Annals of Botany **82**: 481–488, 1998
Article No. bo980704

Seed Structure and Localization of Reserves in *Chenopodium quinoa*

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Received: 12 March 1998 Returned for revision: 28 April 1998 Accepted: 4 June 1998

The three areas of food reserves in quinoa seeds are: a large central perisperm, a peripheral embryo and a one to two-cell layered endosperm surrounding the hypocotyl-radicle axis of the embryo. Cytochemical and ultrastructural analysis revealed that starch grains occupy the cells of the perisperm, while lipid bodies, protein bodies with globoid crystals of phytin, and proplastids with deposits of phytoferritin are the storage components of the cells of the endosperm and embryo tissues. EDX analysis of the endosperm and embryo protein bodies revealed that globoid crystals contain phosphorus, potassium and magnesium. These results are compared with studies on other perispermous seeds published to date.

Key words: *Chenopodium quinoa*, EDX analysis, phytoferritin, phytin, protein bodies, quinoa, seed structure, seed reserves, starch grains.

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd), a seed crop from the Andes region of South America, is also regarded as a crop elsewhere in the world because of its high nutritional value, particularly due to the exceptional seed protein amino acid balance and the quantity of nutritionally favourable lipids. Given the importance of quinoa seeds in the improvement of the food supply in the Andean communities and as an alternative food source in other regions (Castillo, 1995), there is extensive literature on seeds of quinoa and other species of *Chenopodium* covering a variety of aspects including chemical characterization of proteins (Fairbanks *et al*., 1990; Brinegar and Goundan, 1993; Nrisingha and Mandal, 1993), fatty acid composition of seed oils (Daun and Tkachuk, 1976; Wood *et al*., 1993) and nutritional values (Lehmann, Martinod and Moran, 1974; Allred, Mahoney and Hendriks, 1976; Sánchez Marroquin, 1983; Coulter and Lorenz, 1990; Lorenz, 1990; Dini *et al*., 1992; Chauhan, Eskin and Tkachuk, 1992; Prakash, Nath and Pal, 1993; Ranhotra *et al*., 1993; Ruales and Nair, 1993). However, the only information to date on the structural features of quinoa seeds comes from two studies by Cabrera Laverde (1983) and Varriano Marston and DeFrancisco (1984). Both papers give vague, inexact and incomplete information; for example, neither mention the existence of an endosperm and Cabrera Laverde (1983) even confuses it with perisperm. The ultrastructural analysis carried out by Varriano Marston and DeFrancisco (1984) was restricted to the identification of protein and lipid bodies in a undefined embryonic cell and the authors did not report the cells or tissue used in the EDX analysis.

The aim of the present study is to investigate quinoa seed structure and ultrastructure and the spatial localization of food reserves. In order to produce a complete structural study of quinoa seed we have used different but complementary methods. Along with a structural investigation using histochemical methods, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and an EDX analysis of elemental composition of globoid crystals present in the endosperm and embryo, were also carried out. This study was done as a prelude to physiological studies on seed storage behaviour.

MATERIALS AND METHODS

Source of material

Seeds of *Chenopodium quinoa* Willd from Chillan, Chile (1990) were cultivated in INTA, Castelar, Argentina. Seeds were collected in the summer of 1991–1992.

Specimen preparation

Whole seeds and small blocks of tissue cut from regions of the cotyledon, hypocotyl-radicle axis, endosperm and perisperm were fixed at 4 °C for 5–6 h in 2·5% glutaraldehyde in 0·1 M phosphate buffer (pH 6·8).

For light microscopy the fixed tissue was dehydrated through an ethanol series and embedded in butoxyethanol-methacrylate (JB4 Polisciences, Inc., Warrington, PA, USA). Both fresh, unfixed tissue and tissue that had been embedded for TEM were also used for light microscopy. As described by Maldonado and Lott (1991), the staining procedures used included: acid fuchsin (Sigma A3908) and Toluidine blue O (Sigma T 3260 CI 52040) (Feder and O'Brien, 1968); Coomassie brilliant blue (Sigma B 7920 CI 42660) (Gahan, 1984; Pearse, 1985); fast green FCF (Sigma F 7252 CI 42053) (Fulcher, O'Brien and Simmonds, 1972); iodine-potassium iodide (Sigma L 6146) and periodic acid-Schiff (PAS) preceded by aldehyde blockade with dimedone (Sigma D 3504) (O'Brien and McCully, 1981); and Sudan black B (Sigma S 2380 CI 26150) (Bronner, 1975). For

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detection of proteins, bromophenol blue (Sigma B 6131) was also used (Pearse, 1985).

For SEM, unfixed dry seeds were cut longitudinally and transversely, mounted onto aluminium stubs, gold coated and viewed with a scanning electron microscope JEOL JSM-T 100.

For EDX analysis, in order to avoid any fixative-based extraction, powder of dry tissues was used (Lott, Goodchild and Craig, 1984): whole seeds were placed in chloroform-methanol (2:1) for 1 week to extract lipids, then five seeds were dissected into tissue regions that were powdered. The powder was scattered on double sided tape on aluminium stubs. EDX analysis of globoid crystals of the cotyledons, radicle and endosperm were conducted. In each seed, twenty globoid crystals were analysed from each region. EDX analysis was carried out using a Philips SEM-EDX 505 scanning electron microscope. For semi-quantitative analysis, individual globoid crystals were analysed for 60 s at an accelerating voltage of 15 kV and at a count rate of between 1500–2000 cps.

For TEM, tissue fixed only in 2·5% glutaraldehyde, and tissue fixed in glutaraldehyde and post-fixed in 1% OsO_4, was dehydrated in a graded ethanol-propylene oxide series and embedded in Spurr's resin. Sections were mounted on grids coated with Formvar, stained in uranyl acetate followed by lead citrate, and examined under a JEOL 1200EX transmission electron microscope. Unstained sections were also examined for detection of phytoferritin (Robards and Humpherson, 1967).

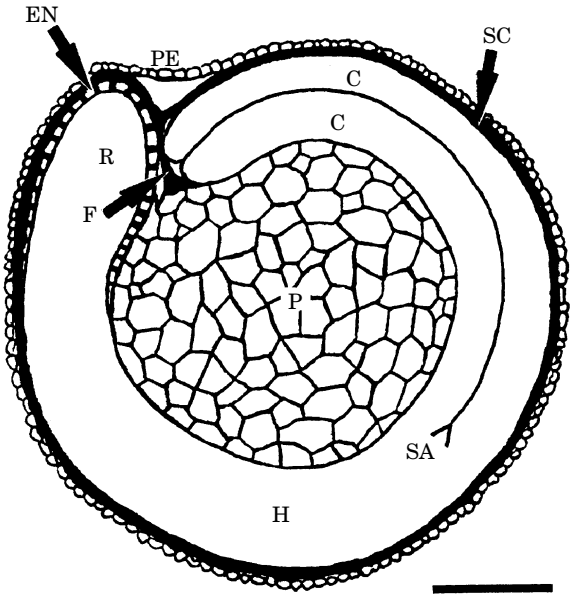


FIG. 1. *Chenopodium quinoa*: median longitudinal section of the grain. Pericarp (PE) covers the seed. The embryo consists of a hypocotyl-radicle axis (H) and two cotyledons (C). Endosperm (EN) is present in the micropylar region. F, Funicle; P, perisperm; PE, pericarp; R, radicle; SA, shoot apex. Bar = 500 μm.

RESULTS

General features

Quinoa seed corresponds to the campylotropous type of Boesewinkel and Bouman (1984) i.e. the embryo is peripheral and a basal body (cf. Bouman, 1984) is present in the seed as a storage tissue or perisperm (Fig. 1). In the mature seed, the endosperm is present only in the micropylar region of the seed and consists of one to two cell-layered tissue surrounding the hypocotyl-radicle axis of the embryo (Figs 2 and 3). A rectilinear vascular strand runs throughout the funiculus (Figs 1 and 2). Quinoa seeds are disseminated with the pericarp covering the seed (Figs 1–4).

The pericarp was two-layered (Fig. 4). The cells of the outer layer were large and papillose in shape. The inner layer was discontinuous and its cells were tangentially stretched.

The seed coat consisted of two layers of cells (Figs 3, 4 and 7): the exotesta and the endotegmen. The cells of the exotesta were large with very thick walls and reduced cytoplasm; starch grains and crystals (Fig. 7) were occasionally observed in the cytoplasm of these cells. The cells of the endotegmen showed small thickenings in the inner walls (Fig. 7). The endotesta and the exotegmen were collapsed, persisting only as cell walls.

Perisperm

The perisperm consisted of uniform, non-living, thin-walled cells. The cells were full of starch grains which were angular in shape (Fig. 5); nuclei and other cytoplasmic organelles were absent at this stage. Simple and compound starch grains occurred in the same cells (Fig. 6). Cell walls and starch grains stained with the PAS reaction; starch grains also stained with iodine-potassium iodide. The single grains varied in diameter from approx. 0·5 to 1·0 μm (Fig. 5). Compound structures, consisting of oblong aggregates of simple grains, were around 20–25 μm in size (Fig. 6).

Endosperm

The endosperm of the mature seed consisted only of one–two cell layers in the micropylar region, enveloping the hypocotyl-radicle axis (Figs 2–4 and 7).

Endosperm cells had thick hard outer-cell walls (Fig. 7). Cell walls stained with the PAS reaction and with Toluidine blue O. The cuticle (Fig. 5), which represents the nucellar cuticle, stained with Sudan black B. The cells have living cytoplasm with round or lobed nuclei. The proportion of normal constituents, i.e. cytoplasm, endoplasmic reticulum, proplastids and mitochondria was very small. Endoplasmic reticulum occurred as closely packed sheets of cisternae (Fig. 8). Proplastids (Figs 9 and 10) were recognized by the presence of deposits of phytoferritin (cf. Robards and Humpherson, 1967).

Protein and lipid bodies occupied most of the cytoplasm (Figs 11 and 12). Lipids were determined by cytochemical staining with Sudan black B. Endosperm protein bodies varied in diameter from 1 to 3 μm and consisted of a proteinaceous matrix containing one or more globoid crystals (Figs 11 and 12). The globoid crystals stained

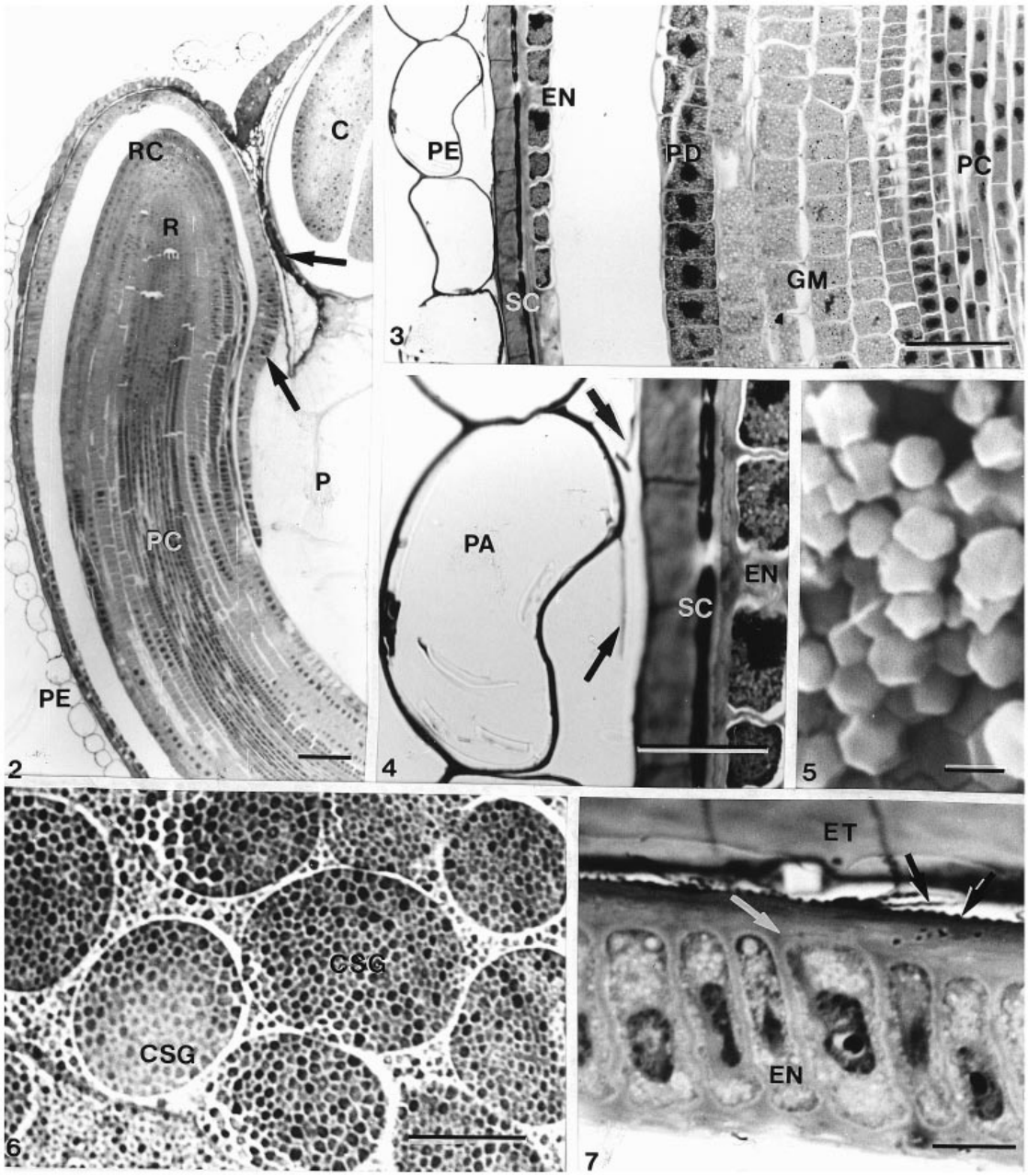


FIG. 2. A section of part of the hypocotyl-radicle axis: C, Cotyledon; P, perisperm; PE, pericarp; R, radicle tip; RC, root cap; endosperm (black arrow). Bar = 100 μm.

FIG. 3. Enlargement of Fig. 2. EN, endosperm; GM, ground meristem; PC, procambium; PD, protoderm; PE, pericarp; SC, seed coat. Bar = 50 μm.

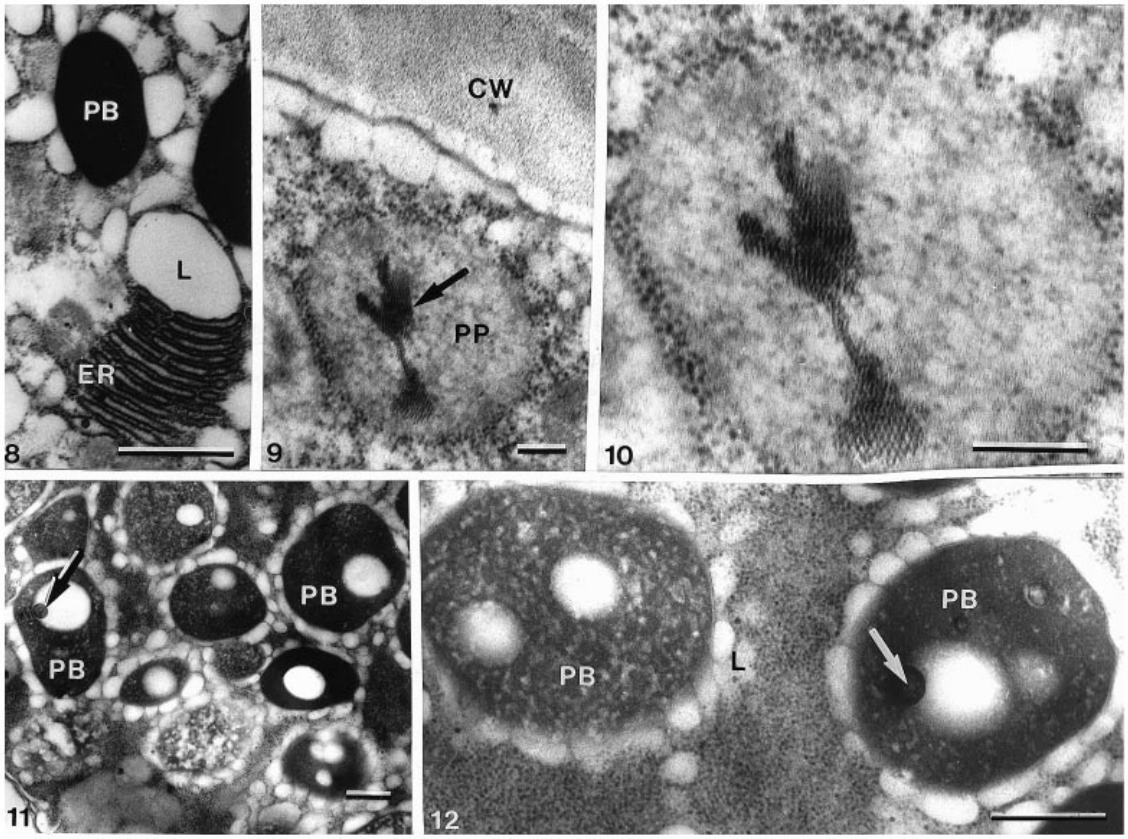
FIG. 4. Detail of pericarp and seed coat. The outer layer of the pericarp with papillose cells (PA) and the inner discontinuous layer with tangentially stretched cells (black arrows) are shown. One-layered seed coat (SC) in contact with the endosperm (EN). Bar = 25 μm.

FIG. 5. Scanning electron micrograph of a perisperm cell showing single starch grains. Bar = 1 μm.

FIG. 6. Light microscopy of a section of a perisperm cell, showing single and compound starch grains (CSG). Section was stained with PAS. Bar = 10 μm.

FIG. 7. Light microscopy of a section of the micropylar endosperm (EN). Endosperm cells have thick outer cell walls (white arrow). Cells of the exotesta (ET) with a crystal. Cells of the endotegmen showed thickenings in their inner walls (black arrows). Section were stained with PAS and Toluidine blue O. Bar = 10 μm.

metachromatically with Toluidine blue O and the proteinaceous region of the protein bodies stained with bromophenol blue, Coomassie brilliant blue, Fast green FCF and acid fuchsin. EDX analysis from powdered preparations revealed that globoid crystals contained P, K, and Mg (Fig. 23).



FIGS 8–12. Transmission electron micrographs of sections of the endosperm. Tissues were fixed with glutaraldehyde and stained with uranyl acetate and lead citrate. Empty areas in Figs 11 and 12 contained globoid crystals before they were chipped out during sectioning. Fig. 8. Section of part of a cell showing endoplasmic reticulum (ER) forming closely packed sheets of cisternae. Bar = 1 μm. Fig. 9. Proplastid (PP) of a cell of the endosperm with clusters of electron dense particles of phytoferritin (black arrow); CW, cell wall. Bar = 1 μm. Fig. 10. Enlargement of Fig. 10. Bar = 1 μm. Fig. 11. Section of an endosperm cell showing protein bodies (PB); black arrow indicates a globoid crystal. Bar = 1 μm. Fig. 12. An enlargement section of a cell of the endosperm showing lipid bodies (L) and two protein bodies (PB), one of them with a globoid crystal (white arrow). Bar = 1 μm.

Embryo

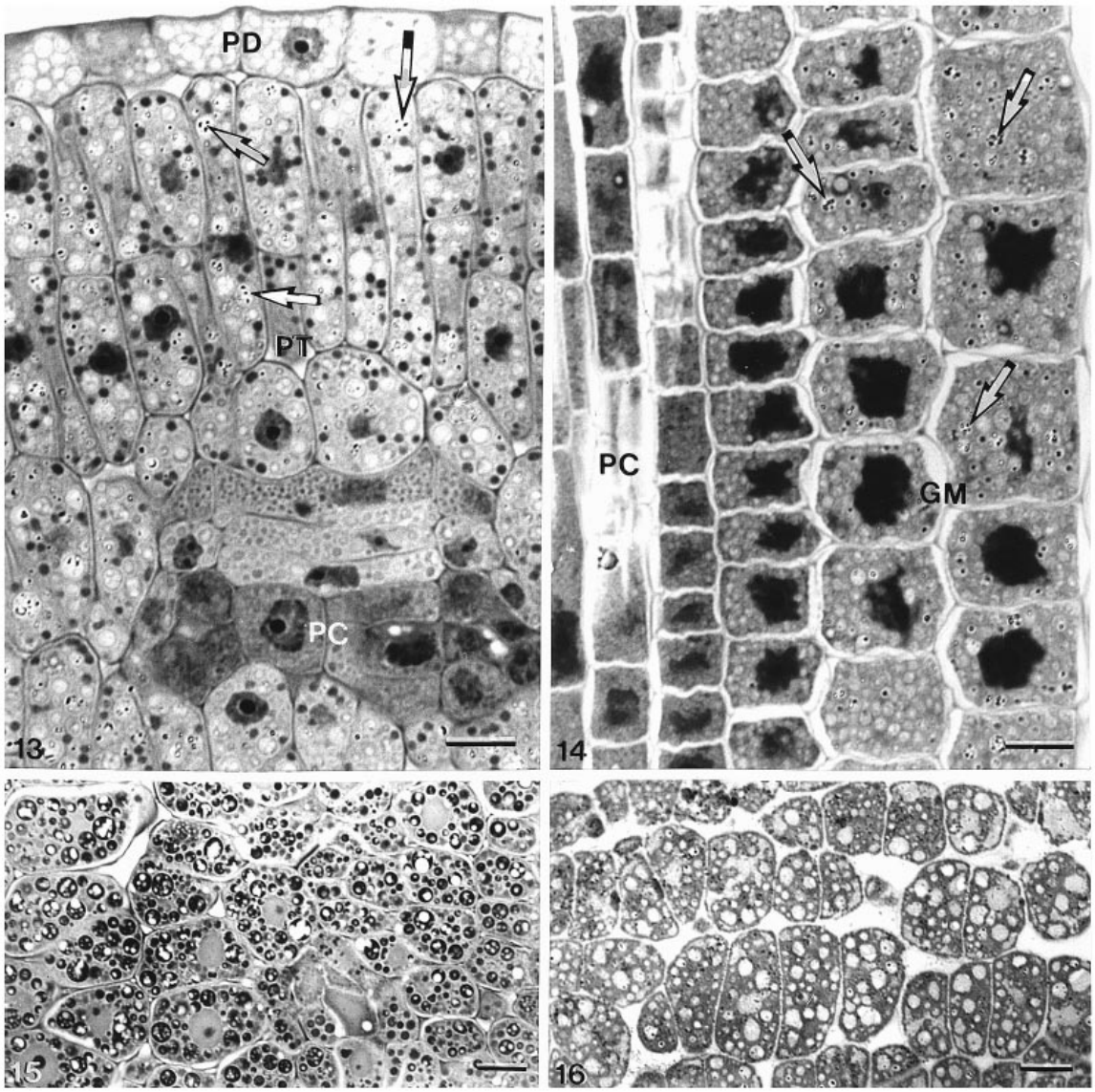
The embryo consisted of a hypocotyl-radicle axis and two cotyledons (Fig. 1); in the axis, the meristem of the root with the root cap (Fig. 2) and the apical meristem of the shoot were distinguishable. Protoderm, procambium palisade and spongy tissues were visible in the cotyledons and protoderm. Procambium and ground meristem were visible in the axis (Figs 3, 13 and 14).

All the embryo cells had thin primary cell walls that stained with PAS, Fast green FCF, acid fuchsin and Toluidine blue O. Nuclei were round or lobed and occupied the cell centre (Figs 13, 14 and 17). The endoplasmic reticulum was present in the form of closely packed sheets of cisternae (Fig. 19). Proplastids occurred in the protoderm and mesophyll of the cotyledons and in the ground meristem of the axis; they contained clusters of dense particles of phytoferritin and, occasionally, starch grains (Figs 20–22). Proplastids were seen to contain vacuole-like structures (Fig. 21).

All embryo cells, including those of the apical meristem, stored abundant protein and lipids in the form of protein and lipid bodies (Figs 15–18). The presence of lipids was determined by staining with Sudan black B. Protein in the protein bodies was determined by staining with bromophenol blue, Coomassie brilliant blue, acid fuchsin and Fast green FCF. Protein bodies contained one or more globoid crystals in the proteinaceous matrix (Figs 15, 17 and 18) and varied in diameter from 0·5 to 3 μm. Procambium and apical meristems contained small protein bodies. The proteinaceous matrix was less electron dense in the protein bodies of the protoderm and frequently also in the smaller protein bodies of the other tissues. Where the protein bodies were smaller, globoid crystals were also smaller. They stained metachromatically with Toluidine blue O (Figs 13 and 14). EDX analysis of globoid crystals from powdered preparation of different embryo tissues revealed that they contained P, K and Mg (Figs 24 and 25). For the three elements, values were not significantly different between cotyledons and axis. Traces of other minerals were not detected.

DISCUSSION

Localization of stored reserves inside the seeds of quinoa showed a marked compartmentation similar to that seen in *Amaranthus hypocondriacus* seeds (Coimbra and Salema,



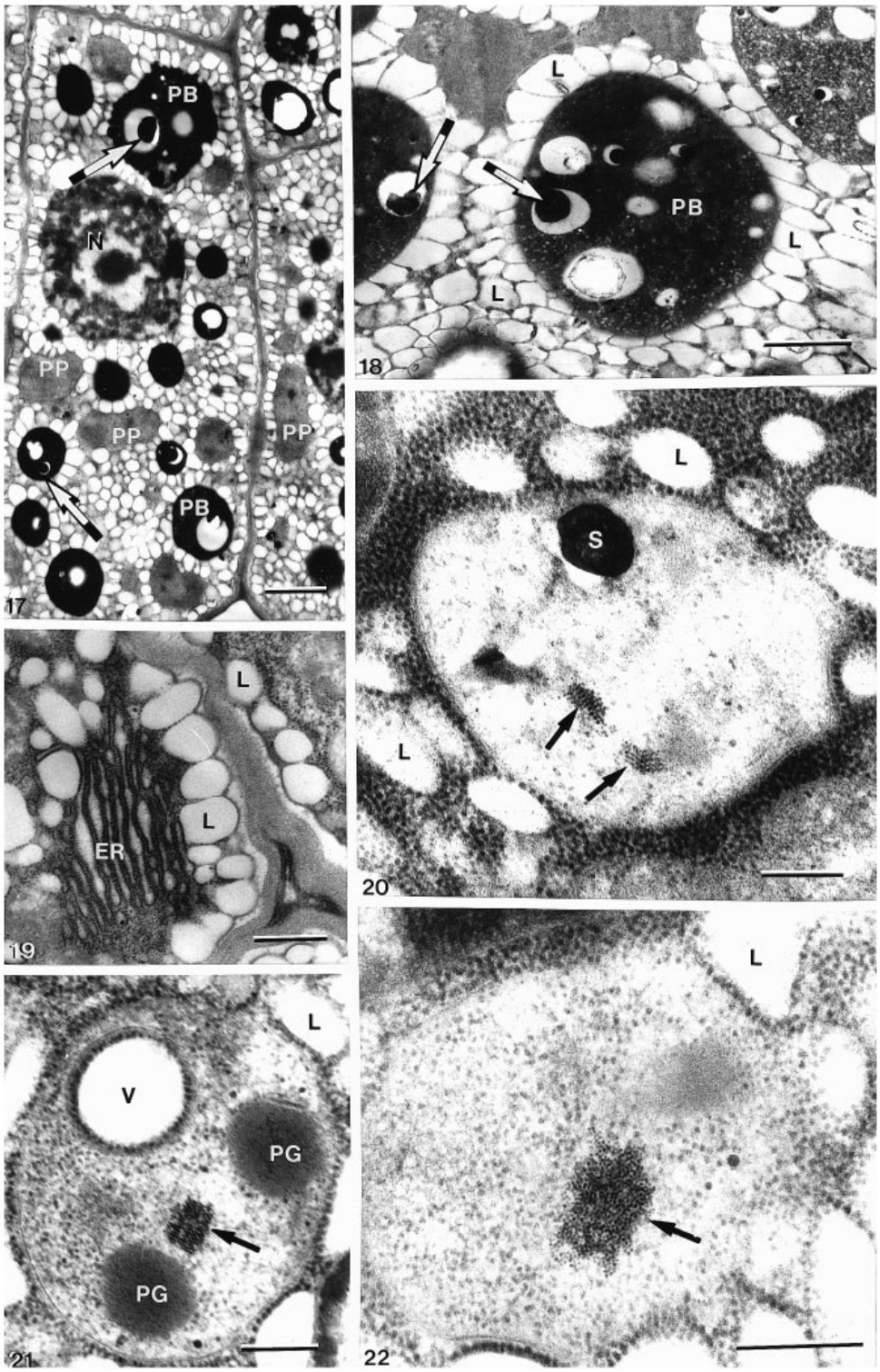
FIGS 13–16. Light micrographs of sections of embryo tissues. Figs 13 and 14. Sections were stained with PAS and Toluidine blue O. Globoid crystals (white arrow) stained metachromatically with Toluidine blue O. Fig. 13. Longitudinal section of a part of a cotyledon showing procambium (PC), protoderm (PD) and palisade tissue (PT). Bar = 10 μm. Fig. 14. Longitudinal section of a part of the hypocotyl-radicle axis showing procambium (PC) and ground meristem (GM). Bar = 10 μm. Fig. 15. Longitudinal section of the spongy tissue of a cotyledon: globoid crystals appear as light regions surrounded by the proteinaceous matrix that stained with Coomassie brilliant blue. Bar = 10 μm. Fig. 16. Longitudinal section of the spongy tissue of a cotyledon; in the cytoplasm, the area occupied by lipid bodies stained with Sudan Black B. Bar = 10 μm.

1994). Carbohydrate reserves are found principally in the perisperm, while proteins, mineral nutrients and lipid reserves are located mostly in the endosperm and embryo.

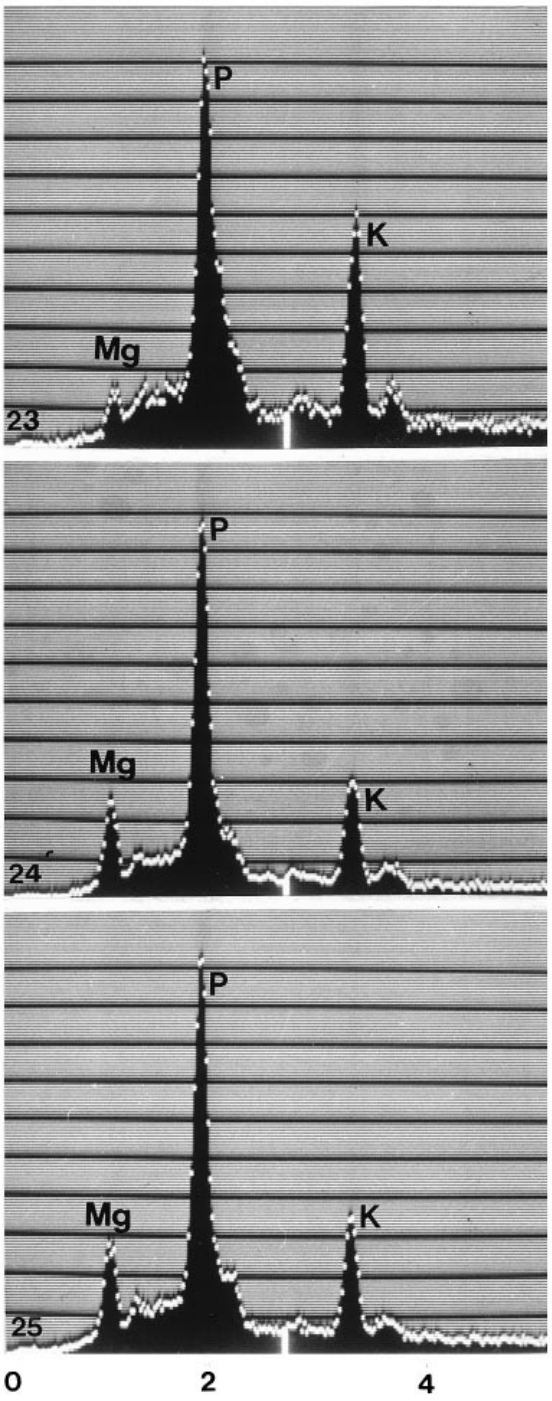
The general structure of quinoa seed, including the presence of one or two endosperm layers around the radicle, corresponds not only with that of other species of *Chenopodium* but also with other genera of Chenopodiaceae (Netolitzky, 1926; Hakki, 1972; Corner, 1976; Johri, Ambegaokar and Srivastava, 1992; Werker, 1997). The present study shows that the two-layer endosperm of quinoa seed consists of living thick-walled cells, rich in proteins and lipids, but without starch. The firm cover of the endosperm which surrounds the hypocotyl-radicle region can be seen as additional protection for the embryo which is only mechanically disrupted during germination; it might also play an important role in the control of germination. We propose to investigate these topics further.

We found proplastids in the cytoplasm of embryo and endosperm cells of quinoa. Proplastids contain clusters of electron dense particles of phytoferritin and, occasionally, starch grains. To date, phytoferritin in seed tissues has been reported in proplastids of cotyledons of some members of Fabaceae: *Pisum sativum* L. (Lobreaux and Briat, 1991), *Vicia faba* L. (Johansson and Wales, 1994) and *Cercis siliquastrum* L. (Baldan *et al*., 1995).

The protein bodies found in the embryo and endosperm



FIGS. 17–22. Transmission electron micrographs of sections of embryo tissues. Empty areas in Figs 17 and 18 contained globoid crystals before they were chipped out during sectioning. Fig. 17. Section of a palisade cell showing the nucleus (N), proplastids (PP), protein bodies (PB) with crystal globoids (white arrows). Bar = 2 μm. Fig. 18. Section of a ground meristem cell showing lipid bodies (L), protein bodies (PB) with crystal globoids (white arrows). Bar = 1 μm. Fig. 19. Section of a cell of the ground meristem showing endoplasmic reticulum (ER) forming closely packed sheets of cisternae; L, lipid body. Bar = 0·5 μm. Fig. 20. Proplastid of a cell of ground meristem. The proplastid contains clusters of particles of phytoferritin (black arrows) and one starch grain (S); L, lipid body. Bar = 1 μm. Fig. 21. Proplastid of a palisade cell. The proplastid contains a cluster of particles of phyoferritin (black arrow), plastoglobuli (PG) and a vacuole-like structure (V); L, lipid body. Bar = 1 μm. Fig. 22. Proplastid of a cell of the spongy tissue with a cluster of particles of phytoferritin (black arrow); L, lipid body. Bar = 1 μm.



FIGS 23–25. EDX analysis spectra of mineral nutrient stores in different tissues of quinoa seeds. Energy levels from 0·0 to 5·0 keV are shown on abscissas of the last spectrum. Fig. 23. Endosperm. Fig. 24. Cotyledon palisade tissue. Fig. 25. Hypocotyl-radicle axis ground meristem.

lack crystalloids and contain one or more globoids. They correspond to the models of protein bodies described by Lott (1981) as Types C, D and E.

P, K and Mg were identified as the major mineral elements of dehulled quinoa by Ranhotra *et al*. (1993). Varriano Marston and DeFrancisco (1984) found P, Mg and K in quinoa embryo based on EDX analysis, although they did not report the organelle, cell or tissue used in the analysis. In the present study, using EDX analysis, the three elements were identified as being the main constituents of globoid crystals in the protein bodies of both the embryo and the endosperm. Values were not significantly different between these tissues. Other minerals, such as Ca or Fe, were not found in the globoid crystals.

The cells of the perisperm have very thin walls and are full of angular-shaped starch grains. These features had been recognized in quinoa seeds by Varriano Marston and DeFrancisco (1984) and agree with previous reports in cells of the perisperm of *Amaranthus* and several other taxa of the Caryophyllales (Netolitzky, 1926; Becker *et al*., 1981; Okuno and Sakaguchi, 1981; Coimbra and Salema, 1994). According to Greenwood (1976) the grain shape depends on the amylose content: the less angular, rounded grains having relatively higher amylose levels. The grain shape of quinoa, like that of *Amaranthus hypocondriacus* seems to indicate that they contain mainly amylopectin.

EDX analysis of globoids have been carried out from perisperm tissues of only five species: *Piper nigrum* L., *Beta vulgaris* L., *Coffea arabica* L., *Zostera capricorni* L. and *Yucca brevifolia* Engelm. (West, Flannigan and Lott, 1993), revealing the presence of P, K, Mg and Ca. Obvious protein bodies containing globoids were found only in *Yucca* perisperm. In the other four species, globoids but not protein bodies, were present. In this study, we found that neither protein bodies nor naturally electron-dense spherical particles occurred in the perisperm.

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