Available online at www.sciencedirect.com
SCIENCE DIRECT®
Flora 200 (2005) 332–338
FLORA
www.elsevier.de/flora

The root-tuber anatomy of *Asphodelus aestivus*☆

Thomas Sawidis*, Sofia Kalyva, Stylianos Delivopoulos

Department of Botany, University of Thessaloniki, GR-54006 Thessaloniki, Macedonia, Greece

Received 5 January 2004; accepted 8 October 2004

Abstract

The root tubers of *Asphodelus aestivus* consist mostly of enlarged fleshy storage tissue. They are bounded by a multiple-layered velamen, responsible for rapid water uptake, water loss reduction, osmotic and mechanical protection. In the cortex area, thin-walled idioblasts contain numerous raphides of calcium oxalate in their large vacuole with a distinctive tonoplast. Wide morphological variations are observed among the raphide cross sections. Electron-dense compounds penetrate the raphide surface and raphide groove. Raphides seem to be vital for the protection of the root tuber parenchyma from herbivores. The cells of uniseriate endodermis are heavily thickened possessing a few plasmodesmata. The vascular cylinder is 20–28-arch and the root xylem consists of vessels in short radial rows, alternating with clusters of phloem cells. The presence of cells, which contain soluble polysaccharides in their large vacuole, is conspicuous after employing the Schiff's reagent. Exodermis cells and all cell walls, especially the thick ones of the endodermis, are also stained. Numerous parenchyma cells, especially those around vascular bundles are stained intensely, when exposed to Sudan Black B. These cells occurring as solitary idioblasts abundantly accumulate oil. Electron-dense remnants are evident within the vacuoles of the storage cells especially near the vascular tissue. The morphological features of *A. aestivus* root tubers and the major part of the total plant biomass are responsible for the species' occurrence and frequent dominance in a wide array of arid environments. *A. aestivus* possessing root tubers is proved to be very efficient in storing water during the long summer drought, less susceptible to climatic stress and well synchronized with the climatic fluctuations of the Mediterranean environment.
© 2005 Elsevier GmbH. All rights reserved.

Keywords: Mediterranean climate; Geophyte; *Asphodelus aestivus*; Root tubers; Raphides

Introduction

Asphodelus aestivus Brot. (*A. microcarpus* Viv.) is a perennial tuberous geophyte of the family Asphodelaceae (Asparagales), widely distributed over the Mediterranean basin (Diaz Lifante, 1996; Polunin and Huxley, 1965). It is a widespread and invasive weed of the calcareous soils of pastures and grasslands, particularly abundant along road sides. It is found both in arid and semi-arid Mediterranean ecosystems (Margaris, 1984) and in certain regions of North Africa (Le Houerou, 1979; Naveh, 1973). These ecosystems, very often referred to as "asphodel deserts", are characterized as the final degradation stages in the Mediterranean regions (Ayyad, 1976; Ayyad and Hilmy, 1974; Le Houerou, 1981). According to the epics of Homerus, the souls of the dead first arrived in underground meadows (*Asphodelos leimon*) on which only asphodels bloomed (Odyssey, XI, 539, 573, XXIV, 13).

☆Dedicated to Prof. Dr. Georg Heinrich, University of Graz, Institut für Pflanzenphysiologie, on the occasion of his retirement.

*Corresponding author.
E-mail address: sawidis@bio.auth.gr (T. Sawidis).

0367-2530/$ - see front matter © 2005 Elsevier GmbH. All rights reserved.
doi:10.1016/j.flora.2004.10.002

A. aestivus, as is the case of other geophytes (cryptophytes), has a considerable importance for vegetation as it has become the dominant life form in some degraded Mediterranean ecosystems. These formations are found on hill sites interspaced among cultivated areas and are used as lowland pastures. In Thessalia plain (Central Greece) "asphodel deserts" are dominated by *A. aestivus*, which participates in the floristic composition and covers 10,000 ha. There are indications that these areas are gradually increasing in size due to overgrazing, frequent fires and soil erosion (Pantis and Margaris, 1988; Pantis and Mardiris, 1992).

A. aestivus has two major phenological phases within a year. An active one (autumn–late spring) from leaf emergence to the senescence of the above-ground structures (photosynthetic period) and an inactive (summer) phase (dormancy), which lasts until the leaves emerge (Aschmann, 1973; Pantis et al., 1994). In the Mediterranean region, geophytes as represented in a prominent way by *A. aestivus* and become the dominant life form in areas degraded by overgrazing and fire. The ability of *A. aestivus,* a native floristic element, to spread and dominate in all those areas, reflects its capacity to face not only the peculiarities of the Mediterranean climate, but also to resist these most common disturbances in its habitat (Pantis and Margaris, 1988).

The over-wintering root tubers of *A. aestivus* develop flat leaves (40–90 cm long 2–4 cm width) from a shoot apex, in February–March and flowering stalks (70–170 cm tall) bearing between 60 and 200 flowers in April–May. The leaves are protected from herbivores by steroid saponins (Dahlgren et al., 1985) and senesce in June, before fruit maturation. The root tubers show lateral growth, and vegetative propagation is frequent in mature plants. However, most of the root tubers remain attached to the mother plant. *A. aestivus* is a sessile organism reproducing by means of root tubers as well as by seeds. These facts are of considerable importance as far as maintenance and even dominance of *A. aestivus* within degraded areas are concerned.

The root tubers, dried and boiled in water, yield a mucilaginous matter which in some countries, is mixed with grain or potato to make Asphodel bread. In Spain and other countries, they are used as cattle and especially for sheep fodder. In Persia, a strong glue is made with the root tubers, which are first dried, pulverized and then mixed with cold water. Under the term "*Tsinisse*" the root tubers of *Asphodelus bulbosus* were used in eastern countries as a mucilage and to adulterate powdered salep.

Given the importance of *A. aestivus* as a consistent component of the Mediterranean vegetation and its dominance over wide areas, we have undertaken the present study focusing on features of the root tubers of the plant. Still, reports of root tuber anatomy in *A. aestivus* and other geophytes are fragmentary. The ultimate goal is to identify the fine structure and function of these organs and thus to explain the remarkable success and abundance of *A. aestivus* in the Mediterranean region. More specifically, the plant's adaptive strategy in the arid environment, the efficiency in water and nutrient storing during the long summer drought and its defense mechanisms against herbivores shall be elucidated.

Materials and methods

A. aestivus plants were collected from a hill about 25 km south-west of Larissa, Thessalia, Central Greece. Root tuber segments were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in 0.05 M cacodylate buffer for 2 h. After dehydration in an ethanol series and post-fixation in 1% osmium tetroxide, the tissue was embedded in Spurr's epoxy resin. Semi-thin sections of 0.5–1.0 mm thickness from resin embedded tissue were heat-fixed to glass slides and stained with 0.5% toluidine blue in 5% borax for preliminary light microscope (LM) observation. Ultrathin sections (0.08 mm) were examined using a Zeiss 9 S-2 transmission electron microscope (TEM).

To stain lipophilic substances, thick sections (1–2 mm) of fixed material or hand cut sections of fresh root tubers were stained with 1% Sudan Black B (Bronner, 1975) and 2% osmium tetroxide (Molisch, 1923), respectively. For the identification of phenolic compounds which, as artifacts, also have a positive reaction to Sudan Black B, the following histochemical reagents were applied on 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 and Howell, 1974). (b) Millon's reagents as modified by Bakker (1956). With this stain, colored nitrosoderivatives of any phenols become evident (Sawidis, 1991, 1998). For polysaccharide staining, sections of fixed or fresh material were treated with the periodic acid-Schiff's reaction (PAS) according to Nevalainen et al. (1972) and examined with LM.

Results

Root tuber morphology

The root tubers of *A. aestivus* are up to 12 cm long and 4 cm thick. They have unlimited growth upwards, while the lower part breaks down. The age of the living part of a root tuber can be determined by the number of thickenings. The above-ground structures (inflorescence stalks and fleshy leaves) are completely dry by

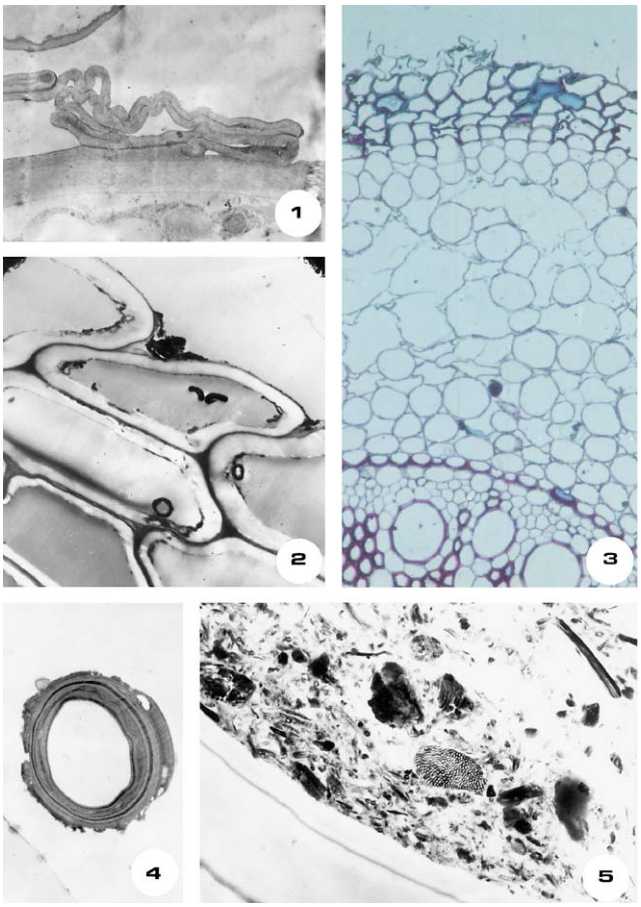

Fig. 1–5. Cell wall of storage tissue revealing densely packed zig-zag-like folding after dehydration during the summer period (10,000). Thick-walled velamen epidermis cells without cuticle on the outer surface (2500). Root tuber cross section showing the cortex area between velamen and central cylinder (160). Myelin-like structures in a velamen epidermis cell (14,000). Amorphous material on the extracellular space of the velamen epidermis (20,000).

June, and only the root tubers survive the dry summer (dormancy). In the morphology of dormant (summer) root tubers indications of intense shrinkage are clearly evident macroscopically in comparison to those of photosynthetic period (autumn–spring). This shrinkage is also visible at LM level for the reason that the cell walls of storage tissue strongly fall back (Fig. 1).

Root tuber anatomy

Root tubers are covered by a multiple-layered velamen, an epidermal system 4–6 cells wide. The velamen epidermis is usually uniseriate and the cells are usually devoid of cuticle. Velamen cells are thick-walled and sometimes single-celled hairs occur (Figs. 2 and 3). The epidermis cells contain myelin-like structures and other peculiar membraneous configurations (Fig. 4). Electron-dense, amorphous material of dead fungi and fungal pelotons appear around the outer surface cells (Fig. 5). Exodermal cells, regularly thin-walled throughout, polygonal and isodiametric sometimes tend to be anticlinally orientated. Many of these cells are frequently smaller than the velamen cells and often nucleated.

The root tuber cortex is more or less 30 cells wide at the central cross section. Cells are circular to oval, thin walled, with triangular intercellular spaces. Large water-storage cells are present. Cortical cells subjacent to the exodermis are smaller, polygonal, isodiametric and lack intercellular spaces. Unmodified, thin-walled crystalliferous idioblasts containing raphide bundles are present among ordinary cortical cells. It is somewhat difficult to illustrate, but under polarized microscopy, the material of these cells reflects the light (Fig. 6). These crystals are stored rather passively in larger vacuoles with a well distinctive tonoplast (Fig. 7). Raphides appear in packs and wide morphological variations are observed among the cross sections (Fig. 8). Some electron-dense substances penetrate the raphide surface and raphide grooves.

The cells of the uniseriate endodermis are periclinally orientated and the cell walls are heavily thickened. The thin wall faces the cortex side while the thick one the central cylinder (Fig. 9). These walls refract polarized light and differ from the neighboring thin-walled parenchyma cells. Endodermis cells possess a few plasmodesmata showing a bulge on their inner side (Fig. 10), especially at the stage of leaf emergence and inflorescence stalks.

The pericycle is uniseriate, while cells are periclinally orientated and isodiametric. The root xylem of the vascular cylinder is 20–28 arch. It consists of vessels in short radial rows, alternating with broadly elliptical to variable shaped clusters of phloem cells. The vascular tissue is surrounded by sharply differentiated, somewhat thick-walled, polygonal parenchyma cells. Sclerenchyma cells are also present (Fig. 11). Vessel end-walls are mostly simple or scalariform. The pith is parenchymatous comprising oval and almost circular, thin-walled cells with triangular, square and rectancular intercellular spaces.

Histochemistry

After employing the Schiff's reagent the presence of cells with a polysaccharidic content is more conspicuous. In hand sections, the red color intensity of the cortex cells is variable and no layered appearance of the content has been observed (Fig. 12). These cells contain soluble polysaccharides in their large vacuole, which in some cases is more clearly seen after being plasmolysed

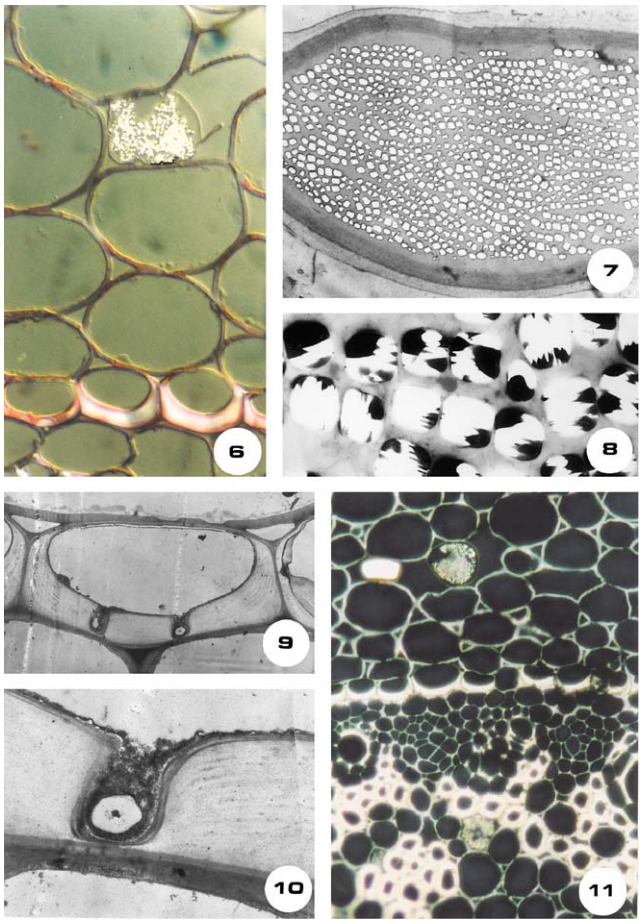

Fig. 6–11. Root tuber cross-section. Cortex idioblast containing densely packed raphides. Staining safranin (green color), polarization optics (3500). Raphides stored in a large vacuole of a cortex idioblast (12,000). Raphides in cross section penetrated by electron-dense material (19,000). Endodermis cell with heavily thickened cell walls at the side of central cylinder (2600). Details of Fig. 9 (15,000). Root tuber central cylinder. Sclerenchymatous tissue. Sudan Black B negative staining (400).

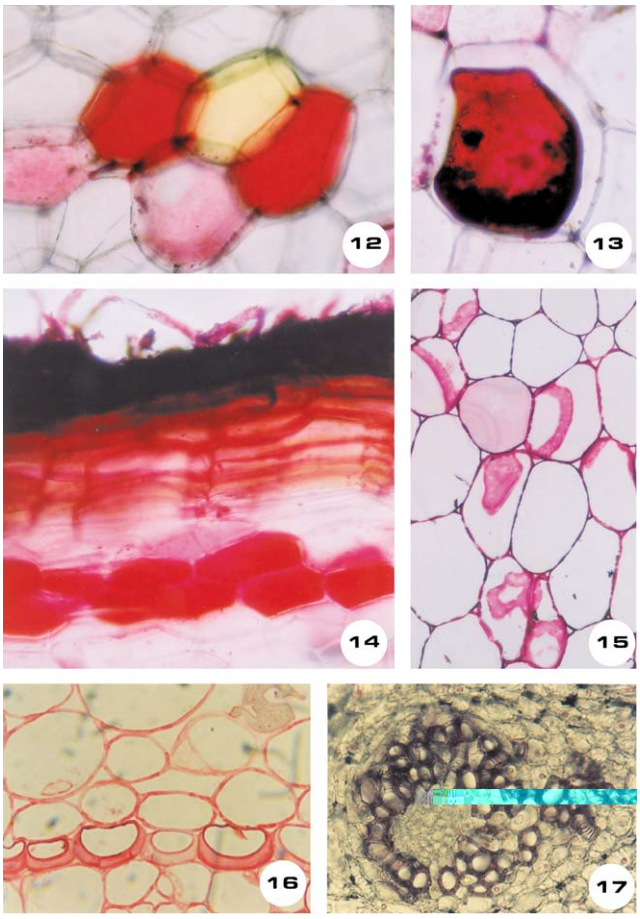

Fig. 12–17. Polysaccharide staining (red; dark in B&W reproduction) in root tuber hand section (160). Polysaccharitic content (electronic version: red/paper version: dark grey) in a plasmolysed vacuole of a root tuber cortex cell (250). Exodermis cells, positively reacted to Schiff' s reagent (electr. vers.: red–paper vers.: dark grey) (160). Stained cell walls of root tuber cortex cells. Some of them tangentially cut (250). Endodermis cell walls, stained intensely with Schiff' s reagent (3500). Staining of xylem vessels at the upper part of the root tuber (dark grey in paper version) (160).

(Fig. 13). All exodermis cells, below the velamen, intensely react to polysaccharide staining (Fig. 14). The extracellular space between the positively reacting and neighboring cells is not penetrated. The positively reacting cells typically do not have a suberized wall layer. The cell walls are also stained which becomes more visible when the cell walls are cut tangentially (Fig. 15). The thick cell walls of the endodermis cells are more conspicuously stained (Fig. 16). Large vessels especially near the upper part of the root tuber, where the xylem is more concentrated, are also red stained (Fig. 17).

When semithin or hand cut sections are exposed to Sudan Black B, numerous parenchyma cells are intensely stained brown to black. In cortex, oil cells occur sporadically as solitary idioblasts and they do not differ greatly by their size from neighboring cells (Figs. 18 and 19). In the central cylinder the oil cells are located around vascular bundles and sometimes are associated with terminal veins. These oil cells accumulate abundant oil insoluble in water. At the stage of leaf emergence (active phase) the oil cells are very large, oval to circular in sectional view. The oil idioblasts are rounded, almost devoid of cytoplasmic content. Plastids are intact, but most of them appear to have degenerated, presumably giving rise to vacuoles. All oil cells possess a few plasmodesmata showing a bulge on their inner side.

Fresh material tested in 2% osmium tetroxide, also show the typical reaction of lipophilic substances whereas both polyphenolic stains applied to sections of fresh material react negatively (with the exception of the