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Detection and subcellular localization of dehydrin-like proteins in quinoa (*Chenopodium quinoa* Willd.) embryos

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Summary. The aim of this study was to characterize the dehydrin content in mature embryos of two quinoa cultivars, Sajama and Baer La Unión. Cultivar Sajama grows at 3600–4000 m altitude and is adapted to the very arid conditions characteristic of the salty soils of the Bolivian Altiplano, with less than 250 mm of annual rain and a minimum temperature of −1 °C. Cultivar Baer La Unión grows at sea-level regions of central Chile and is adapted to more humid conditions (800 to 1500 mm of annual rain), fertile soils, and temperatures above 5 °C. Western blot analysis of embryo tissues from plants growing under controlled greenhouse conditions clearly revealed the presence of several dehydrin bands (at molecular masses of approximately 30, 32, 50, and 55 kDa), which were common to both cultivars, although the amount of the 30 and 32 kDa bands differed. Nevertheless, when grains originated from their respective natural environments, three extra bands (at molecular masses of approximately 34, 38, and 40 kDa), which were hardly visible in Sajama, and another weak band (at a molecular mass of approximately 28 kDa) were evident in Baer La Unión. In situ immunolocalization microscopy detected dehydrin-like proteins in all axis and cotyledon tissues. At the subcellular level, dehydrins were detected in the plasma membrane, cytoplasm and nucleus. In the cytoplasm, dehydrins were found associated with mitochondria, rough endoplasmic reticulum cisternae, and proplastid membranes. The presence of dehydrins was also recognized in the matrix of protein bodies. In the nucleus, dehydrins were associated with the euchromatin. Upon examining dehydrin composition and subcellular localization in two quinoa cultivars belonging to highly contrasting environments, we conclude that most dehydrins detected here were constitutive components of the quinoa seed developmental program, but some of them (specially the 34, 38, and 40 kDa bands) may reflect quantitative molecular differences associated with the adaptation of both cultivars to contrasting environmental conditions.

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Introduction

Dehydrins are a family of extremely hydrophilic late-embryogenesis abundant (LEA) proteins constituting the D-11 subset (Dure et al. 1989). According to Kermode (1997), dehydrins are produced as part of the developmental program of orthodox seeds during the maturation-drying phase. Additionally, certain recalcitrant seeds have also been found to contain dehydrins (Finch-Savage et al. 1994, Farrant et al. 1996, Panza et al. 2007). These intrinsically unstructured proteins are also known to accumulate in response to abscisic acid (RAB proteins) (Koag et al. 2003) and various stress-related factors, which ultimately result in cell dehydration (Allagulova et al. 2003, Rorat 2006). All dehydrins contain one or more copies of a highly conserved lysine-rich 15-amino acid sequence called the K-segment, which has been extensively used to raise antibodies able to recognize proteins immunologically related to the dehydrin family (Close et al. 1989, 1993; Close and Lammers 1993; Gee et al. 1994; Robertson and Chandler 1994; Farrant et al. 1996; Close 1997; Borovskii et al. 2000; Mueller et al. 2003; Turco et al. 2004; Panza et al. 2007).

Although dehydrins often exhibit tissue and organ specificity, immunolocalizations performed in cotton and maize seeds have shown that they are present in all tissue types (Roberts et al. 1993, Asghar et al. 1994, Danyluk

et al. 1994, Close 1996). At the subcellular level, seed dehydrins have been immunolocalized in maize kernels in the cytosol of cells located near the shoot and root apex and in the cytosol, cytoskeletal elements, and nuclei of cells belonging to other seed tissues, including aleuronic and scutellar tissues (Asghar et al. 1994, Close 1996). Dehydrins have also been found to localize to the plasma membrane in wheat seedling (Danyluk et al. 1998), mitochondria in wheat, rye, and maize seedlings (Borovskii et al. 2000), chloroplasts in mature leaves of pea and maize (Mueller et al. 2003), or within the lumen of vacuoles in seedlings and petioles of celery (Heyen et al. 2002). In addition, dehydrins have also been detected in maize embryo tissues, associated with the proteinaceous matrix of protein bodies and membranes of protein and lipid bodies (Egerton-Warburton et al. 1997).

Quinoa (*Chenopodium quinoa* Willd.) is a staple crop of the Andean region of South America, cultivated for the high nutrient content of its seeds (Repo-Carrasco et al. 2003). The species is well adapted to extreme environmental conditions with regard to altitude, amount of annual precipitation, soil salinity, and minimum temperatures, and numerous cultivars have been identified, according to their genotype–environment interaction patterns (Tapia 1999, Bertero et al. 2004).

Quinoa seeds are classified as orthodox according to their tolerance to desiccation (Ellis et al. 1988). Desiccation tolerance, as in the preservation of orthodox seed tissues, has been related to the accumulation of di- and oligosaccharides (Koster and Leopold 1988), the vitrification of the cellular content (Sun and Leopold 1997), the presence of triacylglycerol storage lipids (Crane et al. 2003), and/or the presence of specific proteins such as dehydrins (Dure et al. 1989).

The aim of this study was to detect differences in dehydrin content among mature seeds of two cultivars belonging to distinct genotypic groups (Tapia 1999, Bertero et al. 2004). Cultivar Sajama belongs to group 3 (G3) and is found on the Bolivian Altiplano at 3600–4000 m altitude and is adapted to the very arid conditions characteristic of the salty soils of the Altiplano, with less than 250 mm of annual rain and a minimum temperature of −1 °C. In contrast, cultivar Baer la Unión belongs to group 4 (G4) and grows at sea level in the southern region of central Chile. It is adapted to more humid conditions (800 to 1500 mm of annual rain), nonsaline (fertile) soils, and temperatures above 5 °C. As Sajama and Baer la Unión belong to highly contrasting environments, they are expected to have constitutive differences in protective molecule content, including dehydrins, associated with adaptation to salt, cold, and drought stresses. In order to detect and characterize dehydrins, we performed Western blot analysis of the heat-stable seed protein fraction and in situ immunolocalization microscopy. Both studies used antibodies raised against the mentioned K-segment. This study is part of a monographic treatment on the conservation of quinoa seeds.

Material and methods

Plant material

Two quinoa cultivars from contrasting environments, with regard to altitude (or meters above sea level), amount of annual precipitation, soil salinity, and minimum temperatures, cv. Sajama (Germplasm Bank INTA-Castelar, BBC 15769, series number 21952) and cv. Baer La Unión (Germplasm Bank INTA-Castelar, BBC 6480, series number 6560), were cultivated in a greenhouse from February through July 2004 at 20 °C (day) and 15 °C (night), and water was controlled daily. Grains were sown in pots (volume, 3 liters) with a 2 : 1 mixture of sand and agrolite, an inert, porous (hygroscopic) substrate, and collected at full maturity, cleaned of pericarps, and stored at 4 °C in a container with silica gel. Wheat caryopses (*Triticum aestivum* L. cv. Chinese Spring) were lyophilized and embryos were separated for protein extraction. In additional experiments, we used cv. Sajama and cv. Baer La Unión grains acquired from the Altiplano region (Bolivia) and Fundo La Unión, Perquenco, IX Region (Chile), respectively.

Extraction of heat-stable embryo proteins and Western blot assay

Lyophilized embryos in weighted batches of 20 were ground to a powder in liquid nitrogen and homogenized for 30 min at 4 °C in extraction buffer containing 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5 mM $MgCl_2$, and Protease Inhibitor Cocktail (Sigma Chemical Co., St. Louis, Mo., U.S.A.) at 2 μl/5 ml of extraction buffer, followed by centrifugation at 10,000 **g** for 10 min. Heat-stable protein fractions were prepared following a modified version of the procedure described by Close et al. (1993), immersing extracts in a bath at 92 °C for 10 min, cooling at room temperature, and centrifuging at 10,000 **g** for 10 min at 4 °C. A fraction of the supernatant was separated to estimate heat-stable protein concentration by the Bradford (1976) method using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, Calif., U.S.A.) and bovine serum albumin as a standard. Proteins were resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Laemmli 1970) at a constant voltage of 120 V for 90 min using a Mini-Protean II electrophoresis cell (Bio-Rad Laboratories); heat-stable protein was loaded at 3 μg per lane and benchmark molecular-weight standards (Invitrogen) were used. Following electrophoresis, the fractionated proteins were transferred onto a nitrocellulose membrane (Osmonics, Inc., Minnetonka, Minn., U.S.A.; EP4HY00010) at 200 mA for 75 min using a Mini Trans-Blot electrophoretic transfer cell (Bio-Rad Laboratories). The integrity of blotted proteins was confirmed by Ponceau S staining.

After an overnight blocking with 5% nonfat dried milk in TBS (50 mM Tris-HCl, 150 mM NaCl, pH 8) at 4 °C with shaking, membranes were incubated for 90 min at room temperature with the anti-dehydrin primary polyclonal antiserum at a 1 : 1000 dilution in the blocking solution. The rabbit antiserum had been raised against the carboxy terminus of a consensus sequence common to dehydrins in angiosperms, the K-segment (TGEKKGIMDKIKEKLPGQH) (Close et al. 1993). Membranes were washed three times (10 min each) in TBS–0.05% Tween 20 and incubated for 90 min with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Accurate Chemical and Scientific Corp., Westbury, N.Y.,

U.S.A.) at a 1 : 3000 dilution in the blocking solution. Following three consecutive washes with TBS as described above, incubation with AP buffer (100 mM Tris-HCl, 100 mM NaCl, 5 mM $MgCl_2$, pH 9.5) was performed for 10 min. All incubations and washes were performed with gentle shaking at room temperature. For signal development, membranes were incubated in 10 ml of AP buffer with 66 μl of nitro blue tetrazolium and 33 μl of 5-bromo-4-chloro-3-indolylphosphate (Promega) in the dark. The specificity of the dehydrin detection was confirmed by blocking the primary antiserum for 30 min with an equal volume of the K-segment peptide salt (5 mg/ml) prior to the first incubation of the duplicate membranes. After Western blotting, the relative intensity of each individual band was quantified by the ImageQuant TL program (Amersham Biosciences, Piscataway, N.J., U.S.A.).

Preparation of tissue for light and transmission electron microscopy

Excised embryos were transversally cut in half to obtain separate samples of axes with root apical meristem or shoot apical meristem tissues plus cotyledons. Samples were immediately fixed for 2 h at 4 °C using a mixture of 2% paraformaldehyde and 1% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, washed with deionized water for 30 min, dehydrated in a graded ethanol series with changes every 30 min and embedded in London Resin White resin (Polyscience, Inc.; 17411) according to Harris et al. (1995). Semithin sections (1 μm thick) and ultrathin sections for light (LM) and transmission electron microscopy (TEM), respectively, were obtained with an ultramicrotome (Reichert-Jung, Vienna, Austria) with a glass knife.

Immunolabeling for LM

Tissue distribution of dehydrin-like proteins in embryo sections was observed by silver-enhanced LM. Sections were transferred to clean glass slides, hydrated on a drop of Milli-Q-grade water for 3 min, washed twice with rinsing buffer (0.1% gelatin and 0.05% Tween 20 in 10 mM PBS, pH 7.2) for 3 min, blocked for 90 min with 1% gelatin in PBST (0.05% Tween 20 in 10 mM PBS, pH 7.2) at room temperature, washed with rinsing buffer twice for 3 min, and incubated overnight at 4 °C with the purified rabbit anti-dehydrin primary antibody at a 1 : 200 dilution in the rinsing buffer. Next, sections were washed with three changes of rinsing buffer (5 min each), incubated for 1 h on a drop of colloidal gold (10 nm diameter)-conjugated goat antiserum to rabbit immunoglobulins (G-7402; Sigma Chemical Co.) diluted 1 : 200 in rinsing buffer at 37 °C and washed again with rinsing buffer (three changes, 5 min each). After a 45 min fixation with 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, sections were thoroughly washed with Milli-Q-grade water. Since individual gold particles are not visible at the LM level, a silver enhancement kit (Sigma SE-100) was used to develop a brown-to-black stain in the immunoreactive areas. Finally, the silver deposition reaction was fixed with 2.5% aqueous sodium thiosulfate followed by rinsing in Milli-Q-grade water. Sections were viewed with an Olympus FluoView FV 300 confocal microscope with FluoView 3.3 software (Olympus, Tokyo, Japan). Control sections were treated as above excluding the purified rabbit anti-dehydrin immune serum and also by blocking the primary antiserum with the recognized peptide sequence prior to the incubation step.

Immunolabeling for TEM

Ultrathin sections were mounted on Formvar-coated nickel grids (Polyscience, Inc.; 7550N) for immunolabeling at the TEM level. Sections were first hydrated on a drop of Milli-Q-grade water for 3 min, washed with rinsing buffer twice for 3 min, blocked for 90 min with 1% gelatin in PBS at room temperature, washed with rinsing buffer twice (3 min each) and incubated overnight at 4 °C with the purified rabbit anti-dehydrin primary antibody at a 1 : 100 dilution in the rinsing buffer. Next, sections were washed with rinsing buffer (5 changes, 5 min each), incubated for 1 h on a drop of colloidal gold (10 nm diameter)-conjugated goat antiserum to rabbit immunoglobulins diluted 1 : 150 in rinsing buffer at room temperature and washed again with rinsing buffer (5 changes, 5 min each). After a 10 min fixation with 1% glutaraldehyde in 0.1 M PBS, pH 7.2, sections were thoroughly washed with Milli-Q-grade water. Finally, grids carrying sections were counterstained with uranyl acetate followed by lead citrate (Prego et al. 1998), and examined under a Zeiss EM 109 turbo (Zeiss, Wiesbaden, Federal Republic of Germany) transmission electron microscope operating at an accelerating voltage of 90 kV. Control sections were treated as above, excluding the purified rabbit anti-dehydrin immune serum and also blocking the primary antiserum with the recognized peptide prior to the incubation step (as previously described).

Results

Dehydrin Western blot analysis in quinoa embryos

The pattern of dehydrins in embryos of quinoa cultivars Sajama and Baer La Unión acquired from plants growing under controlled greenhouse conditions is shown in Fig. 1A. Western blot analysis of the heat-stable protein fraction revealed the presence of several dehydrin bands

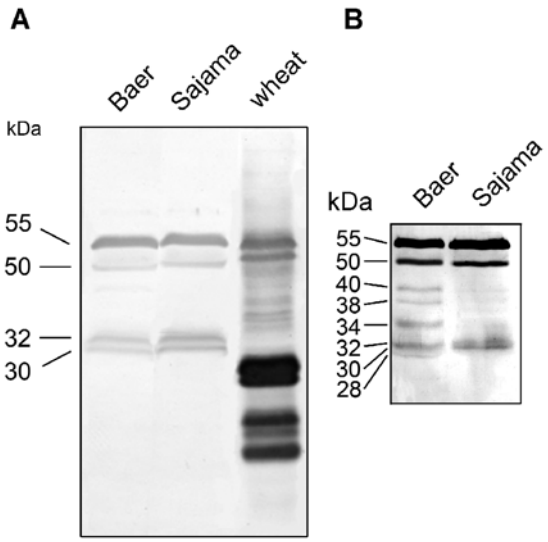

Fig. 1A, B. Western blot analysis of dehydrin-like proteins present in mature seeds of two quinoa cultivars (cv. Sajama and cv. Baer La Unión) and wheat (cv. Chinese Spring). **A** Embryo tissues from plants grown under controlled greenhouse conditions; **B** embryo tissues from grains obtained from their respective natural environments. Heat-stable proteins were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose membranes. The first antiserum was a purified anti-dehydrin antiserum directed against the K-segment common to all dehydrins at a 1 : 1000 dilution. The second antiserum was an alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G at a 1 : 3000 dilution. Protein molecular masses were calculated by the GelCompar, version 4.1 software (Applied Maths, Sint-Martens-Latern, Belgium) and are indicated on the left. The intensity of each band was quantified by the ImageQuant TL program

(at molecular masses of approximately 30, 32, 50 and 55 kDa) common to both quinoa cultivars. The 30 and 32 kDa dehydrin bands of protein fraction from cv. Sajama contained twice as much protein as did those from cv. Baer La Unión, while the 55 and 50 kDa bands were equal.

Dehydrins from wheat (*Triticum aestivum* L. cv. Chinese Spring) embryo tissues were included as a positive control due to their known abundance (Campbell and Close 1997, Werner-Fraczek and Close 1998, Lopez et al. 2003). Only a single band at a molecular mass of approximately 55 kDa was found to be common between quinoa and wheat. In order to characterize the patterns of embryo dehydrins in plants obtained from their respective natural environments, proteins were extracted directly from embryos from grains collected from the Altiplano region of Bolivia (cv. Sajama) and the IX Region of Chile (cv. Baer La Unión). In this case, Western blot analysis revealed the presence of three conspicuous extra bands (at molecular masses of approximately 34, 38, and 40 kDa) in Baer La Unión, which were hardly visible in Sajama, and a weak band (28 kDa) observed only in Baer La Unión (Fig. 1B).

As a control for the specificity of dehydrin detection, duplicate membranes were incubated with primary antiserum preblocked with the consensus K-peptide. No bands were present in the control membranes, indicating that all bands detected are immunologically related to the dehydrin family (data not shown).

In situ immunolocalization of dehydrins in quinoa embryos

The Western blot analysis was complemented by in situ immunolocalization of dehydrin-like proteins in embryos of both cultivars, in order to establish the tissue and subcellular localization of these proteins. They were detected in both cultivars, in all axis and cotyledon tissues (Fig. 2A–F). In apical meristems, dehydrins were scarce in the initial cells but were clearly detectable in derived cells (Fig. 2A, B). In axis and cotyledon tissues, dehydrins were visible especially in the plasma membrane and protein bodies (Fig. 2C, E). At the subcellular level, we detected the presence of dehydrins in the plasma membrane, cytoplasm, and nucleus (Figs. 3 and 4). In the cytoplasm, dehydrins were found associated with mitochondria (Fig. 3A and C), rough endoplasmic reticulum cisternae (Fig. 4A), and proplastid membranes (Fig. 4B). We also confirmed the presence of dehydrins in the matrix of protein bodies (Fig. 4F), as had already been observed by LM (see above). In the nucleus, dehydrins were associated with euchromatin (Fig. 3A, C). Control sections, i.e., sections that had not been incubated with the purified rabbit anti-dehydrin immune serum (not shown) and sections that had been incubated with primary antiserum previously blocked with purified lysine-rich peptide derived from the K-segment common to all dehydrins, were free of labeling (Figs. 2D, F, 3D, and 4C, E, G).

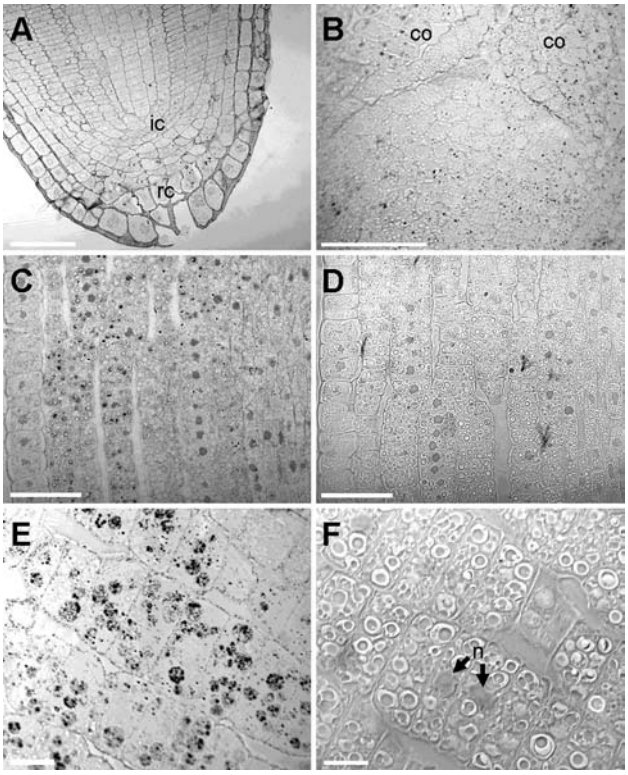

Fig. 2 A–F. Embryo tissue distribution of dehydrin-like proteins in *C. quinoa* cv. Sajama. Silver-enhanced immunogold localization was observed by LM. **A–C** and **E** sections were incubated with the purified rabbit anti-dehydrin primary antibody at a 1 : 200 dilution, washed, and incubated on a drop of colloidal gold-conjugated goat antiserum to rabbit immunoglobulins at a 1 : 200 dilution. Since individual gold particles are not visible at the LM level, a silver enhancement kit was used to develop a brown-to-black stain in the immunoreactive areas. **D** and **F** Control sections treated as above but the primary antiserum was blocked with the purified recognized peptide prior to the incubation step. **A** Root apical meristem. **B** Shoot apical meristem. **C** and **D** Cortex of the root–hypocotyl axis. **E** and **F** Detail of the ground meristem of the axis. In control sections, the absence of an immunoreactive area resulted in transparent sections that are hardly visible. For panel F, it was necessary to use differential interference contrast microscopy to achieve an image. Bars: 50 μm. *co* Cotyledon; *ic* initial cells; *n* nucleus; *rc* root cap. Arrows indicate immunoreactive areas

Discussion

There are a relatively small number of studies of the in situ localization of dehydrins in plant tissues (Svensson 2001, Rorat 2006). Even fewer specifically refer to seeds

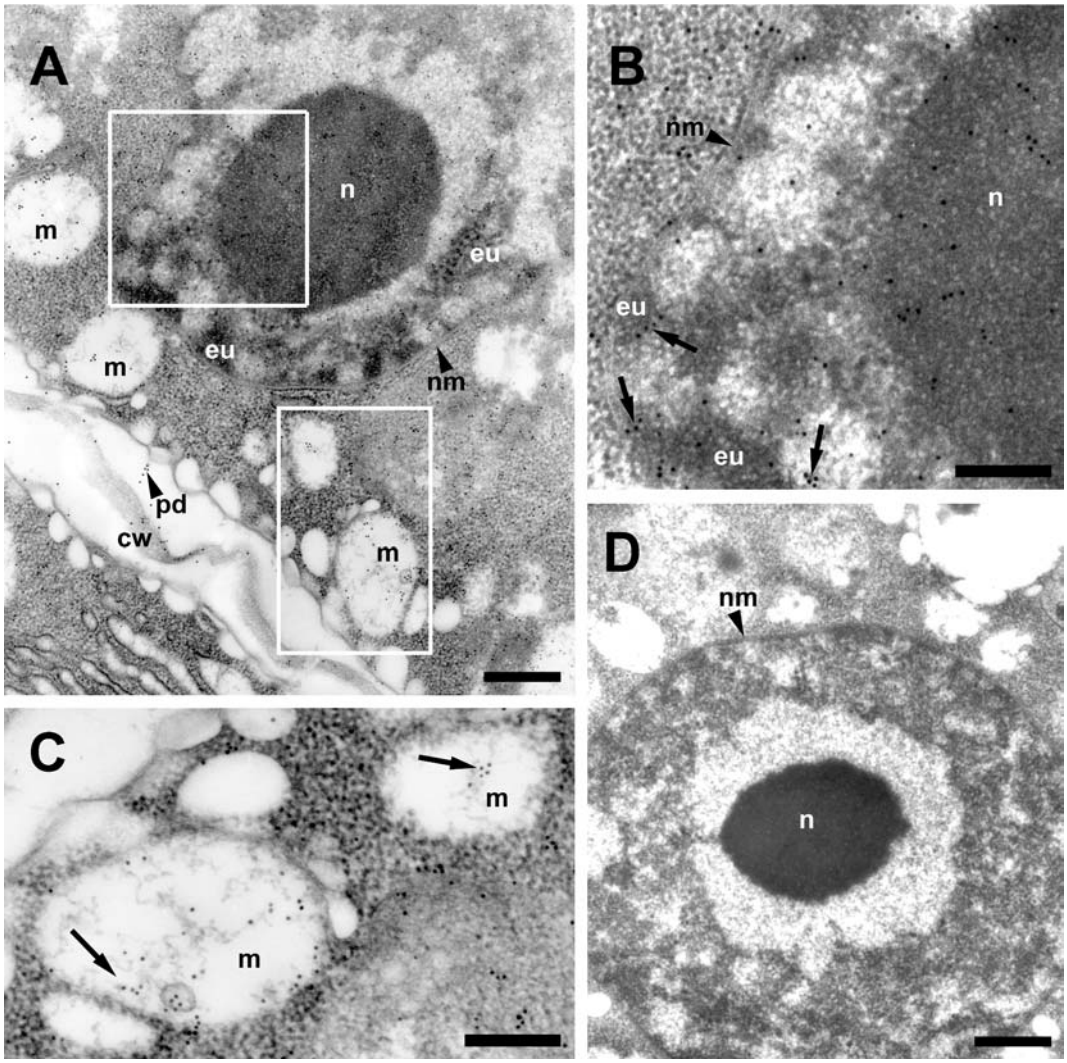

Fig. 3A–D. Shoot apical meristem of a mature embryo (*C. quinoa* cv. Sajama) **A** Ultrathin section incubated with the purified rabbit anti-dehydrin primary antibody at a 1 : 100 dilution and then on a drop of colloidal gold-conjugated goat antiserum to rabbit immunoglobulins diluted 1 : 150. Dehydrins were mainly located on the euchromatin, plasmodesmata, and inner mitochondrial membranes; Bar: 600 nm. **B** and **C** Details of **A**; bars: 400 and 300 nm, respectively. **D** Control section. This section, free of labeling, was treated by blocking the primary antiserum with the peptide sequence prior to the incubation step; bar: 800 nm. *cw* Cell wall; *eu* euchromatin; *m* mitochondrion; *n* nucleolus; *nm* nuclear membrane; *pd* plasmodesma. Arrows indicate gold particle labeling

(Rorat 2006), probably due to the great difficulty of processing tissues that are full of reserves and in a dehydrated state, both characteristics of orthodox seeds like those of quinoa. From these studies it is possible to conclude that (i) different dehydrins can be distributed in different tissues during plant growth and development; (ii) some dehydrins are specific to tissue or cell types, e.g., pollen sac, guard cells, and root meristematic cells; (iii) different types of dehydrins can localize to the same tissue. Two examples of dehydrins present in seeds are RAB18 in *Arabidopsis thaliana* (Nylander et al. 2001) and RAB17 in *Zea mays* (Goday et al. 1994), which exhibit localization in all parts of the embryo and in the endosperm. Other examples are DHN-COG dehydrin, which accumulates in developing cotyledons of pea (*Pisum sativum*) during late embryogenesis (Robertson and Chandler 1994), ECP40, which localizes in carrot endosperm and zygotic embryos (Kiyosue et al. 1993), a 24 kDa (MAT1) and a 26/27 kDa (MAT9) dehydrin from soybean (*Glycine max*) seeds (Momma et al. 1997, 2003), a 35 kDa dehydrin from cowpea (*Vigna unguiculata*) dry seeds (Ismail et al. 1999), and finally, three dehydrins with molecular masses of 16, 18, and 24 kDa in palmito (*Euterpe edulis*) embryos (Panza et al. 2007). Additionally, plants subjected to stress leading to cellular dehydration, such as drought, low temperature, and salinity, show not only a

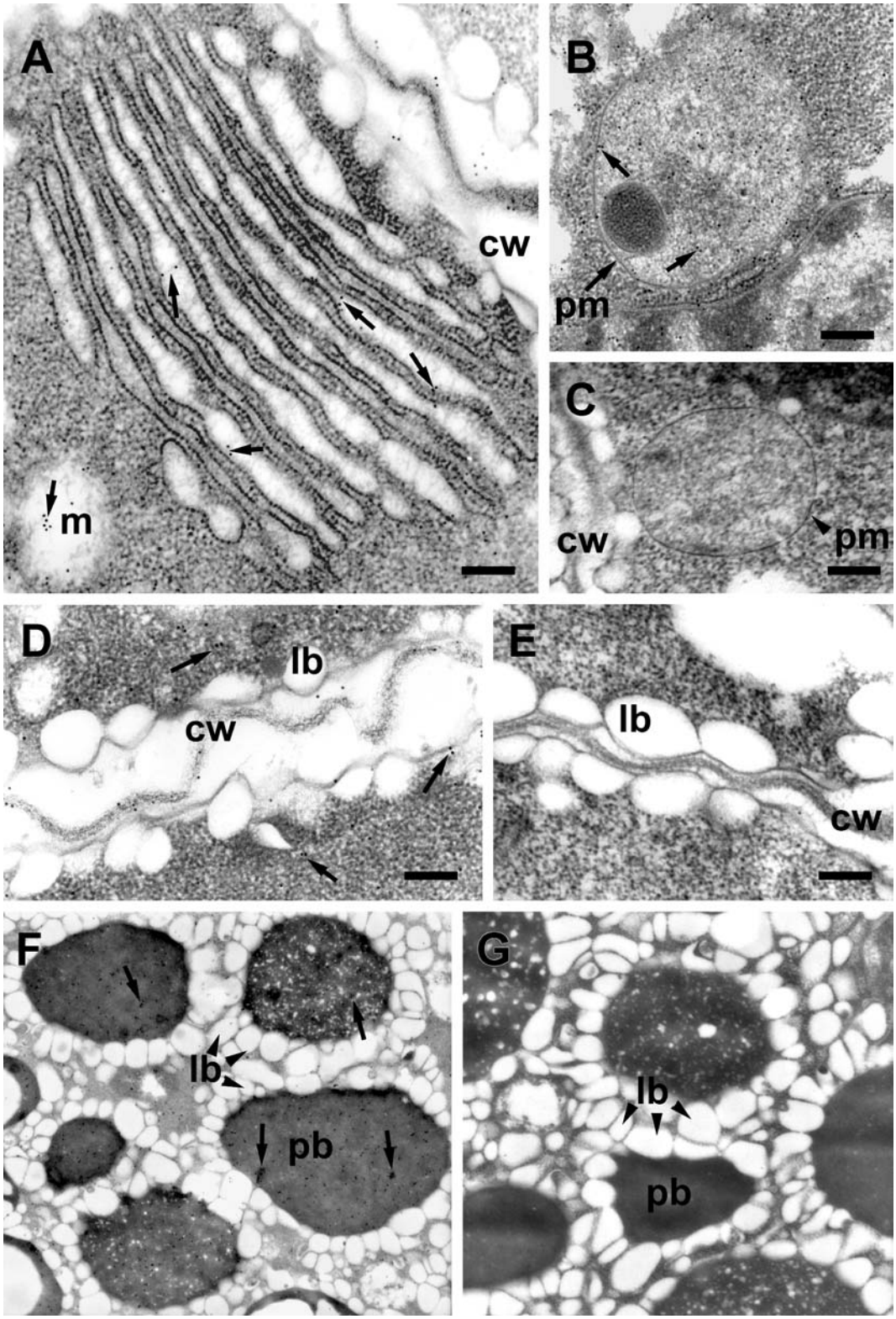

Fig. 4 A–G. Shoot apical meristem of a mature embryo (*C. quinoa* cv. Baer La Unión). **A**, **B**, **D**, and **F** Ultrathin sections labeled for dehydrins treated as the section shown in Fig. 3A–C. Dehydrins were localized at the subcellular level as indicated in the following. **A** Cisternae of rough endoplasmic reticulum; bar: 300 nm. **B** Proplastid endomembranes; bar: 300 nm. **D** Plasma membranes; bar: 300 nm. **F** Matrix of protein bodies; bar: 500 nm. **C**, **E**, and **G** Control sections corresponding to **B**, **D**, and **F**, respectively. The control sections, free of labeling, were treated as the section shown in Fig. 3D. Bars: 300, 300, and 500 nm, respectively. *cw* Cell wall; *lb* lipid body; *m* mitochondrion; *pb* protein body; *pm* proplastid membrane. Arrows indicate gold particle labeling

substantial increase in the content of preexistent dehydrins but often also the synthesis of new dehydrins (Rorat 2006).

To date, dehydrin subcellular immunolocalization studies of seeds have been carried out only on maize seeds and embryos of wheat and *Euterpe edulis*. In maize, dehydrins are present only in the cytosol and nucleus (Ashgar et al. 1994, Goday et al. 1994, Egerton-Warburton et al. 1997, Jensen et al. 1998). In wheat, they are present in nuclei (Brini et al. 2007), while in *Euterpe edulis*, they are found in the cytoplasm and are associated with chromatin, but not with organelles or membranes (Panza et al. 2007). Data on subcellular localization in nonseminal tissues reveal that dehydrins are primarily localized in the cytoplasm and nucleus, but also in various other cell compartments, such as in the vicinity of the plasma membrane (Danyluk et al. 1998), mitochondria (Borovskii et al. 2000), chloroplasts (Wisniewski et al. 1999), endoplasmic reticulum (Neven et al. 1993), vacuoles (Heyen et al. 2002), and protein bodies and amyloplasts (Rinne et al. 1999). The presence of dehydrins in nuclei not only indicates protection of the transcriptional machinery but also raises the question of how these proteins are targeted to the nucleus.

In view of the fact that quinoa is well adapted to extreme environmental conditions, with regard to altitude, amount of annual precipitation, soil salinity, and minimum temperatures (Tapia 1999), the different cultivars seem to constitute an appropriate material to study the potentially protective role of dehydrin in dehydration stress. For this study, we selected seeds from two cultivars from highly contrasting natural environments because the eventual finding of differences between these cultivars would be suggestive of a role for dehydrins in quinoa seed survival. Nevertheless, Western blot (Fig. 1A) and in situ immunolocalization (Figs. 2–4) revealed the same dehydrin pattern and distribution when plants were grown under the controlled conditions of a greenhouse, which suggests that the dehydrins detected here are constitutive components of the desiccation phase in the quinoa seed developmental program. We observed a higher level of the 32 and 30 kDa dehydrins in cv. Sajama compared with cv. Baer La Unión in samples grown under controlled greenhouse conditions. Interestingly, cv. Sajama is the cultivar that grows on the Bolivian Altiplano at 3600–4000 m altitude and is adapted to the very arid conditions characteristic of the salty soils of the Altiplano with less than 250 mm of annual rain and a minimum temperature of -1 °C. It remains to be determined whether this difference is related to the high tolerance of its seeds to such extreme conditions.

However, dehydrin accumulation was clearly differentially altered in both cultivars when grains originated from their natural environments, i.e., cv. Baer La Unión exhibited three extra dehydrin bands, which were scarcely visible in cv. Sajama (the cultivar that grows in more extreme conditions), and a fourth dehydrin band that was not present in cv. Sajama (Fig. 1B). The role of these extra dehydrins in cv. Baer La Unión remains intriguing and warrants further investigations.

Preliminary studies carried out in our laboratory have shown differences in storage behavior and longevity among quinoa cultivars originating from different environments. We also propose to investigate dehydrin differences with regard to these aspects.

Acknowledgments

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