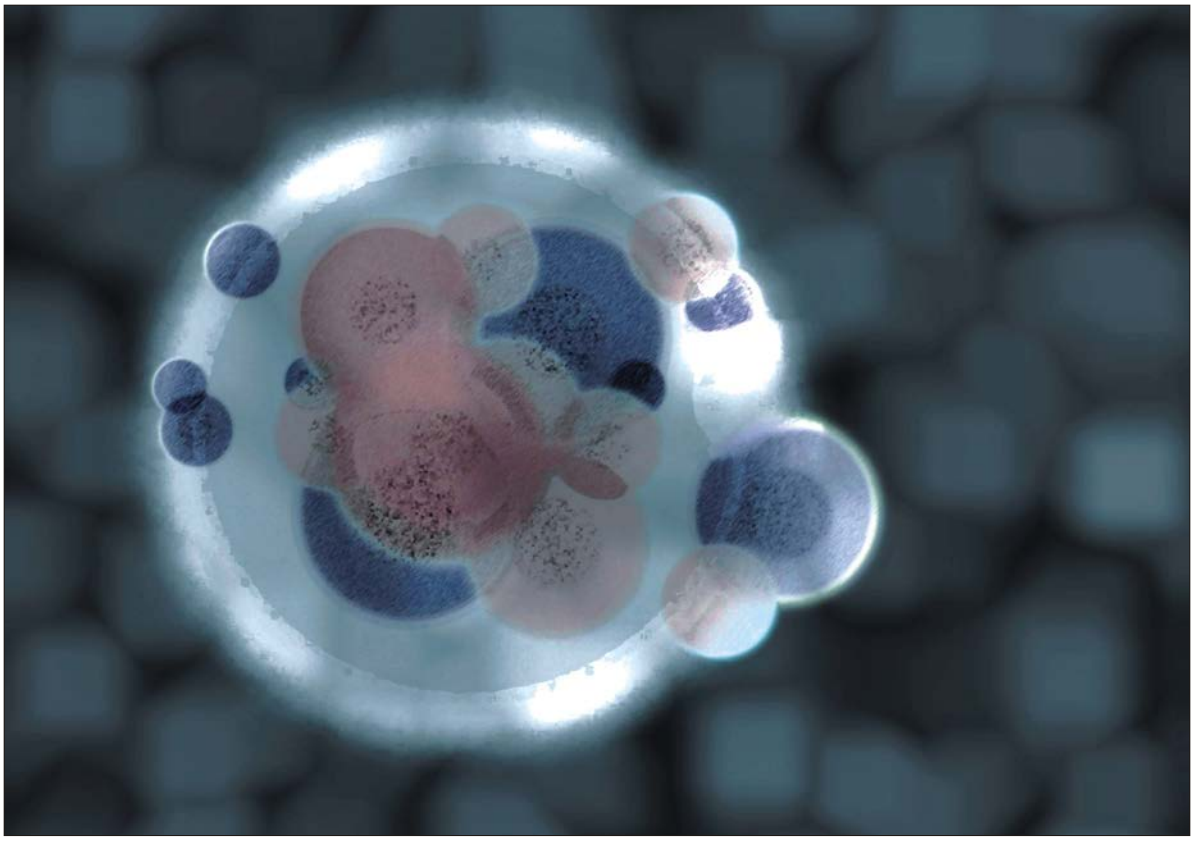

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 7 (6): 848-853, 2007
ISSN 1727-3048

Structural and Developmental Studies on Oil Producing Reproductive Organs in Lime (*Citrus aurantifolia* Swingle)

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Abstract: Anatomical changes in lime (*Citrus aurantifolia* Swingle) reproductive organs were investigated, with emphasis on the ontogeny of essential oil glands and their relation to organ development. Perianth had a leaf-like internal structure, with the developmental changes restricted to the ground system. Ovary diameter increased by cell divisions until fruit color change and by increase in size, wall thickness and intercellular space towards fruit maturity. Development of oil glands in flower and fruit peel revealed a similar pattern and was investigated in the ovary wall. Glands seemed to develop from some epidermal and subepidermal cells, into a conical stalk and a globular or oblong structure consisting of a central cavity surrounded by a protective sheath. Initiation of ovary oil glands started at preanthesis and was restricted to young green fruit. Mature oil glands continued to enlarge throughout fruit growth. Disputes regarding the manner of cavity opening in *Citrus* could be resolved by considering the three dimensional aspect of the oil glands.

Key words: *Citrus aurantifolia* Swingle, development, histology, lime, oil gland

INTRODUCTION

Small shrublike lime tree (*Citrus aurantifolia* Swingle), of the family Rutaceae, is one of the 4 original wild species amongst *Citrus medica*, *C. grandis* and *C. reticulata* from which the main hybrid species are derived (Brown, 2002). Lemons and acid limes are particularly noted for their tendency to flower throughout much of the year (McGregor, 1976).

Lime is nearly similar to other *Citrus* species in its flower and fruit morphology and in the most important anatomic characteristics of the genus: essential oil secretory cavities. Oil glands occur in all parts of the flower (except the stamen) and in the exocarp or flavedo layer of the fruit rind, amongst compact subepidermal parenchyma tissue (Mauseth, 1988).

Lime fruits are used for preserves, granishes and juices. The juice has long been known as a preventive against scurvy and is one of the main sources of citric acid (Sauer, 1993). Lime oil is useful for acne, asthma, chilblains, colds, dull skin, flu, varicose veins (Lawless, 1995) and also Citral oil is extracted for use in perfumes (Sauer, 1993).

Controversial reports are found in the literature regarding the timing of oil gland initiation and development and also the manner of secretory cavity opening in *Citrus* species. Detailed anatomical aspects of *Citrus* glands are mostly confined to fruits, rarely to floral ovaries (Bosabalidis and Tsekos, 1982 a and b) but other oil producing floral organs are neglected. The relationship between oil gland and fruit development has been reported in Washington Navel orange (Knight *et al*., 2001). Liang *et al*. (2006) related the cavity development to the accumulation of essential oil in *Citrus medica* fruits. No study has so far investigated anatomical aspects of gland development in lime.

Studies on lime have so far been restricted to chemical composition of peel and leaf essential oils (Lawrence, 1995 and 1996), physiological aspects of flower and fruit growth (Bartholomew and Reed, 1943) and floral anatomy (Schneider, 1968). Due to the importance of lime as the major commercial crop in south west of Iran, the present study concerns the structural changes associated with the development of oil producing organs and also the relationship between oil gland ontogeny and organ development in lime.

MATERIALS AND METHODS

This study was conducted between 2002-2005 in Shiraz University, Biology Department.

Plant material: Flower and fruits were collected from 20 years old lime (*Citrus aurantifolia*) trees, in Jahrom, south west of Iran. Flowers and fruits were taken at 6 successive developmental stages, based on their morphological aspects.

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Light microscopy: Small pieces of floral organs and fruit peel were prefixed overnight at 4°C in 4% glutaraldehyde, at pH 7.2 in 0.1M cacodylate-buffer. Samples were postfixed in cacodylate-buffered 1% osmium tetroxide, dehydrated in a gradual acetone series and embedded in Spurr´s-resin (Hunter, 1993). Semi-thin transvers and longitudinal sections were cut with glass knives on a LKB ultramicrotome and stained in two ways: (a) with 1% Toluidine Blue, (b) with Methylene Blue, Azure B and Basic Fuchsin (Ruzin, 1999). Micrographs were obtained with a Zeiss photoautomat light microscope.

RESULTS

Anatomy and development

Floral organs: All floral organs were present in the buds of 1.23 mm in average diameter and the sepals were more differentiated than other organs (Fig. 1).

Some cells in the petal upper epidermis, formed papillae and level stomata. Collenchymatous cells gradually differentiated under the lower epidermis. Vascular bundles were parallel in both sepals and petals. Developmental changes in perianth were restricted to the increase in intercellular spaces of spongy parenchymatous cells (Fig. 2). During ontogeny, ovary wall showed divisions in epidermal and parenchymatous cells. Vascular bundles differentiated in three positions: between locules (septal bundles), dorsal bundles and in the center of the ovary on a whorl (marginal bundles) (Fig. 3).

Formation of the juice sacs began in flower buds of 3.25 mm in average diameter as globular structures, by divisions in inner epidermal and subepidermal cells towards the locules (Fig. 4). On the opposite side of the juice sacs, epidermal hairs formed Simultaneously by division and enlargement of the inner epidermis (Fig. 5). Juice sacs and epidermal hairs gradually filled the locules to make the edible portion of the fruit.

After fertilization, divisions in epidermal and parenchymatous cells of the ovary wall continued until fruit color change. Epidermal cells continued their divisions towards fruit maturity but parenchymatous cells stopped dividing and increased in size, wall thickness and intercellular spaces (Fig. 6).

Oil glands: Development of the oil glands was also followed in floral organs and fruit peel. Glands of different size and at variable ontogenic stages, already existed in perianth of the youngest floral bud (Fig. 1). Initiation of oil glands in ovary wall started later, before anthesis. Ontogeny of oil glands in flower and fruit peel showed a similar pattern and we followed it in the ovary wall. Five successive stages of gland initiation and maturation were recognized, based on size, shape and structural aspect. In the earliest stage, a small number of

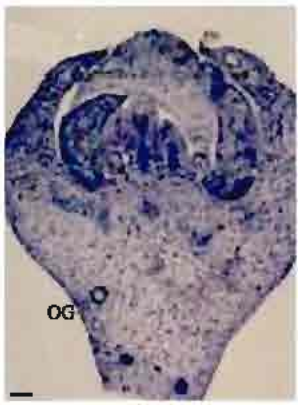

Fig. 1: Light micrograph of longitudinal, Toluidine Blue stained section from *Citrus aurantifolia* floral bud at about the first stage of ontogeny. Carpel(C), Oil Gland (OG), Papillae (Pa), Petal (P), Sepal (S), Stamen (St). Bar = 100 μm

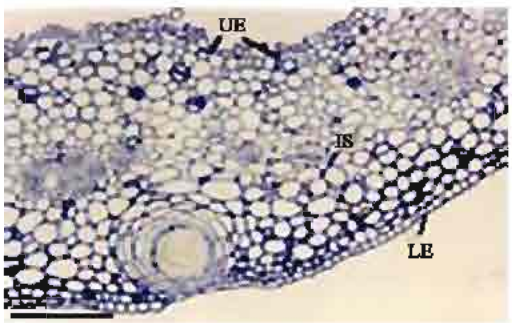

Fig. 2: Cross section of a portion of petal, Toluidine Blue stained. Collenchyma (Co), Intercellular Space (IS), Lower Epidermis (LE), Upper Epidermis (UE). Bar = 100 μm

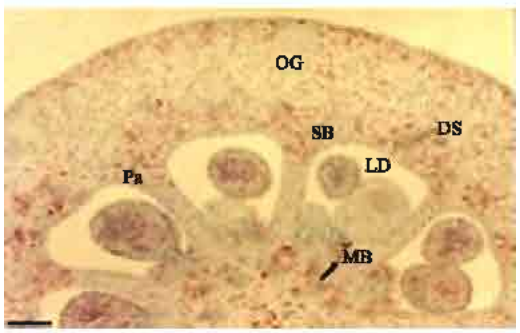

Fig. 3: Cross section of a portion of ovary before anthesis. Methylene Blue, Azure B and Basic Fuchsin stained. Dorsal Bundle (DB), Locule (Lo), Marginal Bundle (MB), Oil Gland (OG), Parenchyma (Par), Septal Bundle (SB). Bar = 100 μm

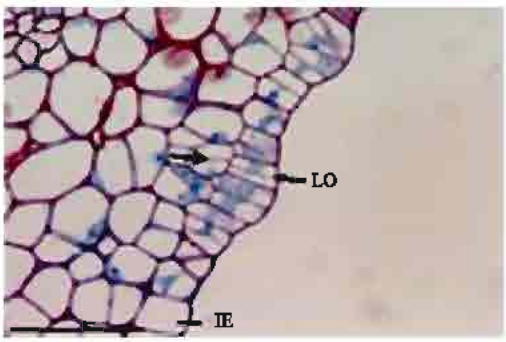

Fig. 4: Cross section of a portion of ovary. Methylene Blue, Azure B and Basic Fuchsin stained. Arrows show divisions. Locule (L), Inner Epidermis (IE). Bar = 20 μm

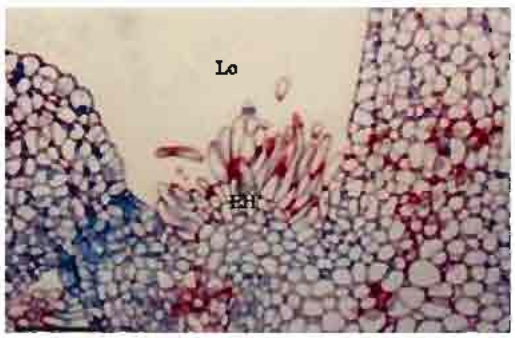

Fig. 5: Formation of Epidermal Hair (EH) in a Locule (Lo). Methylene Blue, Azure B and Basic Fuchsin stained. Bar = 50 μm

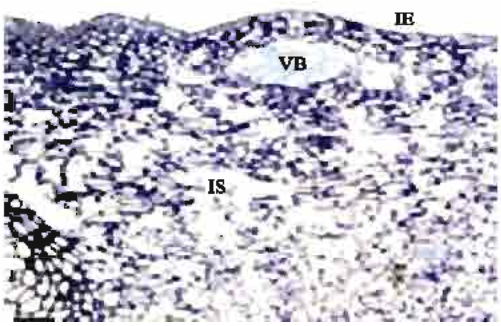

Fig. 6: Cross section of a portion of fruit peel during development. Toluidine Blue stained. Arrows show divisions in Inner Epidermis (IE). Intercellular Space (IS), Vascular Bundle (VB). Bar = 100 μm

outer epidermal and subepidermal cells, divided simultaneously. These cells had larger nuclei than the neighboring cells (Fig. 7). Divisions in subepidermal cells continued to form an undifferentiated spherical or oblong cluster of cells (stage 2) (Fig. 8). Further cell

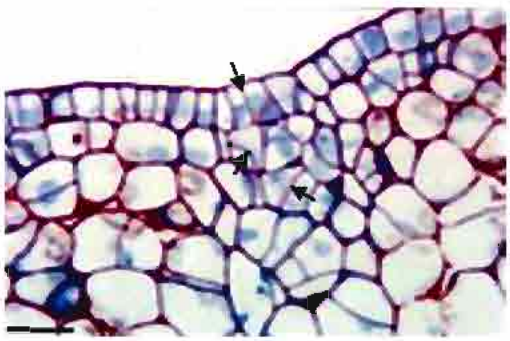

Fig. 7: Development of oil gland in the ovary wall. Stage 1. Gland cells (arrows) have large nuclei and are initiated by divisions in epidermal and subepidermal cells. Bar = 10 μm. Light microscopic section

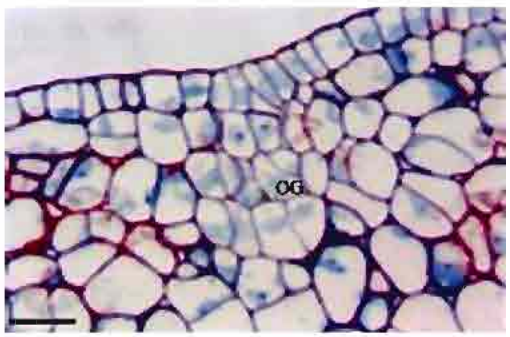

Fig. 8: Development of oil gland in the ovary wall. Stage 2. Formation of spherical or oblong cluster of cells. Oil Gland (OG). Bar = 10 μm. Light microscopic section

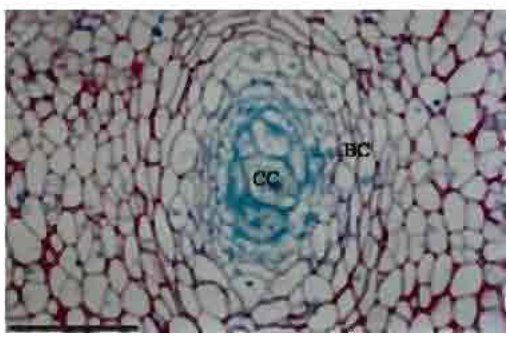

Fig. 9: Development of oil gland in the ovary wall. Stage 3. Flattened Boundary Cells (BC), form around large polyhedral Central Cells (CC). Bar = 50 μm. Light microscopic section

differentiation leaded to the distinction between large polyhedral central cells and flattened peripheral ones of the future gland (stage 3) (Fig. 9). The central cells began

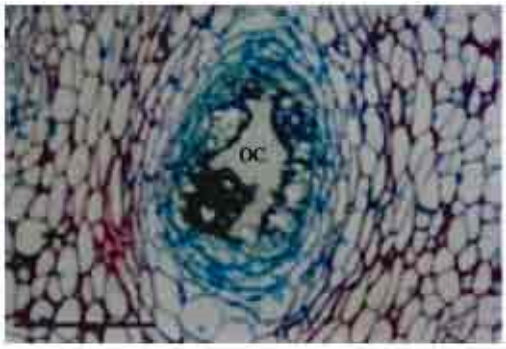

Fig. 10: Development of oil gland in the ovary wall. Stage 4. Formation a small Oil Cavity (OC) by autolysis in central cells. Bar = 50 μm. Light microscopic section

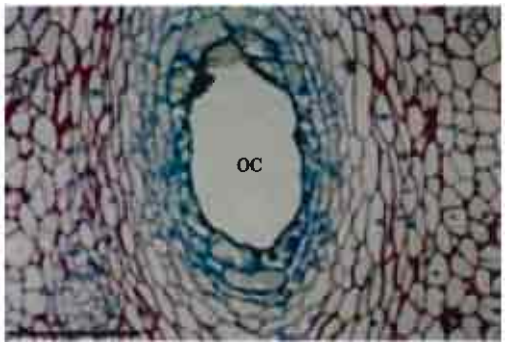

Fig. 11: Development of oil gland in the ovary wall. Stage 5. Mature gland with large central Oil Cavity (OC). Bar = 50 μm. Light microscopic section

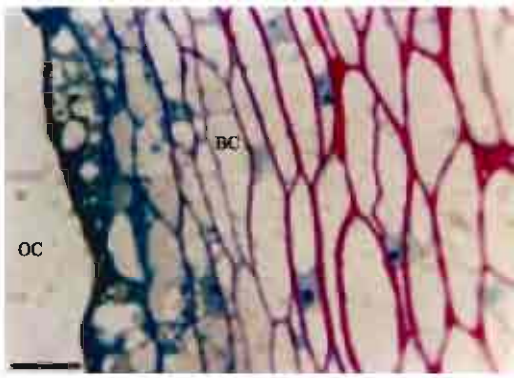

Fig. 12: A part of an oil gland in cross section of petal. Methylene Blue, Azure B and Basic Fuchsin stained. Dense cytoplasm, many small vacuoles and thin wall in Secretory Cells (SC) compared to Boundary Cells (BC). Oil Cavity (OC). Bar = 5 μm

to autolyse and a small cavity formed (stage 4) (Fig. 10). At the final stage (5), lysis continued towards the periphery and the secretory cells covered by a protective sheath, enclosed the central cavity (Fig. 11).

Secretory cells had a dense cytoplasm, many small vacuoles and a wall thinner than the peripheral cells (Fig. 12). Serial sectioning showed that all oil glands are attached to the epidermis by a conical stalk-like structure. The timing of oil gland initiation and development in relation to fruit growth showed that most glands start to initiate before anthesis. Initiation of oil glands continued until the early stage of post anthesis, in the young green fruit peel. After that, former glands continued to enlarge and mature during fruit ripening.

DISCUSSION

Our light microscope findings on lime perianth showed that they resemble leaves in their internal structure, like other flowering plants (Esau, 1965; Fahn, 1990). Stomata were observed in petal adaxial and abaxial epidermis. Presence of stomata in petals has been reported by Esau (1977) and Schneider (1968) too, but Fahn (1990) suggested that petal of flowering plants lacks stomata.

Juice vesicles and epidermal hairs in lime ovary appeared a little before the time the petals opened. Schneider (1968) resulted that formation of juice sacs in *Citrus sinensis* is about the time the petal opened. Burns *et al.* (1992) named both juice vesicles/sacs and epidermal hairs as juice vesicles and reported that they initiate in *Citrus paradisi* at least 2 days before anthesis.

Ovary and then fruit diameter increased by divisions in the epidermal and parenchymatous cells, but divisions in the latter stopped prior to fruit maturation. In contrast, they increased their wall thickness and intercellular spaces. These results are similar to findings in *Citrus sinensis* (Bain, 1958), but Kano *et al.* (1957) reported that ovary wall cells divide only until anthesis and then enlarge to increase the fruit size.

All Oil glands, irrespective of their stage of development, were joined to the epidermis by a conical stalk. This agrees with findings in *Citrus deliciosa* (Bosabalidis and Tsekos, 1982a) and *C. sinensis* (Knight *et al.*, 2001). We found that sepal and petal oil glands have a smaller size and shorter stalk than ovary oil glands. Turner *et al.* (1998) reported that oil glands from the lemon fruit rind have a bigger size than those of the leaf.

Oil gland development in sepal, petal and ovary followed the same pattern and the volume of oil gland was not related to its maturity; an oil gland may be mature but of a small size. These findings have not been reported so far.

Bosabalidis and Tsekos (1982a) and Liang *et al.* (2006) reported that in *Citrus deliciosa* and *C. medica*, respectively, the secretory cavities originate from a pair of meristematic cells: an epidermal and a subepidermal cell which undergo successive divisions resulting in the formation of a conical stalk and a globular gland, respectively. This ontogenic stage was not observed in the present study on lime. Knight *et al.* (2001) have described this process in a series of six different stages from a cluster of up to 10 cells adjacent to the epidermis towards a mature gland with an expanded central cavity. They have reported the same histological events in oil gland differentiation as our findings on lime secretory cavities.

Initiation of oil glands in lime ovary wall was restricted to the second stage of flower development (in flower buds of about 3.25 mm in diameter) towards early stage of fruit ontogeny. Gland initiation in Citrus species has been noted by some to be confined to early stage of fruit development (Knight *et al.*, 2001) but others have suggested continuous formation with fruit growth (Schneider, 1968). Mature lime oil glands continued to enlarge throughout fruit growth. This finding is similar to reports on *Citrus sinensis* (Knight *et al.*, 2001).

There are differences in opinion among investigators about formation of oil gland cavity in members of the Rutaceae family. According to Knight *et al.* (2001) secretory cavities develop schizogenously, while others (Heinrich, 1969) and Fahn (1976, 1990) support the view of a lysigenous process. Buvat (1989), Bosabalidis and Tsekos (1982 b) and Liang *et al.* (2006) consider that the gland is formed schizolysigenously. However, Turner *et al.* (1998) points out that lysigeny concept may be as a result of fixation artefact. Present observations performed on serial sections of the same oil gland in lime ovary wall lead us to the conclusion that all these discrepancies regarding the manner of cavity opening in *Citrus* gland could be resolved by considering the three dimensional aspect of this dynamic structure.

Further ultrastructural and cytochemical studies on developing oil glands will elucidate the mechanisms of active secretion and discharge of *Citrus* essential oil.

ACKNOWLEDGMENT

We acknowledge, with thanks, the financial support provided by the Ministry of Sciences, Research and Technology (Project No. 503494).

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