneously, their original structure on rehydration. In particular, membrane stucture is often regarded as a primary site of desiccation damage; thus, even if this hypothesis needs more subtle analysis by means of immuno electron microscopy, it is likely that nuclear envelope membranes are, like other cell membranes, somehow stabilized by dehydrins in conditions of water deficit. Asghar *et al*. (1994), assuming that dehydrins stabilize the nuclear envelope, proposed that the cell division cycle would be disrupted at the point where degeneration of the envelope normally occurs, so that the tradeoff for a gain in nuclear stabilization would be loss of cell division capability. This hypothesis seems to be supported by our experimental findings that the cell cycle in meristematic root tips of dehydrated pea seedlings is mostly blocked in G2 phase, prior to entry into mitosis. Moreover our findings are in agreement with those of Artlip *et al*. (1995) who noticed that in endosperm of *in vitro* cultured maturing maize kernels, cell division is highly responsive to water deficit, whereas endoreduplication, which does not involve nuclear envelope disassembly, is less so.

Osmotic, cold and ABA treatments, known to mimic water stress effects, and used here with the intention of clarifying the possible interrelationship between nuclear dehydrin production and premitotic block of the cell cycle, failed to provide unequivocal information. All these treatments led to the production of a 40 kDa dehydrin-like protein (see Fig. 5), but only the mannitol treatment induced a G2 block, even if transiently, of the cell cycle, in the same way as drought treatment. It is possible that responses to the different treatments (cold, osmotic stress and exogenous ABA administration) are regulated differently compared to water stress. Inducibility by ABA, for instance, is known to be a feature of only a subset of dehydration induced genes. However, a lower degree of water loss induced by these treatments (cold and mannitol, in particular) compared with water stressed seedlings, could also account for their weaker and less well-defined effects. Unfortunately, a reliable determination of water loss in cold and mannitol treated root apices, and a comparison with water stressed apices, was technically almost impossible to perform owing to the difficulty of uniformly and completely wiping the apices previously immersed in aqueous solutions.

Moreover, it is worth noting that the 40 kDa protein expressed with all three treatments (cold, mannitol and ABA) could correspond to the 40 kDa dehydrin cognate protein from pea reported by Robertson and Chandler (1994) which exhibits an atypical pattern of expression and does not behave as a typical dehydrin molecule. Thus, the only true dehydrins could be represented by the two nuclear dehydrins of lower molecular weight, which are induced only by drought treatment. These two proteins could thus be hypothesized to play some role (perhaps by stabilizing the nuclear envelope) in imposing the strong and persistent accumulation in G2 phase observed only in water stressed root tips.

However several points call for further investigations. For instance (*a*) a hindrance to nuclear envelope disassembly could also result from a stiffening of nuclear membranes, which might not depend on the presence of dehydrins, but on the reduced level of unsaturation of fatty acids of membrane lipids in cells experiencing water deficit. Down regulation of two genes encoding desaturases has been detected during water stress treatments (Leone *et al*., 1994; Grillo *et al*., 1996). In addition, (*b*) microscopic examination of arrested nuclei at 90 % RH has shown that they seem to be mostly in interphase and not in prophase, when nuclear disassembly occurs. Thus some previous process such as the complex pattern of phosphorylation involving key nuclear proteins which is a normal prerequisite for nuclear envelope disassembly (Glass and Gerace, 1990; Nurse, 1990; Foisner and Gerace, 1993), could be impaired. Both these possibilities will be explored in the future.

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