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Essential oil gland number and ultrastructure during *Mentha arvensis* leaf ontogeny

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Abstract

Alterations in essential oil gland number, distribution and fine structure, and the oil content in the leaf of *Mentha arvensis* L. were examined during its growth and senescence. Accumulation of essential oil occurred predominantly during the rapid leaf expansion phase followed by a similar decline. The oil gland (trichome) number increased upto leaf maturation and declined thereafter. Initially, cuticle remains tightly apposed to the secretory head of oil glands but progressively a sub-cuticular space appears to be created for the oil. Considerable enlargement of vacuole with ageing is witnessed, whereas cytoplasm gradually decreases to a thin peripheral layer. Some secretory cells from senescing leaf were found almost empty, having only a few remnant oil droplets.

Additional key words: glandular trichomes, leaf senescence, mint, monoterpenes, oil biogenesis.

Introduction

Mentha arvensis L. (Japanese mint or corn mint) is one of the mint species significant for its high menthol content in the essential oil (80 - 95 %). Other minor constituents of the oil are (iso)menthone and menthyl acetate (Duriyaprapan and Britten 1982). The oil biosynthesis and accumulation takes place in oil glands or glandular trichomes (Maffei *et al.* 1989, McCaskill and Croteau 1995, Lichtenthaler *et al.* 1997). Two types of oil glands, capitate and peltate are recognized (Maffei *et al.* 1986). The former type consists of a basal cell, stalk cell and an apical secretory cell whereas the latter type is represented by one basal cell, stalk cell and an 8-celled secretory head. Several lines of evidences indicate that the secretory structures not only accumulate essential oil but are probably the primary sites of mono- and

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sesquiterpene biosynthesis (Croteau and Johnson 1984, Gershenzon *et al.* 1989, McCaskill and Croteau 1995, Lichtenthaler *et al.* 1997). Direct correlation between the trichome number and oil production has been indicated in some essential oil bearing plants (Maffei *et al.* 1989, Gershenzon *et al.* 1989, McCaskill *et al.* 1992). However, very little is known about the histology, frequency and developmental pattern of the essential oil secretory structures in *M. arvensis* leaf. Hence, the present studies are focused on examining changes in oil gland number, pattern and ultrastructure as compared to essential oil content accompanying the leaf growth and senescence in *M. arvensis*.

Materials and methods

Plants: *Mentha arvensis* L. (cv. Kalka) plants were grown at the Experimental Farm of Central Institute of Medicinal and Aromatic Plants, Lucknow, following the standard agronomic practices. Leaves were sampled 8, 16, 24, 32, 40, 48, 56, 64 and 72 d after unfolding. The last three stages corresponded with the onset of senescence, mid senescence, and full senescence, respectively.

The collected samples were weighed for fresh mass (FM) and dried at 65 °C till constant mass (dry mass, DM). Leaf area was measured using a leaf area meter (*LI-3100, Licor*, Lincoln, USA). Total chlorophyll content was measured by the method of Lichtenthaler (1987). The essential oil was isolated through hydro-distillation as described earlier (Sangwan *et al.* 1994). Light microscope (*Leica MZ-12*, magnification 50×, Heerbrugg, Switzerland) was used to count the number of oil glands.

Transmission electron microscopy: Leaves were cut into 1 - 2 mm^2 pieces and fixed in 3.5 % glutaraldehyde in sodium phosphate buffer (50 mM, pH 7.0) for 4 - 5 h. After thoroughly washing (4 × 10 min) with the phosphate buffer, the tissue was subjected to overnight post fixation in 1.0 % osmium tetraoxide in 50 mM sodium phosphate buffer (pH 7.0). The fixed samples were slowly dehydrated through ethyl alcohol series, cleaned in propylene oxide and embedded in a mixture (1:1) of epoxy resins, *Araldite 502* and *LX -112* (*Ladd Research Inc.*, Vermont, USA). The blocks were cut on an ultra-microtome (*Nova, LKB*, Uppsala, Sweden) fitted with glass knife, and stained in 2.0 % uranyl acetate and 0.2 % lead citrate. The stained sections or blocks were examined under a transmission electron microscope (model *420, Philips*, Eindhoven, The Netherlands) at 60 kV.

Results and discussion

Growth, development and essential oil content: Leaf fresh mass (FM) and dry mass (DM) increased upto 32 d and declined subsequently whilst most of the leaf expansion (*ca.* 90 %) was attained till 40 d (Fig. 1). Content of chlorophylls (per leaf DM) reached the maximum 32 d after unfolding and declined thereafter (data not

presented). However, the pattern of chlorophyll content per leaf area unit,was more or less biphasic (Fig. 1) implying that the leaf expansion rate during 16 to 40 d was much faster than that of chlorophyll accumulation. Depression in the chlorophyll content during post maturation phase was a marked sign of senescence. Essential oil

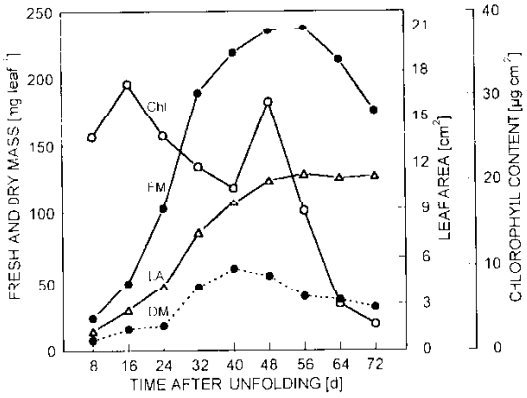

Fig. 1. Fresh mass (FM), dry mass (DM), leaf area (LA) and chlorophyll (Chl) content during different stages of *Mentha arvensis* leaf ontogeny.

content was maximum in young leaves (16 d). A slow but steady decline in the oil content was noticed thereafter (Fig. 2). The decline can be largely due to a very rapid enhancement in leaf fresh mass during maturation with concomitantly less elevation in the oil production. Thus, most of the oil accumulation takes place during the early phases of leaf expansion. During senescence (56 d onwards), lower oil content was recorded (Fig. 2).

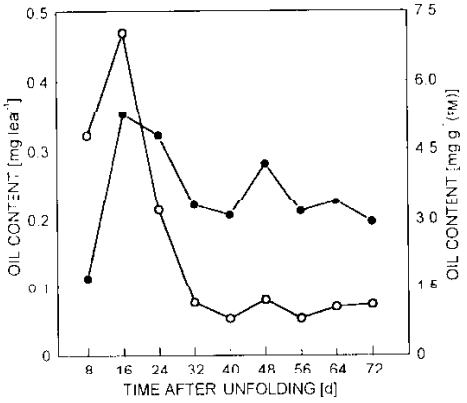

Fig. 2. Changes in essential oil content during *Mentha arvensis* leaf ontogeny. *Closed circles* - [mg leaf^{-1}]; *open circles* - [mg g^{-1}(FM)].

Oil glandular trichome number and density: Total number of essential oil glands (glandular trichomes) on both abaxial and adaxial leaf surfaces increased linearly upto leaf maturation (Table 1). This is in disagreement with the general view that the gland number is fixed at the time of leaf emergence (Ascensao and Pais 1987). Adaxial surfaces of leaves have much lower (almost half) oil gland numbers as compared to their abaxial counterparts (Table 1). On the contrary, in *M. piperita*

Table 1. Changes in the number and average density of essential oil glands during growth and senescence of *Mentha arvensis* leaf. The values are means ± SD of three independent replications.

Leaf age [d]	Number of oil glands [leaf^{-1}]		Oil gland density [cm^{-2}]	
	adaxial surface	abaxial surface	adaxial surface	abaxial surface
16	599 ± 54	1181 ± 129	234 ± 27	461 ± 40
32	2270 ± 293	4316 ± 500	286 ± 23	543 ± 53
48	4067 ± 516	6282 ± 721	413 ± 54	638 ± 59
56	1890 ± 151	4047 ± 476	163 ± 12	350 ± 37
64	1427 ± 134	2672 ± 197	129 ± 11	242 ± 19
72	1061 ± 112	2328 ± 260	95 ± 8	208 ± 23

more oil glands have been observed, during initial phase of leaf development, on the adaxial surface (Maffei *et al.* 1989). The oil gland density on adaxial surface increased upto 48 d and then declined progressively through the senescence. Abaxial surface of leaf followed more or less identical pattern but the number of oil glands were more than twice higher than on adaxial surface at all the stages (Table 1). In addition, the region near midrib had maximum oil gland density and it declined gradually towards the laminar periphery (Figs. 6*A,B*). Also, the leaf basal region had higher number of glands as compared to the apical region. It was observed under light microscope that at the senescent stage, some glands appear empty with brown colour as compared to the totally filled and translucent glands of younger stages (unpublished results). Croteau and Martinkus (1979), have also noticed that undamaged oil glands were devoid of monoterpene oil.

Developmental analysis of oil gland ultrastructure: Though the developmental sequence of the glands co-ordinated with leaf growth and development, the young leaf also possessed mature oil gland and a fully mature leaf also possessed very young glands, although their proportions and frequencies were much less. At all the stages, the capitate (3 celled) as well as peltate (10 celled) types of epidermal glands were observed. Peltate glands were more abundant than capitate. Usually, during the scan only one capitate gland after 4 to 5 peltate glands was found. This is in contrast to *M. viridis lavanduliodora*, where the number of capitate trichomes has been reported to be always greater than peltate trichomes. At the beginning of development, the basal cell of capitate trichome had many large vacuoles with big vesicles (Fig. 3*B*). The stalk cell was narrow and electron dense with scanty dark (oil) droplets. The secretory cell had numerous small vacuoles with more abundant oil

droplets compared to the stalk cell. Nucleus, leucoplasts and mitochondria were visible. The peltate gland (with one basal, one stalk and 8 secretory cells) (Fig. 3A) contained larger and relatively more abundant oil droplets as well as leucoplasts. Few oil droplets were also visible in the cytosol. Similar to capitate gland, stalk cell was

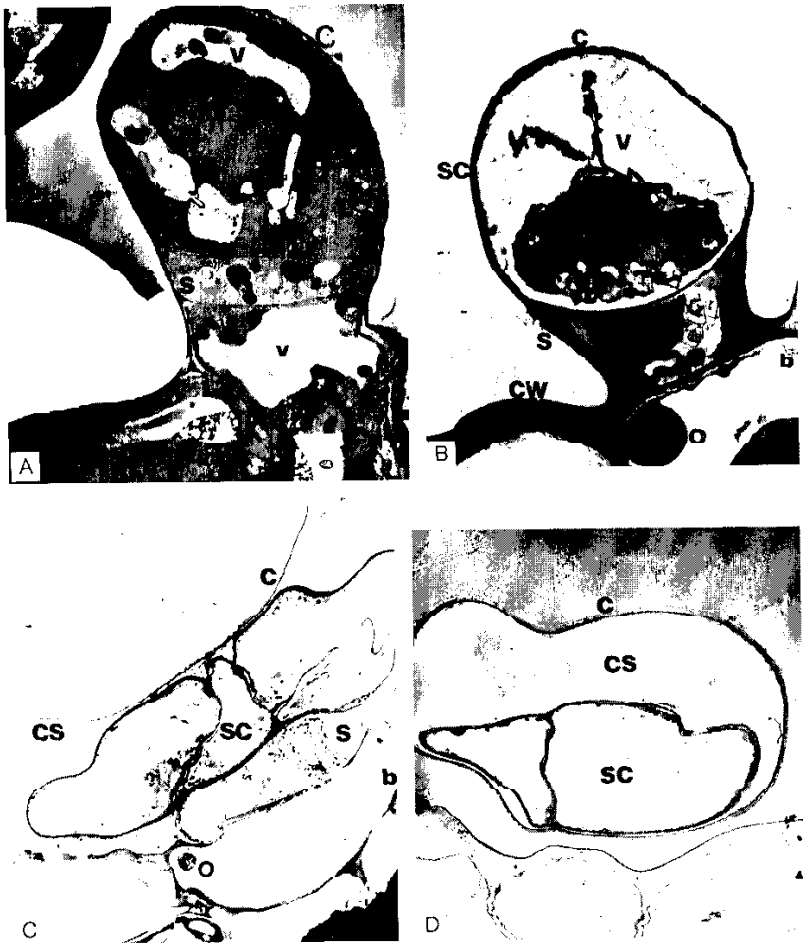

Fig. 3. Changes in ultrastructure of secretory oil glands of *Mentha arvensis* leaf: *A* - peltate gland (8 d, 3 300×) showing tightly apposed cuticle (c), prominent nucleus (n), narrow stalk cell (s) and a basal cell (b); *B* - capitate gland (8 d, 3 300×), cuticle (c) tightly apposed to cell wall, big and prominent nucleus (n), vacuole (v) and few oil bodies present in secretory cell (sc), a prominent oil droplet (o) present at the basal cell (b); *C* - peltate gland (56 d, 1 750×) cuticular space (cs) has been created; *D* - peltate gland (72 d, 1 750×) unbroken cuticle (c) and very big cuticular space (cs) is shown.

narrow but lesser electron dense. Although, the lipid bodies were visible both within and outside the vacuole, their specific identity concerning the volatile or fixed oil can not be stated. Leucoplasts and mitochondria appeared similar to those noted in capitate type gland (Fig. 3*A*,*B*). In both the types of oil glands, the cuticle was tightly apposed to the secretory cell wall and no oil was visible in the intervening space at this stage. Oil inclusions in leucoplasts and a closer association of mitochondria with leucoplasts was found (Figs. 4*A*, 5*D*). Such an association have a functional significance in terms of oil production and/or secretion (Martinkus and Croteau 1981,

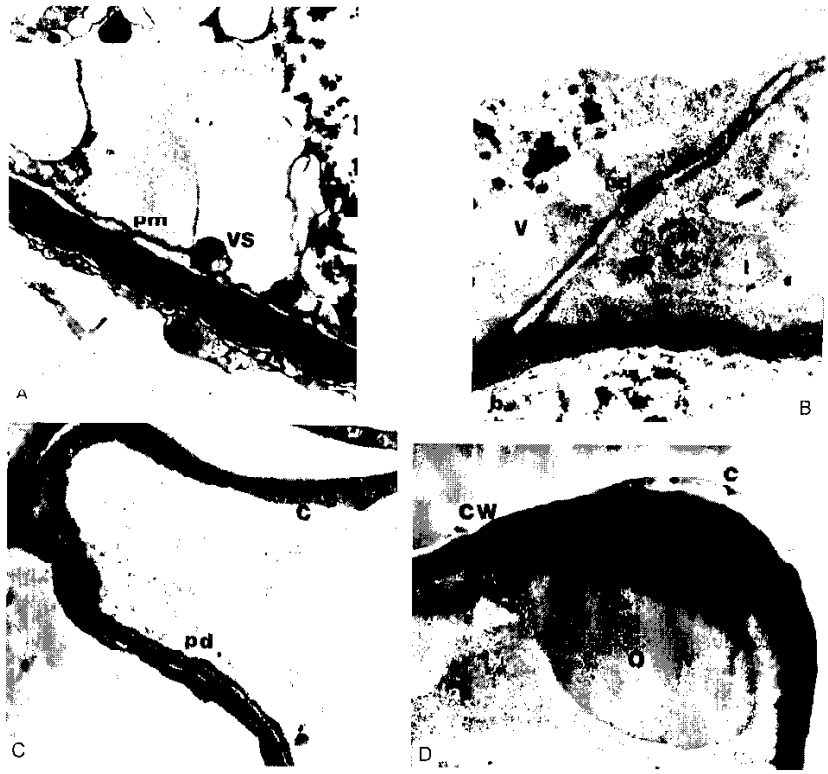

Fig. 4. Ultrastructural changes in stalk and secretory cells of peltate glands: *A* - stalk cell (56 d, 13 500×) exhibiting oil (o), half oil filled vesicles (vs), mitochondria (m) and well defined plasma membrane (pm); *B* - secretory cell of peltate gland (24 d, 10 500×), two cells of secretory head and a basal cell, disintegrating leucoplast (l) with very small oil droplets are seen in picture; *C* - empty secretory cell (72 d, 13 500×) with clear plasmodesmata (pd) and thin layered cytoplasm (c) appressed along the cell wall; *D* - movement of big oil drop (o) into the cuticular space (13 500×) created by expanding cuticle (c).

Croteau and Johnson 1984, Chenieler and Carde 1985, McCaskill *et al.* 1995, Lichtenthaler *et al.* 1997). Preponderance of plasmodesmata (Fig. 4*B,C*) between stalk cell and the secretory cells suggests active intercellular communication within the cellular components. It was a common observation for both the types of oil glands.

Further (24 d), the capitate type gland had largely the same features as in the beginning except that the secretory cell cytoplasm was more shrunken probably due to enlarging vacuole and bigger oil droplets. No oil was visible between cuticle and secretory cell. At onset of senescence (56 d) cuticular space began to appear

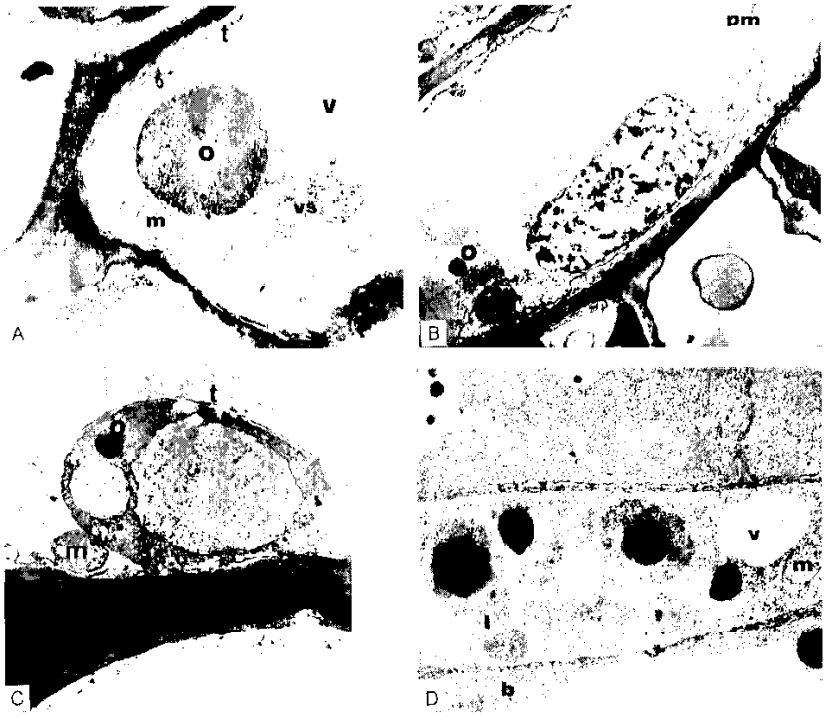

Fig. 5. Ultrastructural changes in basal cell and stalk cell during development and senescence in *M. arvensis*: *A* - basal cell (24 d, 13 500×), vacuole (v) is expanding, tonoplast (t) visible, big oil (o) drop is seen alongwith a mitochondrion (m), vesicles carrying small oil droplets are also visible; *B* - basal cell (56 d, 18 500×), nucleus, plasma membrane and free oil are also seen; *C* - disintegrating leucoplast (18 500×); *D* - stalk cell (8 d, 18 500×) big oil droplets within leucoplast (l), vacuole(v) and mitochondria are seen in picture.

(Fig. 3*C*) and in the fully senescent leaves (72 d), there was a big cuticular space between cuticle and secretory cells accommodating essential oil (Fig. 3*D*). Stalk cell had further narrowed down in the senescent leaves. Nearing completion of

senescence the secretory cells were totally devoid of all the cellular organelles and only a thin layered peripheral cytoplasm was present. The remaining oil droplet was localized on one side of the secretory cell structure (Fig. 5*A*).

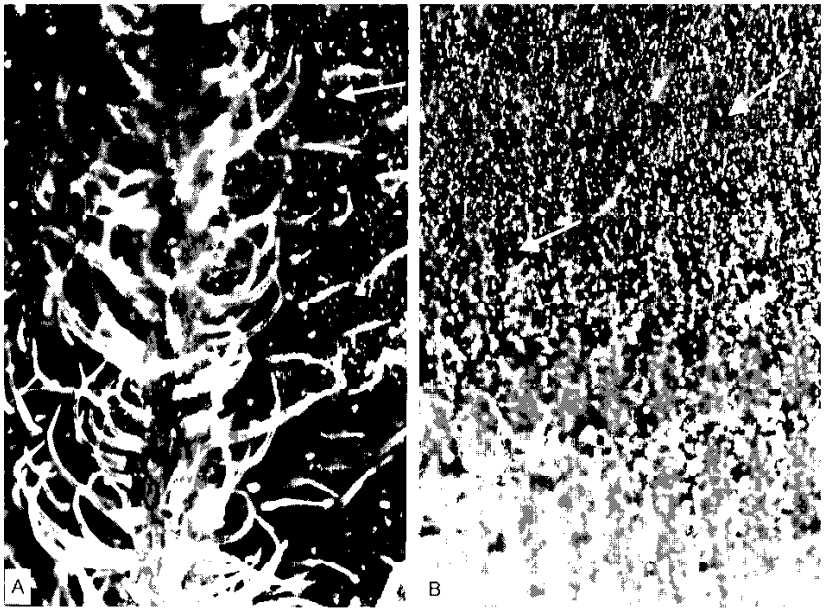

Fig. 6. A representative light microscopic surface scan of *M. arvensis* leaf (abaxial surface). The *arrow* indicates identification of oil glands. *A* - midrib region, *B* - laminar region.

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