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EMBRYO ULTRASTRUCTURE IN *ORIGANUM MAJORANA* L. (LAMIACEAE) AFTER SEED CONDITIONING

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Cytological changes in quiescent or germinated embryos after matriconditioning with Micro-Cel E or osmoconditioning with polyethylene glycol (PEG) were studied in comparison to quiescent or germinated untreated embryos of marjoram (*Majorana hortensis* L.). The sequence of changes related to embryo activation was identical in untreated and conditioned samples, although conditioned embryos underwent activation earlier. In those embryos the degradation of protein and lipid bodies, accompanied by vacuolation and accumulation of starch grains in amyloplasts, was observed even in nongerminated samples, whereas in control embryos the same ultrastructural changes did not occur until germination. The changes in ultrastructure occurred first in the root cap and proceeded towards the shoot meristem. In cotyledons, few symptoms of activation were detected regardless of the treatment. The appearance of Golgi structures in the root cap identified the radicle protrusion stage of germination.

Key words: Embryo ultrastructure, marjoram, matriconditioning, osmoconditioning, priming.

INTRODUCTION

Marjoram (*Origanum majorana* L.) is an aromatic plant native to the Mediterranean and Turkey. In temperate climate the crop is annual as it does not survive the winter outdoors, and plants are obtained either from seed, division, transplants or cuttings. Due to the still-increasing demand for *Herba* and *Oleum Maioranae*, propagation from seeds sown in situ is of great interest (Suchorska and Tołwiński, 1998). In marjoram, mericarps containing a single nonendospermic seed with a spatulate embryo are used for sowing rather than the seed proper. According to the dormancy classification by Baskin and Baskin (2004), a seed that "has the capacity to germinate over the widest range of normal physical environmental factors possible for the genotype" is nondormant. A nondormant seed that does not germinate due to unfavorable environmental factors is in a state of quiescence. Marjoram seeds are nondormant (Duczmal and Tucholska, 2000) and can be stored for 3–4 years in the dry state (=quiescent state), but their germination usually takes several weeks under field conditions. Uneven germination and slow seedling growth in spring are some of the factors limiting marjoram cultivation from sowing in situ. The problems could be overcome by seed osmo- or matriconditioning, which are prehydration seed treatments used to enhance seed quality, providing typical and healthy seedlings, and also to ensure an early and synchronized harvest (Khan et al., 1992).

Osmoconditioning (or osmopriming) (OC) consists in submerging the seeds in salt or polyethylene glycol (PEG) solutions. The osmotic potential of the solution and the time of priming are precisely adjusted to allow the seeds only limited water uptake (Olszewski et al., 2005). During OC, seeds undergo imbibition (first phase of germination), followed by a period in which seed water content is relatively constant (second phase), but radicle protrusion (third phase) is prevented; thus germination is not completed (Bewley and Black, 1994; Bray, 1995). After OC (or MC) the seeds are usually brought back to the quiescent phase by means of dehydration to the original water content (Khan et al., 1992).

In matriconditioning (MC), the carrier of water is a solid substance such as brown coal, charcoal, peat

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Abbreviations: OC – osmoconditioning; MC – matriconditioning; NCQE – nonconditioned quiescent embryos; CQE – conditioned quiescent embryos; NCGE – nonconditioned germinated embryos; CGE – conditioned germinated embryos.

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moss or Micro-Cel E (Dąbrowska and Kolasińska, 1997). To achieve the best seed quality, the proportion (by weight) of seeds, carrier and water should be carefully adjusted (Khan et al., 1992). MC affects seeds in a manner similar to osmopriming, but is considered more effective in improving seed quality (e.g., Dąbrowska et al., 2001). MC is combined with other methods of seed quality enhancement such as pretreatment with fungicide, insecticide, gibberellin or *Bacillus* bacteria (Andreoli and de Andrade, 2002, 2003). Knowledge of changes occurring in the embryo after MC is of increasing importance from the practical point of view.

Seed conditioning is defined as enhancement of its physiological and biochemical events through mobilization of seed reserves to maximize germination and emergence (Khan et al., 1992). The reserves are usually complex water-insoluble compounds, so the question of how their mobilization is affected by conditioning is of interest. Changes such as DNA repair, accumulation of β-tubulin, and accelerated conversion of 1-amino cyclopropane carboxylic acid to ethylene have been found to take place during the second phase of germination after priming. A strong correlation between OC and increased endo-β-mannanase activity has been demonstrated in seeds of tomato and onion (Toorop et al., 1998 Kępczyńska et al., 2003). Compared to the number of agricultural and physiological studies related to the beneficial effects of seed conditioning, there are far fewer microscopy studies in this area. In one such work, conditioning permitted the conversion of protein bodies (also termed protein storage vacuoles; Jiang et al., 2001) into vacuoles, degradation of lipid bodies, accumulation of starch in amyloplasts, and proliferation of rough endoplasmic reticulum (RER) and glyoxysomes within the radicle and lower part of the hypocotyl in carrot seeds (Dawidowicz-Grzegorzewska, 1997). The purpose of this study was to identify the structural features of osmo- or matriconditioned marjoram embryos that could account for the better quality of primed seed, by comparing the structure of conditioned and control (untreated) marjoram embryos before and after germination.

MATERIALS AND METHODS

Marjoram mericarps with mature dry seeds were obtained from a Polish commercial seed supplier (PNOS, Ożarów Mazowiecki, Poland) and osmo- or matriconditioned as described by Pytel (2000). The mericarps were osmoconditioned in polyethylene glycol (PEG–6000, 240 g × kg^{-1}) solution over five days at 15°C under constant white light, then rinsed with distilled water and air-dried at 20°C to their original weight. MC in Micro-Cel E proceeded for six days at 15°C under constant white light. The weight ratio of fruits, carrier and water was 3:1:4. Sieved fruits were dried at 20°C. After dehydration, the mericarps were stored at room temperature for about one year before use in the study.

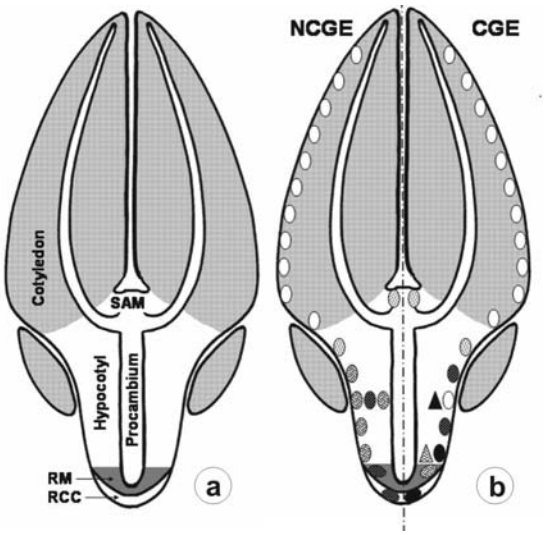

Fig. 1. (**a**) Simplified structure of typical marjoram embryo (longitudinal section). SAM – shoot apical meristem; RM – root meristem; RCC – root cap cells. (**b**) Schematic overview of protein body degradation. Triangle indicates matriconditioning when the outcome differs from osmoconditioning. NCGE – nonconditioned germinated embryos; CGE – conditioned germinated embryos. Five degrees of shading of ovals from black to white indicate the degradation stages: complete, nearly complete, advanced, partial and initiated, respectively.

For germination, control or conditioned marjoram mericarps were surface-sterilized in 70% ethanol for 1 min followed by 15 min in 3.5% aqueous calcium hypochlorite. Next they were incubated in sterile Petri dishes on 1% agar for 24 h. After this, the protruding radicle was just visible in some mericarps (at this stage germination is regarded as finished; Bewley and Black, 1994), and only these were collected for fixation. Unfortunately it was impossible to isolate embryos from dry mericarps. To enable embryo isolation, nongerminated fruits (untreated control, osmo- or matriconditioned) were soaked for 6 h in water. These batches are referred to as quiescent embryos to distinguish them from the germinated ones, although soaking presumably resulted in some physiological activation.

Embryos from quiescent or germinated mericarps were isolated gently from the coat tissues and immediately fixed for 24 h under –0.06 MPa air pressure in a mixture of 5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). After fixation, samples were rinsed repeatedly with the same buffer, postfixed for 2 h with 2.5% OsO_4 in 0.1 M cacodylate buffer, and rinsed again.

Embryos were dehydrated through an ethanol and acetone series. Specimens were embedded in glycid ether 100 epoxy resin (SERVA), hard grade, and polymerized at 60°C. For light microscopy, sections 2 μm thick were cut with a Jung-RM2065-Supercut microtome (Reichert-Jung/Leica) and stained at 70°C with 1% aqueous azure A and 2% methyl blue in 2% borax. Observations and micrographs were made with an Axioskop light microscope (Opton) equipped with Nomarski optics and Contax 167MT camera. Embryos showing symptoms of nonviability in semithin sections were excluded from TEM observations. Five viable embryos per treatment were ultrathin-sectioned with an Ultracut E microtome (Reichert), collected on Formvar-coated slot-grids and contrasted with lead citrate and uranyl acetate. They were viewed with JEM 100C or JEM 1220 (JEOL) transmission electron microscopes (TEM) operating at 80 kV.

RESULTS

NONCONDITIONED QUIESCENT EMBRYOS

The results relate to different embryo regions; for clearer presentation of the results, Figure 1a provides a simplified scheme of typical marjoram embryo structure (longitudinal section).

As a rule, storage organelles (protein and lipid bodies) took up most of the cell volume in all cells of nonconditioned quiescent embryos (NCQE). Soaking marjoram mericarps for 6 h in water did not result in microscopically visible cell activation, as the storage organelles exhibited no signs of reserve degradation. All protein bodies contained globoids, except for those located in root cap cells. Hypocotylar and cotyledonary protein bodies contained globoids and large irregular electron-dense inclusions. Lipid bodies were located at the surface of the protein bodies and along the plasma membrane. The nuclei were small, compact and irregularly shaped, with electron-dense chromatin. Their irregular shape probably was due to the mechanical pressure of large protein bodies. Mitochondria and proplastids were small, and their matrix was usually rather electron-transparent with a central fibrillar domain. Their internal membrane systems were poorly developed (less so in apical meristem cells). In most tissues, small electron-dense organelles, presumably glyoxysomes, were noted in the cytoplasm. Neither Golgi structures nor vacuoles were observed.

Marjoram root cap cells had thin primary cell walls, except that the outer ones were considerably thicker and cuticle-covered (Fig. 2a). Their dense cytoplasm contained sparse, short RER cisternae (Fig. 2a), but there were many unbound ribosomes. The protein bodies were smaller than those in the radicle or hypocotyl tissues, and contained few inclusions. There were fewer lipid bodies than in cells of the adjoining root meristem.

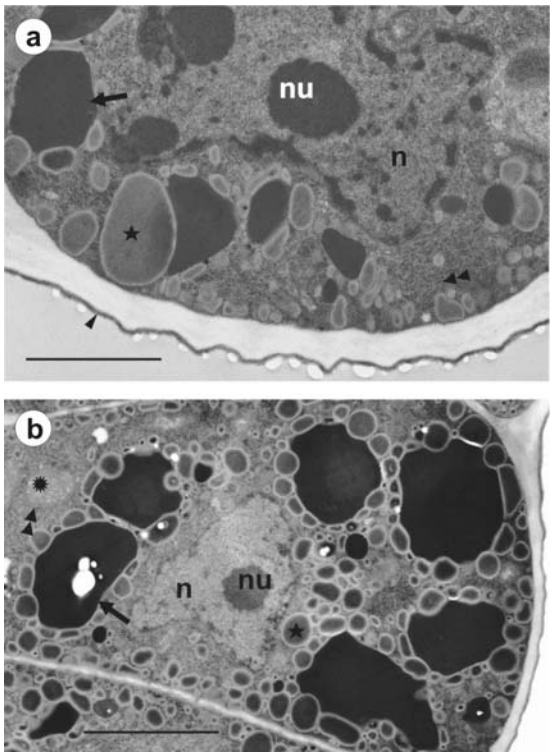

Fig. 2. Untreated marjoram embryo in quiescent phase. (**a**) Marginal root cap cell. In the dense, ribosome-rich cytoplasm, note the presence of lobed nucleus (n) with nucleolus (nu), lipid bodies (star) and protein bodies (arrow). Double arrowhead indicates rough endoplasmic reticulum. Single arrowhead shows cuticle on outer side of cell wall. Bar = 1.8 μm, (**b**) Epidermal cell near the rim of root cap. Lobed nucleus (n) with nucleolus (nu), lipid bodies (star), protein bodies (arrow), mitochondrion with electron-transparent regions in the matrix (rosette), rough endoplasmic reticulum (double arrowhead). Bar = 3.3 μm.

The root meristem, which in control embryos was entirely ensheathed by the root cap, exhibited closed structure, with clearly delineated tiers of the initial cells of the plerome, periblem and dermatocalyptrogen. In meristem cells the ultrastructural organization was generally similar to that of the root cap cells, but the fine structure of plastids and mitochondria was more differentiated.

In the hypocotyl, protoderm cells contained fewer but bigger protein bodies than in the other hypocotylar tissues (Fig. 2b). The protoderm outer cell walls were thicker and cuticle-covered. Sparse ribosomes and many large protein bodies were visible in the thin-walled cortical parenchyma cells. The elongated procambium cells were rich in ribosomes; their protein bodies were small and rich in globoids.

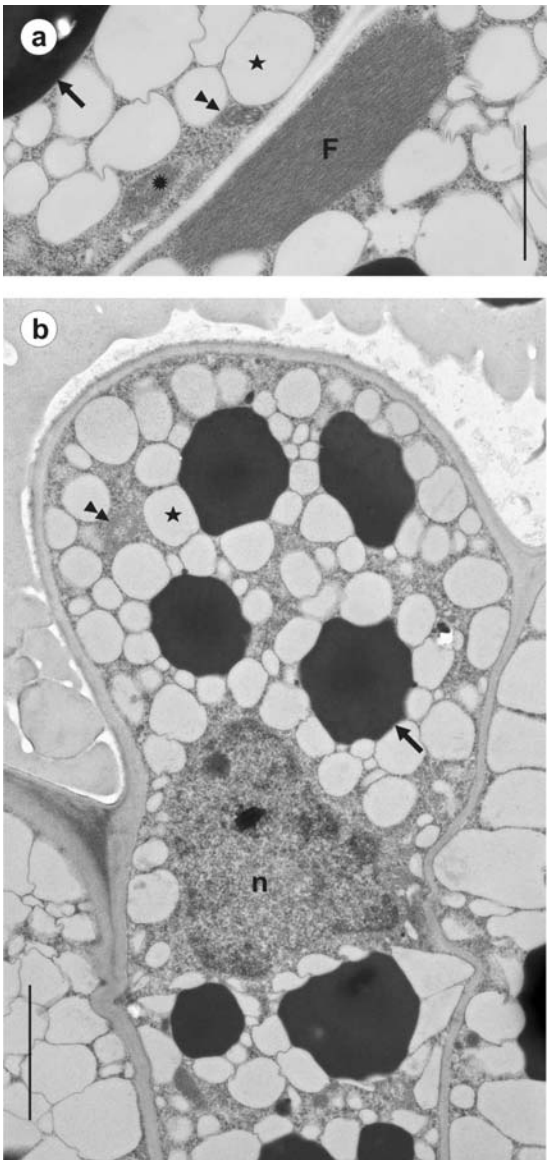

Fig. 3. Untreated marjoram embryo in quiescent phase. (**a**) Bundle of fibrillar protein (F) in the cytoplasm of cotyledon mesophyll cells. Star – lipid body; arrow – protein bodies; rosette – proplastid; double arrowhead – mitochondrion. Bar = 1.7 μm, (**b**) Unicellular trichome primordium near shoot apical meristem. n – nucleus; star – lipid body; arrow – protein bodies; double arrowhead – mitochondrion with fibrillar domain in matrix. Bar = 1.8 μm.

In the shoot apical meristem a uniseriate tunica was discernible. The meristem was composed of thin-walled cells, with smaller protein bodies and fewer lipid bodies than in root and hypocotyl tissues.

In cotyledons, compact mesophyll was differentiated into palisade and spongy tissues, both lacking intercellular spaces. Locally, the otherwise thin cell walls were thickened and contained numerous deposits of electron-dense material. Exclusively in mesophyll cell cytoplasm, thick bundles were found of probably proteinaceous fibrillar material, not bound with the membrane (Fig. 3a). In marjoram embryo mesophyll, protoplasts were filled with very large protein bodies containing globoids and extremely electron-dense irregular inclusions, tightly surrounded by lipid bodies (Fig. 3a). Protodermal cells were similar in ultrastructure to mesophyll cells except for having smaller protein bodies and thicker outer cell walls covered with cuticle. Cotyledonary procambium was composed of narrow cells rich in lipid bodies. Protein bodies were smaller in these cells. Fewer ribosomes were noted than in the hypocotylar procambium.

In the protoderm of the cotyledons and hypocotyl, unicellular and bicellular trichome primordia were found (Fig. 3b). At this stage (and at the other stages examined in this work) it was not possible to distinguish the type of hair, secretory or structural, that would have developed from them. Their protoplasts contained numerous ribosomes but relatively small protein bodies (Fig. 3b). Lipid bodies were fewer than in the hypocotyl or cotyledons. RER cisternae were short and sparse.

NONCONDITIONED GERMINATED EMBRYOS

Figure 1b presents a schematic overview of protein body degradation in nonconditioned germinated embryos (NCGE) and conditioned germinated embryos (CGE). After germination occurred in untreated embryos of marjoram, visible changes were observed in their fine structure as compared with quiescent (control) embryos. The changes varied between individuals and between particular regions of the embryo. The most conspicuous and most generally expressed change was degradation of protein bodies and their concurrent conversion to vacuoles, usually with some fibrillar and electron-dense remnants of the storage material still present at the time of fixation. Degradation started in the root cap cells and proceeded successively towards the shoot apical meristem. Lipid bodies were usually scattered in the cytoplasm in NCGE. A transitional stage was observed in some cells, however, in which lipid bodies were no longer associated with the membrane of protein bodies but were grouped together, occupying particular cytoplasmic domains. The cell nuclei were usually less electron-dense than in control embryos, and round in section. The proliferation (or appearance) of RER was initiated, as well as the differentiation of proplastids into amyloplasts and differentiation of mitochondria. The latter process was less evident than expected. The microbodies easily found in quiescent marjoram embryos were no longer discernible in germinated ones.

In the periplasmic space of marjoram root cap cells, many small, homogenous and electron-dense

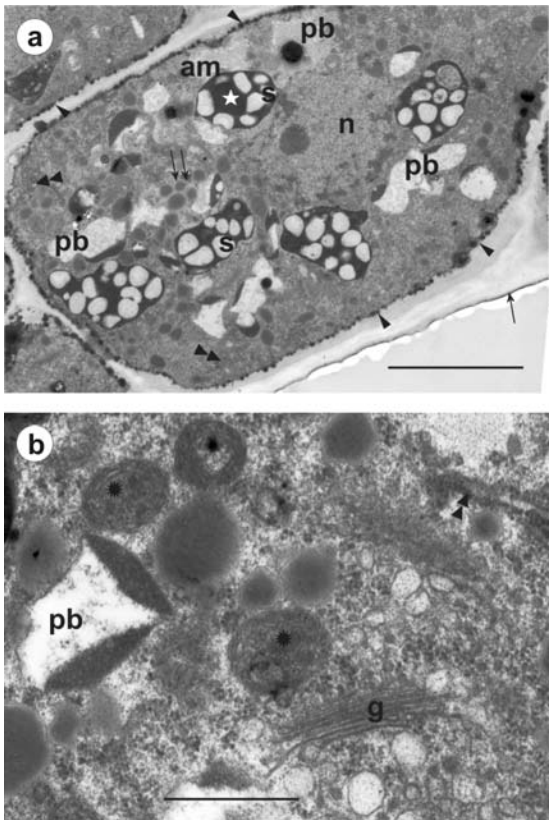

Fig. 4. Germinating untreated marjoram embryo. (**a**) Apical cell of root cap. n – nucleus; double arrow – lipid bodies scattered in the cytoplasm; pb – degraded protein bodies; am – amyloplasts with starch grains (s) and electron-dense inclusion (white star); double arrowheads – rough endoplasmic reticulum; arrowheads – osmiophilic globuli in periplasmic space; arrow – cuticle. Bar = 4.2 μm, (**b**) Root cap cell. pb – partly degraded protein bodies; double arrowhead – rough endoplasmic reticulum; rosettes – mitochondria with developed cristae; g – Golgi body. Bar = 0.6 μm.

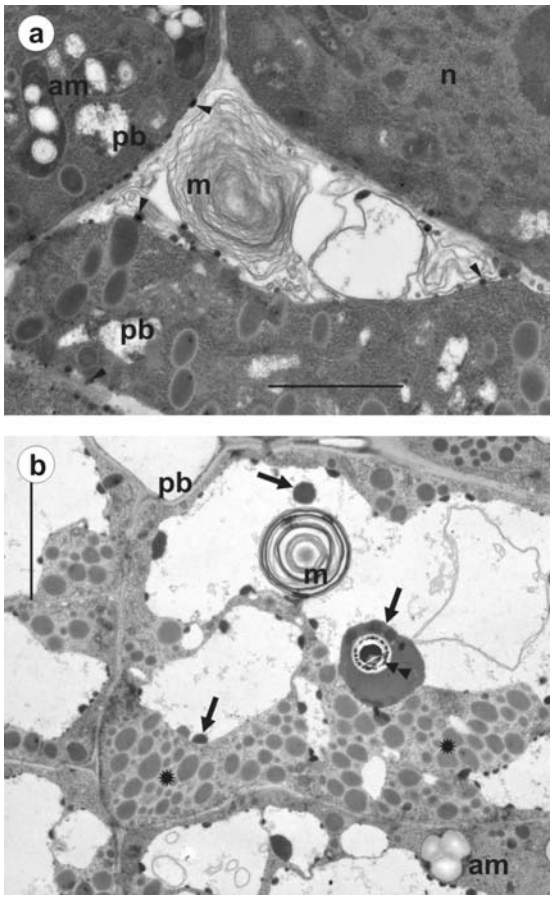

Fig. 5. Germinating untreated marjoram embryo. (**a**) Root meristem cells. n – nucleus; pb – degraded protein bodies; am – amyloplasts with starch grains; arrowheads – osmiophilic globuli in periplasmic space; m – myelin-like membranous structures between cell wall and plasma membrane. Bar = 1.5 μm, (**b**) Cortical parenchyma cells midway along hypocotyl length, on convex side. arrows – electron-dense remnants of storage protein in vacuole; double arrowhead – remnants of globoid; m – myelin-like membranous structure in vacuole; rosettes – cytoplasmic domain rich in lipid bodies; am – amyloplast. Bar = 4.7 μm.

globules appeared (Fig. 4a). Their frequency decreased towards the proximal rim of the root cap. The breakdown of protein bodies was nearly complete; vacuoles with few remnants of storage proteins were observed. Lipid bodies became randomly scattered in the cytoplasm. New structures appeared during germination: many vesicles, Golgi bodies and amyloplasts (Fig. 4b), and their starch grains were the largest of all the ones in embryo tissues. The inner membrane of the amyloplast envelope invaginated into branched tubules, and inclusions of an electron-dense substance were often visible within the invagination lumen.

In the root meristem, the transformation of protein bodies into vacuoles was similar to the process observed in the root cap (Fig. 5a). However, some lipid bodies that no longer adjoined protein bodies were clustered in particular cytoplasmic domains. Some electron-dense globuli were found in the periplasmic spaces, as in root cap cells. A conspicuous feature of this region was slight plasmolysis of some cells, with prominent, tubular or vesicular membranous structures revealed between the cell wall and plasma membrane (Fig. 5a). In some sections the structures were very extensive and three-dimensionally complex.

In the hypocotyl, the breakdown of protein bodies was most advanced midway along its length, although less than in the radicle (Fig. 5b). It was most evident in the middle layers of the cortical parenchyma, and on the convex side if the hypocotyl was bent. Here, vacuoles were already fused into a single one. In other parts of its parenchyma as well as its epidermis and procambium, protein bodies were only partially degraded, but lipid bodies were no longer associated

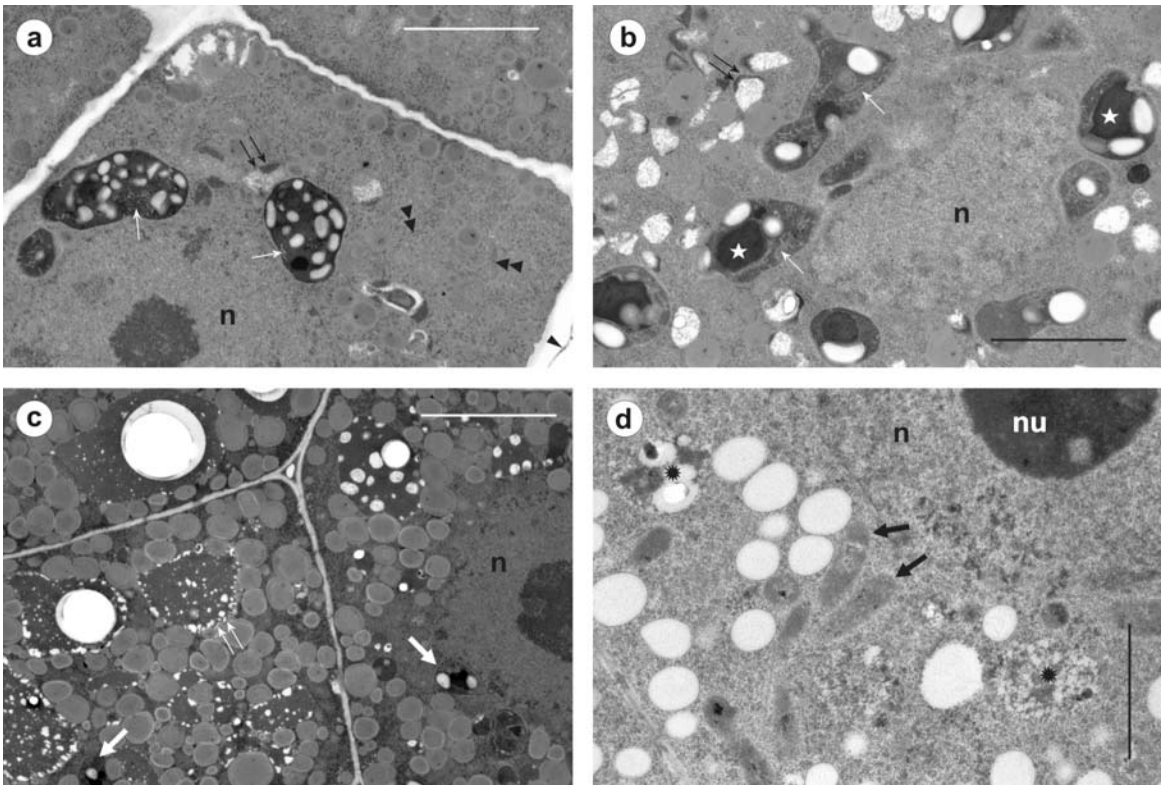

Fig. 6. Matriconditioned marjoram embryo in quiescent phase. (**a**) Root cap cell. n – nucleus; double arrow – degraded protein bodies with osmiophilic remnants of storage protein; white arrows – tubular network in amyloplasts; double arrowheads – rough endoplasmic reticulum; arrowhead – cuticle. Bar = 2.5 μm, (**b**) Hypocotyl epidermal cell at boundary with root cap. n – nucleus; double arrow – degraded protein bodies with electron-dense remnants of storage protein; white arrows – well-developed tubular network and osmiophilic inclusions (white stars) in amyloplasts. Bar = 2.2 μm, (**c**) Hypocotyl cortical cell at boundary with root meristem. n – nucleus; double arrow – partly degraded protein bodies with osmiophilic remnants of storage protein; arrows – amyloplast with osmiophilic inclusions and starch grains. Bar = 4.5 μm, (**d**) Tunica cell from shoot meristem. n – nucleus with nucleolus (nu); rosettes – partly degraded protein bodies with fibrillar remnants of storage protein; arrows – proplastids. Bar = 1.1 μm.

with their membrane. At the root/hypocotyl boundary, the epidermal cells contained small electron-dense globuli in the periplasmic space. The amount of globuli rapidly diminished basipetally. Epidermal and cortical amyloplasts (Fig. 5b) formed tubular invaginations of the envelope's inner membrane and accumulated electron-dense inclusions. Long RER cisternae appeared in epidermal cells, in contrast to the short ones within the cortical parenchyma. The changes within procambial cells in the hypocotyl (and cotyledons) followed the same course as described for parenchyma cells, but no starch grains were found in their enlarged and very electron-dense plastids, and invaginations of the inner membrane of plastids were rarely seen.

In the shoot apical meristem the protein bodies were partly degraded, and only small amounts of starch were accumulated in plastids. Mitochondria and RER were still relatively sparse.

In the cotyledonary mesophyll, the structural changes due to germination were limited to differentiation of cristae in mitochondria and the appearance of a few small starch grains in amyloplasts. Even when starch was absent, however, plastids formed some tubular invaginations, and electron-dense inclusions, although small, were usually present. In epidermal cells only, degradation of protein bodies was just beginning. As usual, it coincided with the differentiation of amyloplasts. In the cotyledonary procambium, differentiating sieve tubes were found, distinctive for their thickened cell walls. In these cells, protein bodies were completely degraded and few lipid bodies were found. The inner membranous systems in mitochondria and plastids were poorly developed, and usually instead of starch some electron-dense inclusions accumulated in plastids.

In the hypocotylar and cotyledonary trichome primordia, polysomes and many short RER cisternae appeared. Lipid and protein bodies were almost completely degraded. The very electron-dense plastids were enlarged, and developed branched tubular invaginations with electron-

dense inclusions, but the presence of starch grains varied between primordia.

CONDITIONED QUIESCENT EMBRYOS

The structural changes observed in all conditioned quiescent embryos (CQE) were similar. Most of them did not depend on the conditioning method, and generally they had structure very similar to that of nonconditioned germinated embryos. Below are described the most significant features, as well as the differences observed between the osmo- and matriconditioned ones.

Regardless of the treatment, in root cap cells no electron-dense globuli were observed in the periplasmic space (Fig. 6a), and no Golgi bodies were found.

In matriconditioned embryos, the root meristem cells contained few, small and in most cases undegraded protein bodies, while in osmoconditioned ones only the remnants of degraded protein were observed near the protein body membrane.

Regardless of the treatment, cortical parenchyma and epidermal cells in the hypocotyl underwent similar changes (Fig. 6b,c). In matriconditioned embryos, small amyloplasts with starch grains were observed only in cells in which degradation of protein bodies was initiated, whereas in osmoconditioned ones fine starch grains were found in epidermal and cortical plastids regardless of the occurrence or lack of protein body degradation. In the hypocotylar (and cotyledonary) procambium, some cells began their differentiation into sieve tubes. The protoplasts of such cells, joined by broad plasmodesmata encircled by a callosic layer, lacked vacuoles and had only a few lipid bodies. Their nuclei were compact, and the inner structure of plastids and mitochondria was simplified.

The ultrastructure of shoot meristem cells (Fig. 6d) was very similar to that of NCGE.

In the epidermis and spongy parenchyma of cotyledons, cells generally did not differ in ultrastructure from those of NCQE (Fig. 7). In a few protein bodies, however, degradation was initiated near the membrane. The only difference between osmo- and matriconditioned embryos was the presence of starch grains in plastids of matriconditioned ones.

Conspicuous changes in the fine structure of the hypocotylar trichome primordia were noted after conditioning. In these cells the protein bodies were almost completely degraded, unlike the neighboring cells in the upper part of the hypocotyl. In plastids, numerous electron-dense inclusions accumulated, but no starch. Unlike the hypocotylar primordia, cotyledonary primordia did not degrade storage protein, and their mitochondria and plastids were poorly differentiated. In these unicellular primordia, polarization of the protoplast was observed: large protein bodies were located proximally, and small ones distally.

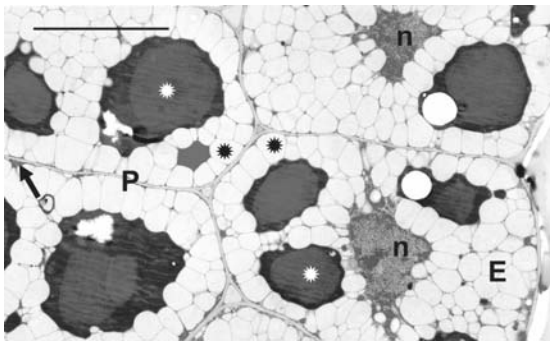

Fig. 7. Osmoconditioned marjoram embryo in quiescent phase. Epidermal (E) and parenchymatous (P) cotyledon cells. n – nucleus; black rosettes – lipid bodies; white rosette – protein bodies; black arrow – proplastid. Bar = 6.7 μm.

CONDITIONED GERMINATED EMBRYOS

In conditioned germinated embryos (CGE), the ultrastructural changes were more advanced than in corresponding tissues of CQE, and the developmental stages were more advanced than in NCGE. The most significant features of CGE are characterized below.

Strongly electron-dense globuli were observed in the periplasmic space of the root cap of both matri- and osmoconditioned embryos (Fig. 8a). In that tissue, partial plasmolysis of some cells was evident (Fig. 8b), with membranous structures revealed in the periplasmic space. Protein bodies were degraded and converted to vacuoles with loosely fibrillar remnants of storage material. The structure and location of lipid bodies did not change during germination. In a few cases, amyloplasts were lobed and spatially associated with mitochondria.

In the root meristem, due to partial plasmolysis of cells, membranous vesicles, tubules and coils were revealed in the periplasmic space (Fig. 8b). Unlike in untreated germinating embryos, electron-dense globuli did not occur in the periplasmic space (Fig. 8b). Protein bodies were less degraded than in NCGE but more degraded than in CQE. Lipid bodies, still numerous, were associated together within particular cytoplasmic domains (Fig. 8b).

In the hypocotyl, epidermal cells exhibited high variability of protein body degradation. In matriconditioned embryos, the least degraded ones were observed near the root cap cells, and the most degraded protein bodies in cells midway along the length of the hypocotyl (Fig. 9a). In osmoconditioned embryos, complete degradation of storage proteins was observed in cells close to the root cap; the degree of degradation rapidly decreased basipetally, becoming imperceptible midway along the length of the hypocotyl. Myelin-like structures were often found in the vacuoles formed from the degraded protein bodies. The structures contained extra membranes left

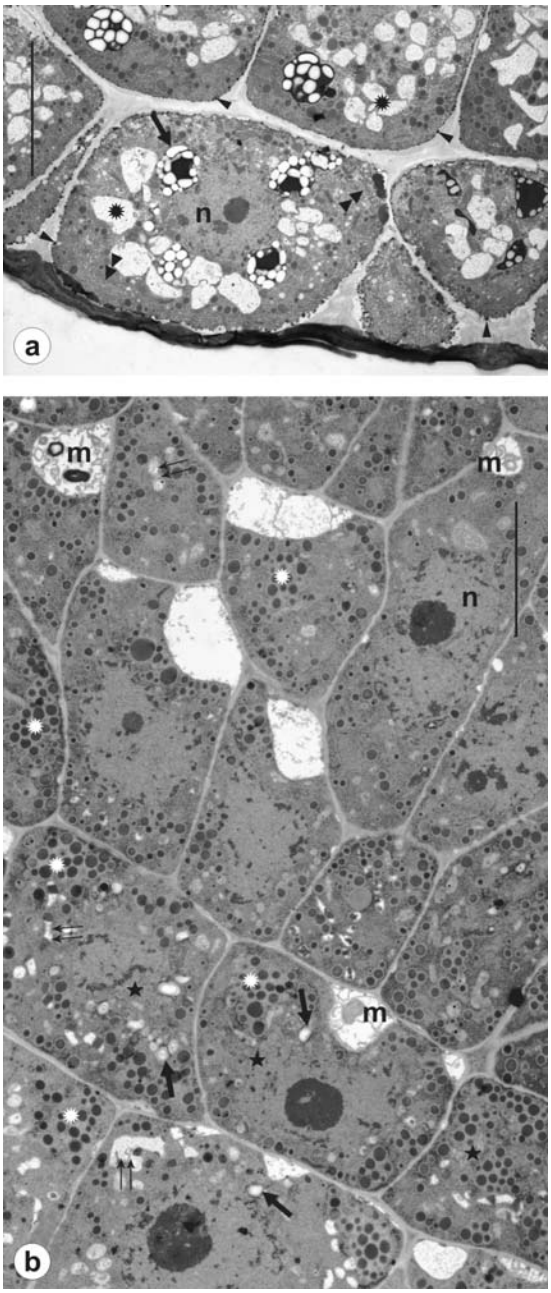

Fig. 8. Geminating conditioned marjoram embryos. (**a**) Osmoconditioned germinating embryo. Root cap cells. n – nucleus; rosettes – vacuoles derived from degraded protein bodies; arrow – amyloplast with starch grains and electron-dense inclusion; arrowheads – electron-dense inclusions in periplasmic space; double arrowheads – areas in cytoplasm rich in cisternae of rough endoplasmic reticulum. Bar = 5.5 μm, (**b**) Matriconditioned germinating marjoram embryo. Root cap and root meristem cells (stars indicate initial cells of periblem). n – nucleus; double arrows – degraded protein bodies; m – membranous structure in periplasmic space; white rosettes – cytoplasmic domains of lipid bodies; arrows – amyloplasts; arrowhead – mitochondrion. Bar = 5.6 μm.

after the protein bodies coalesced into a large vacuolar compartment (Bethke et al., 1998). In every epidermal cell, starch grains in amyloplasts were small, regardless of the degree of protein degradation. In cortical cells, the size and number of starch grains in amyloplasts were positively correlated with the extent of protein degradation. Within the stroma of plastids, a tubular network with small electron-dense inclusions appeared. In the hypocotylar procambial tissue, developing sieve elements had significantly thickened cell walls. Their long RER cisternae were often already arranged into packets, or else they surrounded plastids which had simplified fine structure. The differentiating sieve elements lacked protein bodies, lipid bodies and vacuoles, and their mitochondria tended to be located parietally. In other procambial cells, plastids contained electron-dense inclusions rather than starch grains. A few dividing plastids were recognized.

In shoot meristem cells, the structure of protein bodies was nearly the same as in quiescent ones. However, amyloplasts with starch grains appeared during germination. The tubular system in plastids, when present, contained small electron-dense globuli in the lumen of invaginations. A few metaphase cells were found, indicating cell cycle activation in the meristem.

In cotyledons, the fine structure of most protein bodies remained unchanged in the mesophyll cells; their structure was similar to that of untreated quiescent embryos (Fig. 9b). Only a few small protein bodies underwent degradation, and some electron-dense inclusions appeared within the lumen of plastids' inner membrane network. In cotyledonary procambial strands, differentiated sieve tubes were found. Developing tracheary elements were also observed; their protein bodies were completely degraded and only sparse lipid bodies were found in them. In the other procambial cells, degradation of protein bodies was just beginning. No starch grains were found in procambial plastids.

Hypocotylar trichome primordia were unicellular or bicellular, while only unicellular ones were observed on cotyledons. Metaphases or anaphases were found in some primordia. Compared to CQE, more RER cisternae were found in the cytoplasm, and conversion of protein bodies to vacuoles was advanced. In bicellular primordia the basal cell was more vacuolated. This probably was already preprogrammed in the unicellular primordia of CQE, according to the polarization pattern of their unicellular primordia.

DISCUSSION

As described in this work, the ultrastructure of non-conditioned quiescent embryos of marjoram corresponds with descriptions of dry embryo ultrastructure (DeManson, 1988; Werker, 1997; Weng, 1998; Fiordi et al., 2001). The cell ultrastructure of quies-

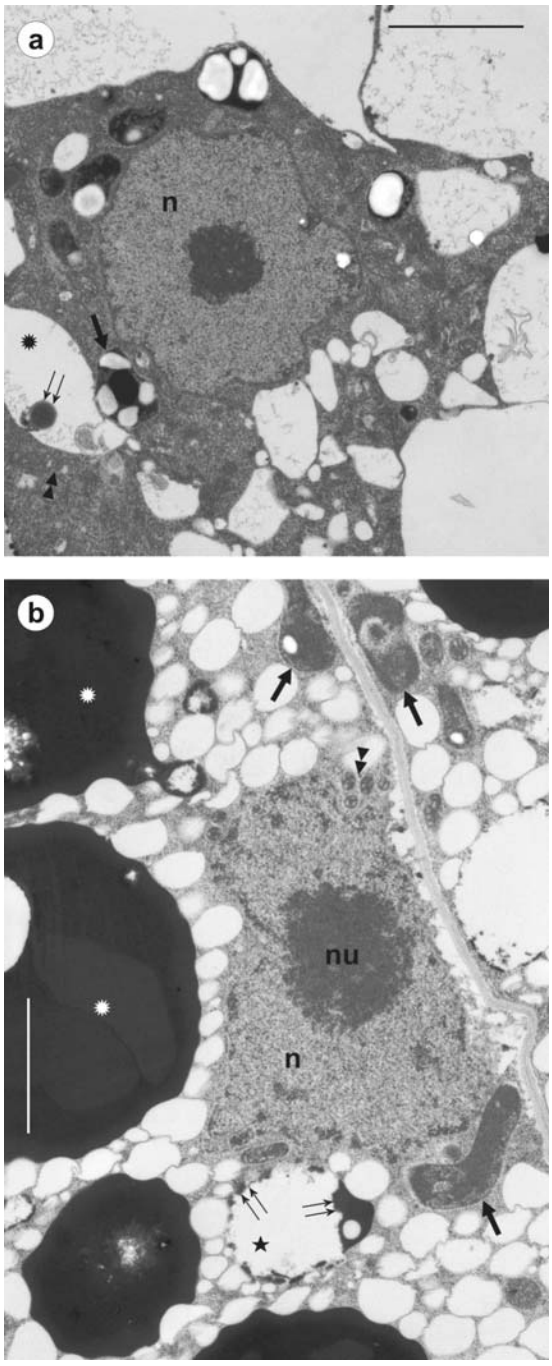

Fig. 9. Germinating matriconditioned marjoram embryo. (**a**) Hypocotyl epidermal cell. n – nucleus; black rosette – vacuole derived from degraded protein bodies with remnants of storage protein (double arrow); double arrowhead – mitochondria; arrow – amyloplast with starch grains and electron-dense inclusion. Bar = 2.9 μm, (**b**) Cotyledonary mesophyll cells. n – nucleus with nucleolus (nu); star – partially degraded protein body with remnants of storage material (double arrows); white rosettes – protein bodies devoid of protein degradation symptoms or in initial stage of degradation; arrows – proplastids; double arrowhead – mitochondria. Bar = 2.1 μm.

cent embryos is often referred to as simplified. In *Brassica napus* dry embryos, cell organelle ultrastructure was not equally simple in all embryo tissues and organs (Kuraś, 1984). Cells of shoot and root meristems had less simplified structure than cells from other embryo parts. Such was the case in the studied marjoram as well. While in marjoram embryos the cells of the respective meristems contained relatively fewer protein and lipid bodies, the fine structure of their plastids and mitochondria was more differentiated. However, only short cisternae of RER and no Golgi structures were found in these meristems, a trait indicating low metabolic activity.

In marjoram NCQE, every cell of the embryo accumulates reserves in specialized organelles. It is typical for seed reserves to be accumulated and mobilized not only in specific storage tissues (cotyledons are usually mentioned) but also in the embryonic axis (Tiedemann et al., 2000). Also typically, variability of protein body ultrastructure within the embryo is observed in different plant species, for example in *Washingtonia filifera*, Arecaceae (DeManson, 1988).

The thick bundles of fibrillar material found in mesophyll cell cytoplasm of marjoram NCQE were similar to bundles described earlier in mature nongerminated rice seeds (Bechtel and Pomeranz, 1978), *Washingtonia filifera* cotyledons (DeManson, 1988), *Vicia faba* embryos (Briarty et al., 1970) and *Nicotiana alata* pollen (Cresti et al., 1985). Although the function of these structures was not determined, it was supposed that they represented another form of reserve protein or stored enzymes used later in germination.

The ultrastructural changes observed in nonconditioned germinated marjoram embryos are generally similar to those reported earlier (Werker, 1997) in different plants. The ultrastructural changes are evident but unevenly expressed between individuals or between embryo tissues. The most general change is degradation of protein bodies and their simultaneous conversion to vacuoles. A similar process was elegantly documented in germinating seeds of *Tillandsia*, Bromeliaceae (Fiordi et al., 2001). Differentiation of mitochondria was less evident than expected in marjoram. On the other hand, even the mitochondria of dry seeds contain enough Krebs cycle enzymes and terminal oxidases to provide ATP to support metabolism for several hours after imbibition (Bewley, 1977). Microbodies easily discernible in nonconditioned quiescent embryos of marjoram were no longer discernible in conditioned germinated embryos. This was contrary to expectations, as the glyoxysomes are involved in β-oxidation of storage fatty acids, and thus proliferate during germination (Kilarski, 2003). The discrepancy probably was due to the resemblance between sections of glyoxysomes and leucoplasts after hydration of marjoram cells, making the organelles poorly distin-

guishable, especially when starch or envelope invaginations are absent from the section. Histochemical labeling of glyoxysomes would be needed to follow their differentiation in germinating embryos.

In some parts of the activated marjoram embryo, many small, homogenous and electron-dense globuli appeared in the periplasmic space. The chemistry of these globuli cannot be determined from conventionally contrasted sections, but two possibilities should be mentioned. First, deposits of phenolics appear electron-dense and homogenous after osmification and lead contrasting (Nielson and Griffith, 1978), and such compounds occur in embryos (Wronka et al., 1994; Kuraś et al., 1999; Stefanowska et al., 2003). On the other hand, in auxin-treated coleoptiles of *Zea mais*, globuli of similar ultrastructure were cytochemically determined as (possibly glycosylated) proteins (Kutschera and Edelmann, 2005, and literature cited therein), and the appearance of the structures was interpreted as associated with the initiation of cell wall extension, and maintenance of it. A conspicuous feature of root meristem in germinated untreated marjoram embryos was slight plasmolysis of some cells, with membranous structures between the cell wall and plasma membrane. Two questions arise. First, why did plasmolysis occur? Since the mericarps were placed on aqueous agar for the germination, the influence of medium osmolarity can be discounted. Fixation is also doubtful as a cause, because it did not occur in already vacuolated cortical cells of the hypocotyl, which would be much more susceptible to osmotic disturbances. Possibly cells within the plasmolyzed region leaked osmotically active substances to the apoplast. It is known that the influx of water into cells of quiescent seeds during imbibition results in temporary perturbations to membranes, leading to immediate and rapid leakage of solutes into the surrounding imbibition solution (Bewley, 1997). Within a short time the membranes return to their more stable configuration and the leakage is curtailed. Second, what was the origin of the membranous structures revealed in the periplasmic space of plasmolyzed cells? The accumulation of periplasmic membranes in the form of lomasomes or other structures has usually been associated with exocytosis (Battey et al., 1999). Within the root meristem of the untreated marjoram embryo, which at the time of fixation was still inactive mitotically, this process could hardly be considered intense enough to produce such amounts of excessive membranes. Possibly the membranes remained there from the stage of premature embryo. It has been shown that auxin, which has a central role in the patterning of root meristems (Jiang and Feldman, 2002), enhances membrane flow from the endoplasmic reticulum to the plasma membrane, possibly via activation of a cluster of genes responsible for induction and acceleration of exocytotic processes connected with cell wall metabolism (Hager et al., 1991).

The present study, the first carried out on marjoram embryos, showed that conditioned quiescent embryos differed from unconditioned ones. The period of increased metabolic activity of the embryo (5 or 6 days, depending on the conditioning method) resulted in ultrastructural differentiation that was analogous, with some exceptions, to that occurring during germination. When after conditioning the quiescent phase was restored by dehydration, differentiation was maintained during about a year of seed storage. This accords with the aim of conditioning, since both osmoconditioning and matriconditioning of seeds allow water uptake followed by biochemical and physiological activation, but prevent protrusion of the radicle (Khan, 1992; Bewley and Black, 1994; Bray, 1995). The metabolic activation of marjoram embryos resulting from conditioning, as inferred from the ultrastructural changes, started from the root cap and proceeded towards the hypocotyl and plumule. Degradation of reserves was more advanced in conditioned quiescent embryos of marjoram than in nonconditioned germinated ones. This observation is generally consistent with those from other plants, for example germinating seeds of apple (Dawidowicz-Grzegorzewska, 1989), vetch (Schlereth et al., 2000) or bean (Müntz et al., 2001). However, the wave of activation must have proceeded unevenly, resulting in unequal progress of structural changes in various embryo tissues. This was most evident in the case of protein body degradation, where the differences in nonconditioned germinated or conditioned but still quiescent marjoram embryos involved not only different organs of the embryo but also different tissues of the same organ. This seems typical, as similar phenomena have been noted in plants distantly related to marjoram such as *Vicia sativa* and *Phaseolus vulgaris* (Müntz et al., 2001; Schlereth et al., 2000; Tiedemann et al., 2000, 2001). In these plants, reserve mobilization started in the radicle and proceeded acropetally. The species-specific pattern of protein body degradation during germination depends on the composition of proteinaceous reserves (globulins vs. other proteins, with globulins degraded first) in particular tissues, and the amount of stored proteinases. In *V. sativa* the first tissues to undergo globulin breakdown were the epidermis and the procambium of the embryonic axis, and the differentiation of functional conductive tissue started there during germination. In *P. vulgaris*, globulins were absent in the embryonic axis procambium, and thus the degradation of protein bodies was less expressed in this tissue than in *V. sativa*. In *V. sativa* cotyledons, unlike in the embryonic axis, storage globulins strongly predominated over stored proteinases, explaining why protein degradation was nearly blocked until the postgerminative stage, when *de novo* synthesized proteinases began their activity. However, globulin breakdown in cotyledonary procambium preceded that in the other cotyledonary tissues, and co-occurred with the formation of

functional vascular tissues. In marjoram embryos, early differentiation of conductive elements was also observed. Unlike in the other mentioned plants, it was more evident in the cotyledonary than in the hypocotylar procambium.

In conditioned quiescent marjoram embryos and in both types of germinated ones, accumulation of starch was concomitant with the progress of protein degradation. Generally, starch synthesis was positively correlated with the degree of protein body degradation, but starch appeared before the degradation of protein bodies became noticeable in some cells. In *Brassica napus*, Kuraś (1986) made a similar observation, describing the appearance of starch grains as the first symptom of metabolic activity. This process, like protein body degradation, was also expressed differentially between embryo organs/tissues, both in marjoram and *B. napus*. In marjoram, an exception to the positive correlation of protein body degradation and starch synthesis was in procambial strands (especially differentiating sieve elements), which accumulated little or no starch, while protein hydrolysis in them was advanced or complete.

Regardless of the organ, plastids in every activated marjoram embryo contained highly electron-dense substance accumulated within the invagination of the inner membrane of the plastid envelope. In view of the location of that substance and its homogenous ultrastructure, it is doubtful that the substance is proteinaceous. Presumably the inclusions accumulated electron-dense components of thylacoid membranes or compounds usually located in chloroplastic plastoglobuli. Early accumulation of mono- or diterpenes, which are known to be synthesized in plastids (McCaskill and Croteau, 1995; Turner et al., 1999; Bouvier et al., 2000) should also be considered, however remote that possibility might seem in nonsecretory embryonic cells. In marjoram embryos, two ultrastructural components (Golgi structures and periplasmic electron-dense globular deposits) were found only in germinated embryos, regardless of the treatment. The appearance of Golgi bodies, as well as numerous vesicles in the root cap cells of germinated embryos, was probably a symptom of the taking on of the secretory function by this tissue. During the stage of radicle protrusion and its subsequent growth, the root cap would secrete polysaccharides to facilitate the root's mechanical penetration of the soil, and it would produce border cells, aiding in the development of specific rhizosphere conditions. As the root cap is the first part of the germinating plant to emerge into the soil environment, accumulation of phenolic compounds within the apoplast of the root cap – assuming that such was the chemical composition of the electron-dense globuli – is probably a symptom of the activation of an early defense system against soil phytopathogenic microorganisms.

Another interesting feature of the investigated material was the presence of trichome primordia in quiescent embryos already, both control and conditioned ones. At this early developmental stage it was not possible to identify the kind of trichome, structural or secretory, into which they would eventually differentiate. Regardless of the specific structure, after differentiation they would function within the plant's protection system against various unfavorable environmental conditions such as ultraviolet radiation or excessive feeding by herbivores. Since marjoram originates from a dry and warm climatic zone, early formation of a protective system should be very important for the development of the aerial organs of this species.

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