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Anatomical studies on the adventitious roots of the geophyte *Urginea maritima* (L.) Baker

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The anatomy and histochemistry of the adventitious roots of *Urginea maritima* (L.) Baker and the plant's adaptive strategies to the Mediterranean climate were investigated. The adventitious roots of *U. maritima* are bounded by a multiple-layered velamen, responsible for rapid water uptake. Both the epidermis and endodermis of the root are uniseriate. The cortex is 741.3 μm thick and is composed of numerous large parenchyma cells with storing character. In the cortex, scattered idioblastic cells containing numerous raphides of calcium oxalate exist. The average length of the calcium oxalate needles is 72 ± 22 μm. The presence in the cortex of cells containing in their vacuoles soluble polysaccharides is conspicuous after employing the Schiff's staining technique. Also, numerous cortical cells are filled with lipids and become intensely stained brown to black when treated with sudan black B. Morphometric studies have shown that idioblastic cells occupy 19.83% of the cortex relative volume, cells containing lipids 14.38%, and cells containing polysaccharides 11.27%. The cortex storage cells occupy 34.11% of the total root volume. The average volume of the cortical cells is 73143 μm^3. The vascular cylinder is usually 10-arched. The root xylem consists of vessels in short radial rows. The phloem consists of sieve elements located between the vessel rays. *Urginea maritima* possessing adventitious roots proves to be efficient in storing water during the long summer drought, less susceptible to climatic stress and well synchronized with the climatic fluctuations. Specialized features of *U. maritima* adventitious roots are, at least in part, responsible for the species' occurrence and frequent dominance in a wide array of semiarid ecosystems of the Mediterranean region.

Key words: adventitious root, anatomy, geophyte, Mediterranean climate, morphometry, *Urginea maritima*.

INTRODUCTION

Urginea maritima (L.) Baker is a perennial bulbous geophyte (a herbaceous plant with an underground storage organ) of the family Liliaceae (Bruneton, 1996), native to the Mediterranean basin and well-adapted to its type of climate (Kopp *et al*., 1996). It generally occurs in the slopes of hills, the sandy grounds near the Mediterranean Sea (Battandier, 1893; Gentry *et al*., 1987) and in certain regions of Northern Africa (Cuenod *et al*., 1954; Rogues, 1959; Bellakhdar, 1997), Middle East, and Europe.

Urginea maritima has two major phenological phases within a year. An active one, (autumn-spring) from leaf emergence to senescence of the aerial parts (photosynthetic period), and an inactive one (summer), which lasts till the leaves emerge (dormancy). The over-wintering bulb develops leaves from the shoot apex from December to March and flowering stalks from August to September. The plant has ornamental value, flowering after several years when the bulb reaches a considerable size (Pascual-Villalobos & Fernandez, 1999).

Urginea maritima has two varieties: red and white. The red variety (red squill) is predominant in Tunisia (Cuenod *et al*., 1954; Makhlouf, 1978), Algeria (Battandier, 1893), and Greece. The white variety is predominant in Morocco (Bellakhdar, 1997). The plant can reach 1 m in height. The leaves are wide (3-10 cm), long (30-100 cm), lanceolated, somewhat undulated, shining and dark-green. The bulb is tunicated,

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mostly globular. It can reach the size of a child's head, consisted of fleshy coats, which overlap with each other. The flowers are white, small and numerous; they are arranged in terminal racemes. The fruits are loculicidal capsules containing numerous flattened seeds (Cuenod *et al.*, 1954).

From the phytochemical point of view, it has been reported that the major constituents of *U. maritima* bulbs are glycosides (cardiac glycosides) of the bufadienolide type (Kopp *et al*., 1996). Anthocyanins (Vega *et al*., 1972), flavonoids (Fernandez *et al*., 1972), fatty acids, polysaccharides (Spies *et al*., 1992) and calcium oxalate are also present (Cogne *et al*., 2001). The cardiac glycosides (scillaren and scillarenin) are used as a cardiotonic diuretic for the treatment of cardiac marasmus and edema (Harvey & Champe, 1992; Mitsuhashi *et al*., 1994). Scilliroside, the major toxic glycoside, occurs in all plant parts including the leaves, flowers, stalks, scales, and especially the roots and the core of the bulbous part (Verbiscar *et al*., 1986b). Poisoning occurs frequently in autumn; there are marked differences in the susceptibility of livestock to the *U. maritima* (Fitzpatrick, 1952). Young calves are more susceptible, while goats (Blood & Radostits, 1989) and wild boars (Makhlouf, 1978) are less. The scillioside is further used as a rodenticide (Verbiscar *et al*., 1986a), while the bulb extract is a strong insecticide (Pascual-Villalobos & Robledo, 1999). Rivera & Obon de Castro (1991) mention that *U. maritima* bulbs have been planted in some cases touching the roots of fruit trees in Spain to avoid ant infestations. Raphides of calcium oxalate (3%) have an irritant action on mucosa (Makhlouf, 1978).

There is little information on *U. maritima* from the biological point of view, except for the morphological and caryological studies by Pfosser & Speta (2004), a few phytochemical studies (Verbiscar *et al*., 1986a,b; Kopp *et al*., 1996; Krenn *et al*., 2000) and some pharmacological studies (Pascual-Villalobos & Robledo, 1999). *Urginea maritima* is a consistent component of the Mediterranean vegetation. It is dominant over wide areas and important for homeopathic therapy. In this first paper of a series we focus on the anatomical study of the adventitious roots of the plant. The ultimate goal is to identify the structure and function of the root and give some points on the abundance of *U. maritima* in the Mediterranean region. More specifically, we have tried to elucidate the plant's adaptive strategy to semiarid environments, its efficiency in water and nutrient storing during the long summer drought and its defense mechanisms against herbivores.

MATERIALS AND METHODS

Urginea maritima (L.) Baker plants were collected from a hill about 10 km north-west of Chania, on the island of Crete, southern Greece. Adventitious root segments (2-3 cm from the root tips) were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer for 2 h. After post-fixation in 1% osmium tetroxide and dehydration in an ethanol series, the tissue was embedded in Spurr's epoxy resin. Semi-thin sections (1 μm thick) from resin embedded tissue were obtained with a Reichert OM U_2 ultra microtome; they were stained with 0.5% toluidine blue O in 5% borax and photographed using an inverted photomicroscope ECLIPSE TE2000-S (Nikon). Ultrathin sections (0.08 μm thick) were stained with uranyl acetate and lead citrate and examined using a Zeiss 9 S-2 transmission electron microscope.

To stain lipophilic substances, semi-thin sections of fixed material or hand-cut sections of fresh adventitious roots were stained with sudan black B (Bronner, 1975). A freshly saturated solution of sudan black B (1%) in 70% ethanol was prepared and kept in a closed container for 12 h at 37°C, then filtered. Glass slides with semi-thin sections were immersed in 70% ethanol for 1-2 min, and then transferred into the freshly filtered saturated solution of sudan black B at 60°C in an oven for 35 min. The slides were rinsed in 70% ethanol for one minute, and then washed with water. For the identification of phenolic compounds which, as artifact, also have a positive reaction to sudan black B, the following histochemical reagents were applied to fresh hand-cut sections: a) DMB reagent (0.5% solution of 3, 4 dimethoxybenzaldehyde in 9% HCL). This forms a red reaction product with condensed tannin precursors (Mace & Howell, 1974); b) Millon's reagent as modified by Bakker (1956). With this stain, colored nitrosoderivatives of any phenols become evident (Sawidis, 1991, 1998). For polysaccharide staining, semi-thin sections of fixed material were treated with periodic acid-Schiff's reagent (PAS) according to Nevalainen *et al*. (1972) and examined with a light microscope (LM). Root water content was measured (RWC, fresh root mass – dry root mass / fresh root mass × 100) according to Cappelletti (1954). For the morphometric evaluation of the relative volume of the histological com-

ponents of the adventitious root, a transparent sheet bearing a square lattice of point arrays, 10 mm apart, was laid over light micrographs of root cross-sections (×200). The point-counting technique analysis was then applied (Steer, 1981).

RESULTS

Adventitious root morphology

The adventitious roots of *U. maritima* are up to 20 cm long and about 1 cm thick. They have unlimited growth downwards, while the lower part breaks down. The age of the living part of the adventitious root can be determined by the number of thickenings. The above-ground structures (inflorescence and fleshy leaves) are completely dry by June, but the adventitious roots and the bulb survive the dry summer (dormancy). The average contents of root total water during the photosynthetic period (autumn-spring) and dormancy period (summer) are 91.2 and 87.27%, respectively. In the morphology of dormant (summer) adventitious roots, indications of intense shrinkage are clearly evident macroscopically in comparison to those of the photosynthetic period (autumn-spring). This shrinkage is also visible by microscopy (Figs 1, 2).

Adventitious root anatomy

The adventitious roots of *U. maritima* are covered by a multiple-layered velamen, an epidermal system 2 - 4 cell wide. The velamen epidermis is usually uniseriate, with no cuticle, thick-walled and sometimes bears hairs (Fig. 1). The epidermal cells contain different myelin-like structures and other peculiar membraneous configurations. Putative bacteria are occasionally present within the vacuoles of the epidermal cells (Fig. 3). Electron-dense material of putative dead fungi and fungal pelotons also appears inside the epidermal cells (Fig. 3) and inside the inercelluar spaces (Figs 4, 5). Exodermal cells, regularly thin-walled throughout and elongated tend to be anticlinally orientated. Many of these cells are frequently smaller than those of velamen and are often nucleated (Fig. 1).

The adventitious root cortex is in cross-section approximately 20 cells wide. The cortical cells are globular to oval in shape, thin-walled, with a large size reflecting a water-storing character (Fig. 6). Sheath-walled idioblastic cells containing raphide bundles (profiles) are present among ordinary cortical cells (Fig. 7). Raphides occur within the central vacuole. Under the electron microscope, the vacuolar content appears foamy and each crystal needle is embedded in a translucent homogeneous substance (Fig. 8). The cortical cells often contain myelin-like structures and irregularly shaped nuclei (Fig. 9).

The cells of the uniseriate endodermis are rather oval and the cell wall does not greatly differ from that of the neighboring tissues (Figs 10, 11). The pericycle is also uniseriate and its cells are more elongated (Fig. 10).

In the root vascular cylinder, the xylem is ordinarily 10-arched (Fig. 10). It consists of vessel members in short radial rows, alternating with clusters of phloem (sieve element) cells (Fig. 12). During the active phase of plant life (autumn-spring), cells in the central cylinder possess nuclei of different shapes (Fig. 12). Moreover, many organelles, such as mitochondria, Golgi bodies, endoplasmic reticulum elements etc., accumulate close to the intercellular space, which is filled with a homogeneous substance. The parenchyma cells of the central cylinder possess a few plasmodesmata showing a bulge on their inner side, especially at the stage of leaf emergence and inflorescence stalk. Myelin-like structures are also present inside the cells.

Histochemistry

After employing the Schiff's reagent, the presence of cortical cells with a polysaccharidic content is evident in the adventitious roots (red-stained cells, arrows) (Fig. 13). The positively reacted cells typically do not have a peculiar wall layer like that of the raphide idioblastic cells.

When semi-thin or hand-cut sections are treated with sudan black B, numerous cortical parenchyma cells appear intensely stained brown to black (Fig. 14). This staining is due to the presence of lipids, since application of the test for identification of phenolics did not give positive results. In the cortex, cells with lipids are scattered and they do not differ in size from ordinary cortical cells. Observations of hand-cut sections under the polarized light reveal open bundles of calcium oxalate needles 72 ± 22 μm long (Fig. 15).

Morphometry

In order to investigate quantitatively the histological components of the adventitious roots, a morphometric study was applied to the cortex region (Tables 1,

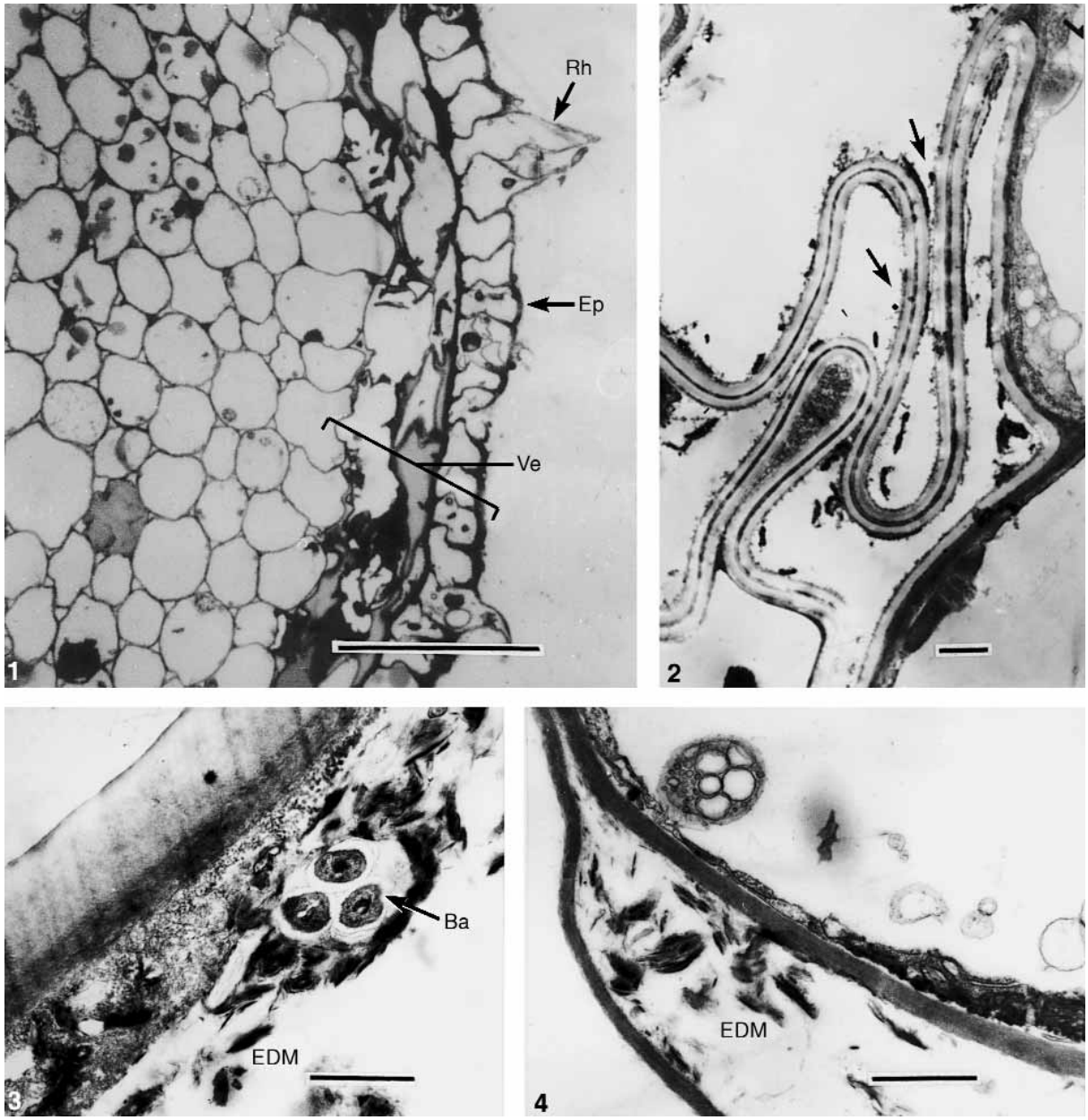


FIG. 1-4. Adventitious root cross section showing the velamen**.** Scale bar = 1 μm except for FIG. 1, Scale bar = 100 μm.

FIG. 1. A multiple-layered velamen bearing hairs.

FIG. 2. Cell walls of storage cells revealing densely packed zig-zag-like foldings during the summer period.

FIG. 3. Putative bacteria and electron dense material inside the velamen epidermal cells.

FIG. 4. Electron dense material in an intercellular space of the velamen epidermis.

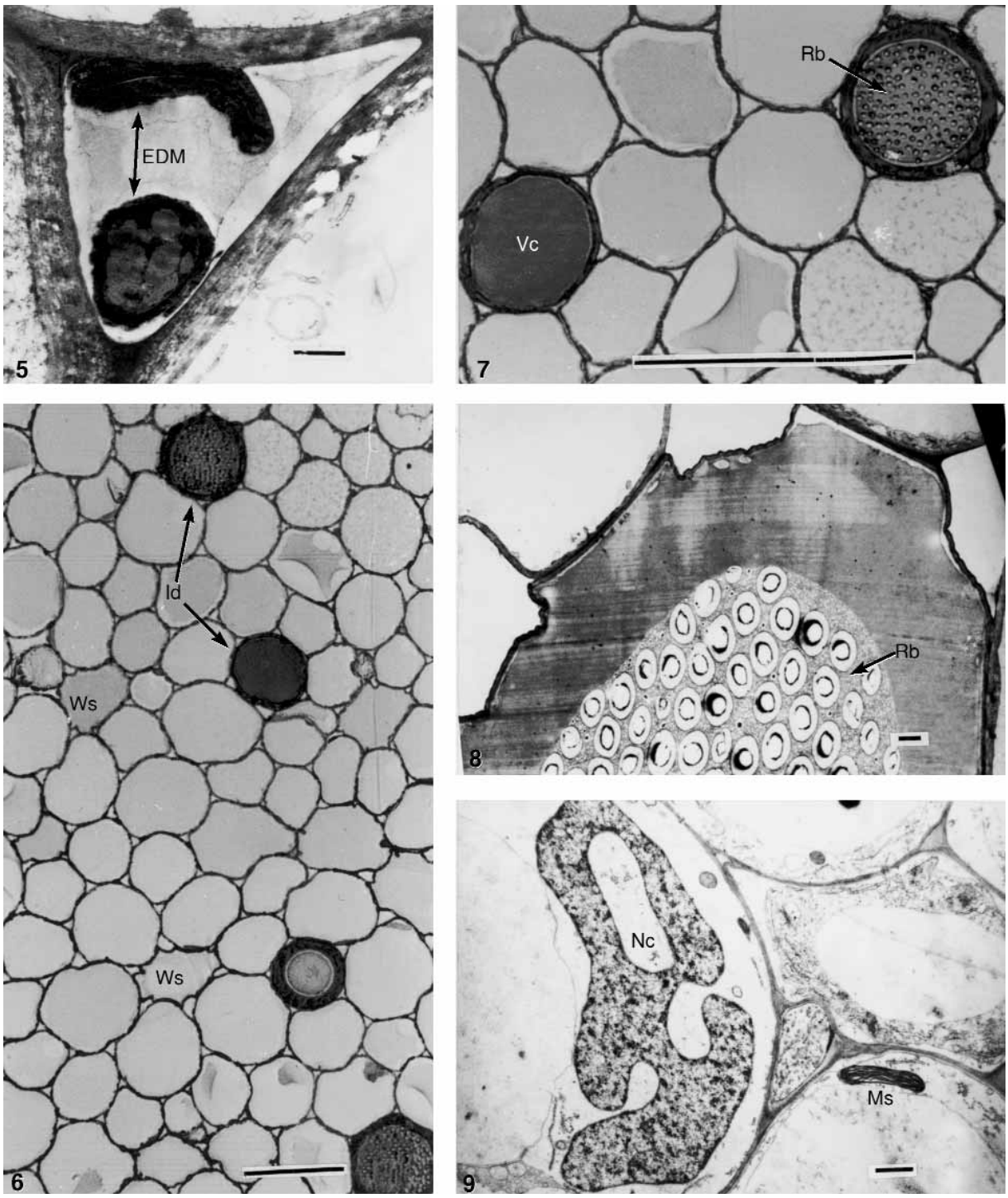
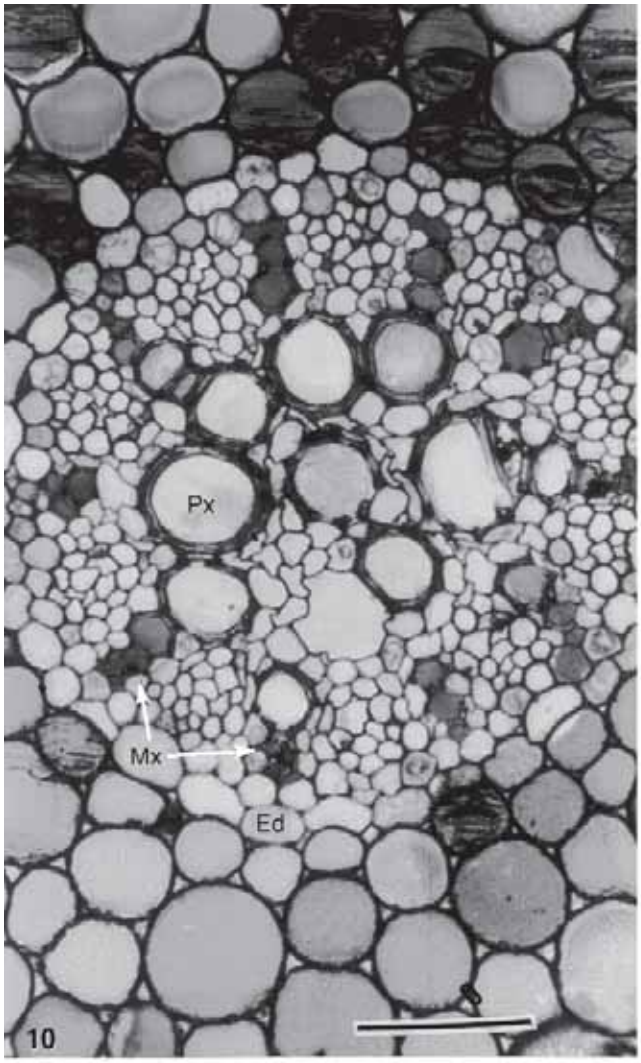


FIG. 5. Electron dense material in an intercellular space of the velamen cells. Scale bar = 1 μm.
FIG. 6. Cortex region indicating water-storing character. Scale bar = 100 μm.
FIG. 7. LM micrograph illustrating raphides stored in a large vacuole of a cortical idioblastic cell. Scale bar = 100 μm.
FIG. 8. EM micrograph illustrating a cortical idioblastic cell containing densely packed raphides within the vacuole (raphides are embedded in a homogenous material). Scale bar = 1 μm.
FIG. 9. A myelin-like structure and a wrinkled nucleus in cortical cells. Scale bar = 1 μm.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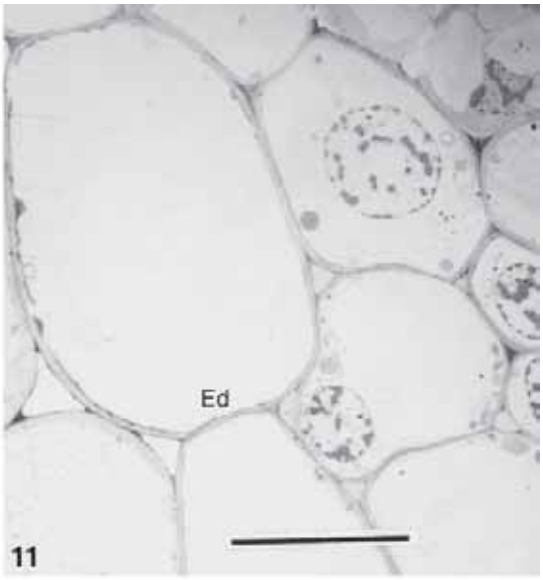
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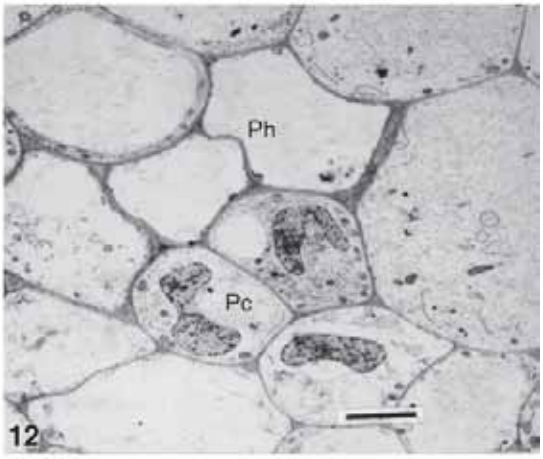
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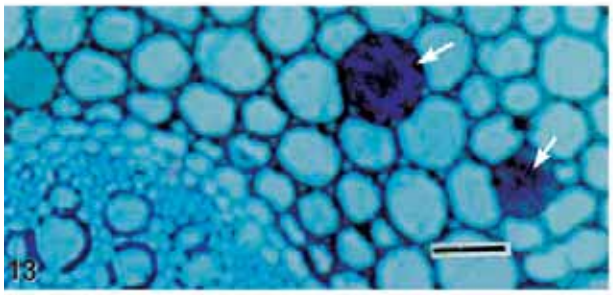
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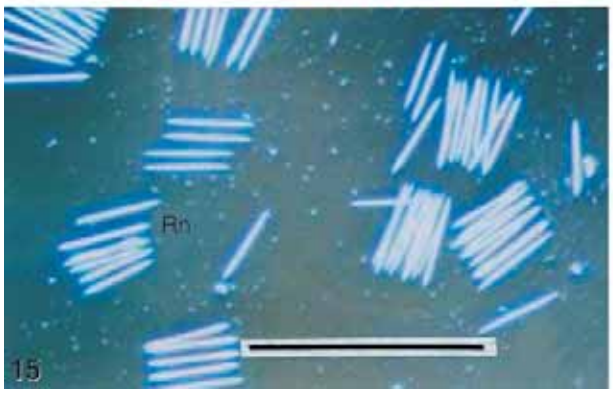
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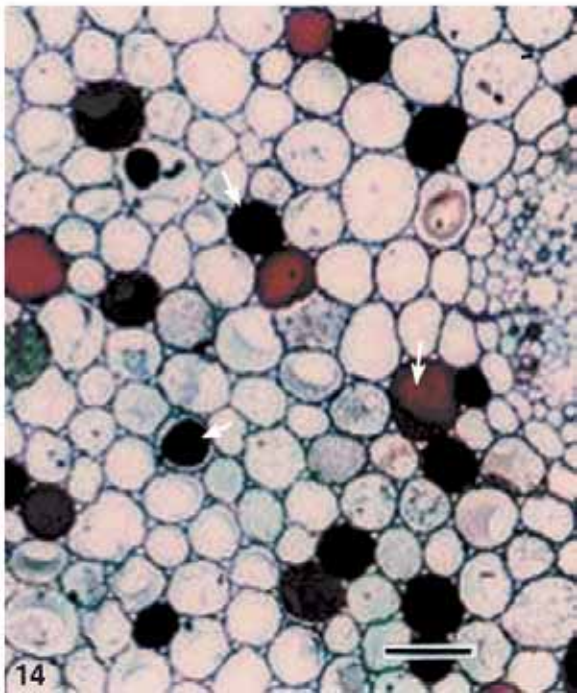
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FIG. 10. 10-arched xylem in central cylinder. Scale bar = 100 μm.
FIG. 11. The uniseriate endodermis. Scale bar = 25 μm.
FIG. 12. Phloem cells connected to active parenchyma cells. Scale bar = 25 μm.
FIG. 13. Scattered cortical cells (arrows) stained with Schiff's reagent for polysaccharide identification. Scale bar = 100 μm.
FIG. 14. Scattered cortical cells containing lipids stained brown to black with sudan black B. Scale bar = 100 μm.
FIG. 15. Hand-cut section revealing an open bundle of calcium oxalate needles. Scale bar = 100 μm.

Abbreviations used in figures

Rh: root hair
Ep: epidermis
Ve: velamen
Ms: myelin-like structure
Edm: electron dense material
Ba: bacteria
Id: idioblastic cell
Ws: water-storing cell
Vc: vacuole
Rb: raphide bundle
Nc: nucleus
Ed: endodermis
Px: protoxylem
Mx: metaxylem
Ph: phloem
Pc: parenchyma cell
Rn: raphide needles
EM: electron microscope
LM: light microscope

TABLE 1. Relative volumes (%) of the histological components for the root cortex (± SD, n = 10)

Histological component of the root cortex	Relative volume %
Parenchyma cells	43.99 ± 3.30
Idioblastic cells containing raphides	19.83 ± 1.10
Cells containing lipids	14.38 ± 0.71
Cells containing polysaccharides	11.27 ± 1.62
Intercellular spaces	10.53 ± 1.28

TABLE 2. Average thickness (μm) of the histological components of the adventitious root (cross section) at the region 3 cm above the root tip (± SD, n = 10)

Parameters	Mean ± standard deviation (μm)
Thickness of velamen	69.25 ± 9.23
Thickness of cortex	741.30 ± 51.34
Diameter of central cylinder	318.58 ± 5.26
Diameter of an endodermal cell	18.44 ± 1.06
Diameter of a cortical cell	52.32 ± 1.71
Periclinal wall thickness of epidermal cells	0.86 ± 0.30
Wall thickness of cortical cells	1.41 ± 0.69
Wall thickness of endodermal cells	0.50 ± 0.16
Wall thickness of nacreous vessel members	2.89 ± 0.74

2). The relative volume of the idioblastic cells containing raphides is the highest (19.83%), that of the cells containing polysaccharides the lowest (11.27%), while the corresponding one of the cells containing lipids is intermediate (14.38%). The cortex storage cells occupy in total 45.48% of the root cortex volume.

The cortical cells are mostly globular to oval, with an average diameter of 52.32 μm (Table 2) and an average volume of 73143 μm^3. Measurements revealed that the cortex is the thickest tissue (741.3 μm) being almost twice thicker than the central cylinder (318.58 μm). The sum of thicknesses of the root partial tissues results in a total thickness of the adventitious roots of *U. maritima* of 1976.56 μm (0.198 cm) at the region 3 cm above the root tip. Consequently, dividing the cortex thickness by the root radius and multiplying by 100 yields the cortex contribution to the total root thickness (75%) and that of the central cylinder (16.12%). Correspondingly, the cortical storage cells occupy about 34.11% of the total root volume. The thickest wall is the nacreous wall of the vessel members (2.893 μm), whereas the thinnest one is that of the endodermal cells (0.5014 μm).

DISCUSSION

A characteristic feature of the *U. maritima* adventitious roots is the presence of a multi-layered epidermal structure, the velamen. The velamen is responsible for rapid water uptake and loss, and osmotic and mechanical protection (Raven *et al*., 1981). During the dry period, the velamen cells are filled with air, while during raining period they become filled with water acting as an absorptive tissue. Since the adventitious roots of *U. maritima* explore shallow soil horizons (10-30 cm in depth), it is likely to be vulnerable to dehydration, in the upper soil profile, as witnessed by a visible shrinkage of the older portions of the adventitious roots. This shrinkage probably reflects a hydraulic effect with water moving from the non-growing to the growing plant regions (Matyssek *et al*., 1991; Sawidis *et al*., 2005). The presence of a velamen is an adaptive strategy of the plants in the arid Mediterranean region, aiming to use up short seasonal rainfalls. The velamen structure of some taxa is associated with mycorrhiza (Pridgeon & Chase, 1995). In the case of *U. maritima*, uptake of nitrogen by adventitious roots might be the result of a mutual plant-fungus interaction and/or plant-bacterial nitrogen one.

The pathway followed by the water in the adventitious roots is most probably the apoplast. The increased intercellular spaces of the root probably increase the apoplastic volume and then the apoplastic movement. Therefore, the apoplastic pathway seems to be related to water economy in order for the plant to survive the very dry summer. At the endodermis, however, the water is forced to traverse the plasma membranes and protoplasts of tightly packed endodermal cells. Therefore, the endodermis forms an osmotic barrier between the cortex and the vascular cylinder of the roots.

Urginea maritima proves to be efficient in storing water during the long summer drought. When values of water content in the upper part of the soil profile vary around zero, the adventitious roots remain hydrated and turgid with a total water content of about 87.27%. They are less susceptible to climatic stress and constitute a rather energetically stable system. This adaptation synchronizes the plant's phenological development with the seasonality of the unstable Mediterranean climate (Pantis, 1993).

Crystalline inclusions of different chemical composition and shape are found in many plants. Calcium oxalate needles in cells of *U. maritima* adventitious roots are typical for raphide bundles found in many monocots. Accumulation of oxalic acid in tissues, which is not readily metabolized, may cause osmotic problems. Therefore, precipitation of calcium oxalate in the form of crystals, as a metabolic waste or by-product, seems to be an appropriate way for the plant to avoid these undesirable situations. The relationship between calcium-ion absorption and oxalic acid synthesis in plants is most probably established in order for the ionic balance in tissues to be maintained (Bosabalidis, 1987). On the other hand, calcium content in dormant adventitious roots may be viewed as an osmoregulatory adaptation to drought during the dry-warm summer period (Levitt, 1980). Raphides of calcium oxalate are, at least in part, responsible for producing mild inflammation and itching when rubbed on the skin (Cogne *et al*., 2001). Therefore, raphides take part in both mechanical and chemical irritation when they come into contact with tender tissues of soil-living worms and herbivores. However, the mechanical irritation could be vital for the parenchymatous tissues of the adventitious roots of *U. maritima*. The defense mechanisms could be viewed by other stored compounds to act against microbial agents, herbivores, rodents, fungi and insects (Fitzpatrick, 1952; Miyakado *et al*., 1975; March *et al*., 1991; Hoffmann *et al*., 1993; Sathiyamoorthy *et al*.,

1999; Heth *et al*., 2000; Civelek & Weintraub, 2004).

From the histochemical point of view, the presence of specialized storage cells for polysaccharides and lipids in the adventitious roots of *U. maritima* is evident in the present study. Presence of carbohydrates (Praznik & Spies, 1993), lipids, flavonoids (Fernandez *et al*., 1972) and anthocyanins (Vega *et al*., 1972) has also been reported. It is believed that the highest amount of polysaccharides accumulates in the underground tissues in late spring-early summer. This is obviously an adaptive strategy for the plant to survive during dormancy. Furthermore, the plant exhibits not only quantitative but also qualitative differences in the bufadienolide (glycosides) composition according to its nativity (Krenn *et al*., 1994).

The adventitious roots of *U. maritima* show a narrow fluctuation of total water content between the photosynthetic period (91.2%) and the dormancy period (87.27%), which is a sign that the roots have developed good water-storage characters. Our morphometric evaluations (Tables 1, 2) revealed correspondences with the anatomical (Figs 6, 7) and histochemical (Figs 13, 14) results. Therefore, one could be led to the assumption that most of the root occurs as a storage tissue, which, in its turn accounts for the meaning of the term 'geophyte'.

In conclusion, the adventitious roots of *U. maritima* play an important role in storing and utilizing water and nutrients, thus protecting the plant from drought stress and environmental hazard. Furthermore, they constitute a means of synchronization of the plant with the seasonality of the Mediterranean climate. Structurally, these functions are based on: (a) the multiseriate epidermis (velamen) enabling quick gain of only transiently available soil water, (b) the tightly packed endodermis possessing a few plasmodesmata, (c) the large parenchyma water-storing cells, (d) the cortical cells containing soluble sugars, (e) the cortical cells containing lipids, (f) the cortical cells containing starch, and (g) the idioblastic cells containing raphide crystals.

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