Mesophyll structure during leaf development in *Ballota acetabulosa*

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**SUMMARY**

Mesophyll structure and water relations were studied in expanding and expanded dorsiventral leaves of *Ballota acetabulosa* Benth., a wild perennial shrub from the Mediterranean. The spongy mesophyll was developed earlier than the palisade in expanding leaves and exhibited a larger internal exposed surface. By contrast, in fully expanded leaves the internal exposed surface was much larger in the palisade than in spongy mesophyll. The development of chloroplasts in cells of both mesophyll tissues, as well as their arrangement along the cell walls exposed to intercellular canals, coincided with the formation of intercellular spaces. Leaf expansion appears to be positively related to increasing internal exposed surface and declining water potentials. As a result, mature leaves possess a high ratio of internal exposed surface per leaf area and low values of turgor. It is considered that an increase in the internal exposed surface might be an adaptive feature for small-leaved shrubs, grown under water deficiency.

Key words: *Ballota acetabulosa*, intercellular space, leaf development, palisade and spongy mesophyll, water relations.

**INTRODUCTION**

The regulation of CO₂ transfer from the ambient air into the leaf and concomitant loss of water from the leaf is a major concern of those interested in plant growth in dryland areas and has been reviewed repeatedly (Meidner & Mansfield, 1968; Meidner & Sheriff, 1976; Davies, 1986; Schulze, 1986; Parkhurst, 1994). The traditional view is that the bulk of water transpired from a leaf is evaporated from its mesophyll cells (Turrell, 1936; Jarvis & Slatyer, 1970; Boyer, 1985; Nonami & Schulze, 1989), although it has also been argued that most of the water is lost from the cells lining the substomatal chambers (Sheriff & Meidner, 1974; Meidner, 1975; Edwards & Meidner, 1978; Tyree & Yiannoulis, 1980, 1982; Meidner, 1990). Mesophyll structure and water status are both related to leaf expansion (Dale, 1988; Parkhurst, 1994). The surface area of mesophyll cells is 10 (or more) times that of the cross-sectional area of the leaf available for intercellular gaseous diffusion (Jarvis, 1971) and about 7–30 times its external area (Meidner & Sheriff, 1976; Raven, 1984; Bolhar-Nordenkampf & Draxler, 1993). However, as Raven (1993) noted, observations quantifying the internal area per area of leaf are relatively few.

*Ballota acetabulosa* is a common herbaceous, xerophytic species in south and east Greece (Heywood & Richardson, 1990). Adaptations of xerophytes involve small and thick leaves, with high specific dry weight, small volume of internal air space and a high percentage of palisade mesophyll tissue (Shields, 1950; Parkhurst & Loucks, 1972). In an earlier work, Psaras (1986) reported that mature, hairy leaves of *Ballota* possess a thin mesophyll where the chloroplasts were distributed along intercellular canals. The aim of this study, was to identify structural and functional features in *Ballota* that might influence leaf development, in the context of Mediterranean environment. We focused, in particular, on the structure of the mesophyll and leaf water relations.

**MATERIALS AND METHODS**

Shrubs of *Ballota acetabulosa* Benth. (Labiateae) 40–50 cm tall, were grown in a field, north-east of Athens, at about 50 m above sea level. Shoots bearing up to eight–nine pairs of leaves, of all developmental stages [L1 (youngest)–L9 is the leaf position on axis] were collected early in the morning, during March 1991 and 1992. The developmental stage of leaves was expressed by leaf plastochron index (LPI) using...
Erickson’s formula (Silk, 1980) and 11.65 mm as baseline length. Specific leaf area (SLA) is the ratio of the leaf area, measured with a Delta-T area meter (Delta-T Devices), per unit d. wt (80 °C, 48 h). D. wt per unit leaf area is the specific dry weight (SDW). Chlorophyll content was determined after Linder (1974) and expressed on a f. wt basis.

Plant material was cut and fixed immediately in 5% glutaraldehyde, buffered at pH 7, at room temperature for 2 h, post-fixed in 1% OsO₄ at 4 °C for another 2 h, dehydrated in a graded ethanol series then embedded in Spurr epoxy resin. Semi-thin sections (1-2 μm; LKB Ultrotome III microtome) were stained in 1% Toluidine Blue ‘0’, in 1% borax solution and photographed. Images from cross and paradermal sections of leaves were used for quantitative anatomical measurements. The number of mesophyll cells per unit surface (a surface area of 5400 μm² was considered) as well as the total leaf cell number were measured on micrographs. To determine the total air space per leaf, the area of the intercellular space (ICS) as it appeared in the micrographs of the paradermal sections, was traced and blackened, on a transparent overlay, and measured (Delta-T area meter). The air space of each type of mesophyll tissue was obtained by multiplying the area of ICS by the height of each of the mesophyll types. The length of the internal exposed surface (IES) of mesophyll cells was estimated using the area of ICS and the height of each of the cell layers. The measurements were made on 100 leaf sections taken from five different leaves.

Leaf water potential (ψ) was determined on 6 mm diameter leaf discs placed in C-52 psychrometric chambers (Wescor Inc. Logan, Utah) attached to a dewpoint microvoltmeter (HR-33T, Wescor Inc.). All thermocouples were calibrated using standard salt solutions (Wescor) and were kept in polystyrene boxes for extra temperature insulation (air temperature: 21-26 °C). Solute potential (ψₛ) was measured in the same leaf discs after freezing and thawing. The equilibration time of leaf samples in the chambers had been previously found to be 2 h for each of the water potential components. Turgor potential (ψᵣ) was calculated by difference.

RESULTS
Leaf expansion

Measurements of structural characteristics of successive leaves along a shoot are given in Figure 1. The area per leaf increased from L1 to L6, concurrently with the increase in d. wt and LPI, whereas the minimum SLA was detected for L3.

Chlorophyll content increased substantially from L4 to L5, but decreased slightly from L6 to L9 (Fig. 1).

Tissue differentiation

The dorsiventral leaves of Ballota consist of five cell layers, i.e. the upper and lower uniseriate epidermes and three layers of mesophyll (Figs 2, 3). In L1 (LPI: — 5.06) all cells possessed a dense cytoplasm and their cross-sectional dimensions were similar in all cell layers (Fig. 2a-d). In the upper epidermal cells, vacuoles were slightly larger, indicating that differentiation in the upper epidermis started earlier than in the other tissues. Several multicellular branched hairs (Fig. 2a), abundant stalked glands (Fig. 2a) and immature stomata were observed (Fig. 2b). Young cells destined to differentiate into palisade tissue were longer than mesophyll cells destined to differentiate into spongy tissue (Fig. 2a). Although mitotic figures were not frequent, the meristematic structure of the young palisade was
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Figure 2. Leaf structure in developing leaves. (L1 (a–d) and L2 (e–h); cross sections (a, b and e, f). On the lower epidermis, young (b) and mature (f) stomata are marked by open arrowheads and the substomatal chambers by asterisks. Parademial sections of palisade (c, g) and spongy (d, h) tissue are shown; in L1 (c, d) both mesophylls are highly meristematic with negligible ICS (solid arrowheads), whereas in L2 (h) the differentiation of the spongy tissue is illustrated.

obvious from the newly-formed cell walls, the extremely small vacuoles and from the absence of ICS (Fig. 2c, d, g). Ground tissue cells destined to develop into spongy cells were more or less isodiametric, forming small ICS (Fig. 2d).

Epidermal cells of L2 (LPI: −406), rectangular in cross section, exhibited several small vacuoles (Fig. 2e, f). Mature stomata were found on the lower epidermis (Fig. 2f). Palisade cells, either with or without a few small vacuoles, possessed a dense
cytoplasm (Fig. 2e, g) and small ICS, triangular in cross section (Figs 2g, 5). Spongy cells (Fig. 2h) exhibited several small vacuoles, convex surfaces, plastids and numerous ICS, indicating that they had differentiated earlier.

In L3 (LIP: -3.06, Fig. 3a-c), epidermal cells were highly vacuolated. Rounded spongy cells contained large vacuoles, pronounced ICS (Fig. 3c), and developed chloroplasts, the majority of which were arranged along the intercellular canals. Cylindrical
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Leaf position on axis

Figure 4. Cell dimensions and cell number, of successive leaves along a shoot. (a) Height of palisade (●) and spongy (○) cells, and of upper (■) and lower (▲) epidermis; △, leaf thickness. (b) Diameter of palisade (●) and spongy (○) cells. (c) Number of cells per leaf (—) and per leaf surface (---) in palisade (●) and spongy (○) tissue. The inset illustrates the normalized values vs. IES. Values are means ± SD (n = 100 measurements).

Palisade cells, with small vacuoles and small immature and undifferentiated plastids, were densely packed and exhibited small ICS (Fig. 3a–b).

In L4 (LP1: −2-06), epidermal cells were vacuolated and mature stomata were found on both surfaces (Fig. 3d). Substomatal cavities were always present, but they were more developed when associated with the stomata of the lower epidermis. Cylindrical palisade cells, with a central vacuole and several plastids in the parietal cytoplasm, formed either triangular or polygonal intercellular canals.

Figure 5. Internal exposed surface (IES) and intercellular space (ICS) in palisade (●) and in spongy (○) mesophyll, from L1 to L7. Values are means ± SD (n = 100 measurements); the inset shows the normalized values.

Expanded leaves (L5, LP1: −1-06, Fig. 3g–i; L7, LP1: 0-94, Fig. 3j–k) possessed epidermal cells with thick, convex external walls and stomata that were raised above the level of the rest of the epidermal cells (Fig. 3g). Cylindrical palisade cells, with the chloroplasts parietally distributed along the intercellular canals, touch each other on a narrow line along their side walls and most of their surface is exposed to the ICS (Fig. 3i–k). Spongy cells, different in shape, formed a large ICS (Fig. 3k–j). The striking feature is that chloroplasts were not found at wall areas in contact with the neighbouring cells.

Quantitative anatomy

The height of both types of mesophyll cells increased from L1 to L5, concurrently with a substantial increase in leaf thickness (Fig. 4); from L1 to L4 the height of spongy cells was twice that of palisade cells but the number of palisade cells per unit surface was about twice that of spongy cells (Figs 3e, i–k, 4). Although mitotic figures were not frequently seen in sections, an increase in the total leaf cell number
from L1 to L4 was observed (Fig. 4). The increase in cell number per unit surface (Fig. 4) as well as the increase of their diameter from L2 to L5 (Fig. 4) by c. 14% for spongy cells and 17% for palisade cells coincided with the formation of the ICS (Fig. 5). Our results show that 7% (±0.48) of the total volume in L5 is occupied by ICS of the palisade and 15% (±0.29) by ICS of the spongy mesophyll, both being substantially increased throughout leaf development (Fig. 5). The mesophyll of L1 possessed negligible ICS and IES and in L2 and L3 the spongy mesophyll exhibited a much larger IES than did the palisade. In L3 IES of spongy was c. two-fold higher than that of palisade (Fig. 5). At stage L4, IES of spongy was equal to that of palisade and from L5 to L7 IES of palisade was larger than that of spongy (Fig. 5, inset). The ratio of IES vs. leaf area and SDW was substantially increased from L2 to L3 and started to decrease one plastochron later (Fig. 6).

**Water relations**

ψ and ψ c. gradually declined from L1 to L4 (Fig. 7); thereafter, quite similar values of ψ and ψ c. were recorded. High turgor values (0.5 MPa) were calculated for L1 and L2, where ICS was trivial. Turgor decreased by c. 0.3 MPa from L1 to L3, while it remained constant (c. 0.12 MPa) from L5 to L7. When turgor was plotted vs. leaf area, a reciprocal relationship was detected (Fig. 7a, inset). IES was extended regardless of the declining water potential (Fig. 7b, inset).

**DISCUSSION**

In the early expanding stages (L1–L2), the increase in leaf area of *Ballota* was associated with a slightly higher number of palisade than spongy cells. The dimensions of mesophyll cells (i.e. diameter and height) reversed later, being higher in spongy cells, resulting in a larger IES in the spongy mesophyll (Figs 4, 5). In L4, the number of palisade cells (5 × 10⁶) was greater than that of spongy cells (2 × 10⁴). This might indicate an earlier differentiation of spongy cells, as argued by Maksymowych (1990). Dale & Milthorpe (1983) noted that ICS are formed first in the spongy parenchyma and some two plastochrons later in the spongy. Our results show that in mature leaves of *Ballota* (L5–L8), the increase in area was a result of an increase in the cell number of both mesophyll tissues, as well as an increase of ICS in the palisade (Fig. 5). The volume of ICS (c. 22%) and the ratio of IES vs. external leaf area (c. 10), though, are not representative of xeromorphic leaves (Turrell, 1936; Sifton, 1945; Fahn, 1982).

The structural characteristics of mesophyll cells affect the photosynthetic capacity of the species (Pyke, Jellings & Leech, 1990). In the photosynthetic cells of *Ballota*, the chloroplasts are arranged just beneath those parts of cell walls exposed to the internal leaf atmosphere and the IES might be the real photosynthetic surface, as suggested by Jarvis & Slatyer (1970). Raven (1993) has pointed out the problems of increasing the rate of photosynthesis per unit area of cells exposed to the gas phase, because of the volume of the chloroplasts and the low diffusion coefficient of CO₂ in aqueous solutions, whereas Parkhurst (1986; 1994) argues that limited intercellular diffusion might partly explain the existence of distinct palisade and spongy mesophyll tissues. It is likely that the trivial ICS of young tissues reduces the diffusion path of CO₂ in the gaseous-phase, whereas it facilitates the transport of water that largely bypasses most mesophyll cells, where it evaporates from their wet cell walls into the intercellular spaces in its movement from the xylem to the stomata (Matsuda & Riazi, 1981; Taiz & Zeiger, 1991). This might be advantageous for the photosynthetic requirement of the early stages, where there is a major net requirement for solutes (Pate & Layzell, 1981). At the early stages of leaf development in *Ballota*, the formation of ICS in both types of mesophyll cells appeared concurrently with the development of the chloroplasts and the vacuole, and the lowering of ψ c. ψ and ψ c. of expanding leaves (L1 and L2) were substantially higher than in expanded leaves; this is in agreement with results from evergreen sclerophylls grown in the same environment (Rhizopoulou & Mitrakos, 1990). In *Ballota*, enhanced turgor values were mainly a result of the decline of ψ c.; measurements, though, were not made on individual cells. This might indicate an osmotic adjustment that enables the tissue to extract more water and to satisfy the demand in the enlarging regions. Leaf growth in this, Mediterranean, environment might be related to a compromise between structural and functional characteristics. Further investigation will be required fully to test these findings.
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Figure 7. Water ($\psi_w$, $\Omega$), solute ($\psi_s$, $\bullet$) and turgor ($\psi_t$) potentials, of successive leaves along a shoot. Values are means ± SE ($n = 3$ samples). The insets show: (a) turgor vs. leaf area and (b) a linear regression between $\psi_t$ and IES ($r = 0.805$).

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REFERENCES


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