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ANATOMICAL AND PALYNOLOGICAL STUDIES OF *OLEA EUROPEA* L.

CHNAR NAJMADDIN*

College of Sciences, Biology Department, Salahaddin University, Erbil, Iraq

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Abstract

A study was made on the anatomy of stem, petiole and leaf as well as pollen morphology of *Olea europaea* L. collected from Irbil, Iraq. There were druses crystals in lamina and peltate trichomes in the petiole and lamina. The shapes of the epidermal cells of the leaf were usually polygonal and the anticlinal walls were straight. The stomatal apparatus presented on the abaxial side of the leaf was actinocytic type. Pollen grains were tricolpate. Its shape in equatorial view is subprolate while that of circular and the ornamentation of pollen grain is reticulum cristatum type.

Introduction

The olive tree *Olea europea* L. (Fam.: Oleaceae) is one of the earliest fruit crops to be domesticated (Alche *et al.* 2007) and valued. It is a long lived evergreen tree (Rkhis *et al.* 2011) or shrub that has major economic, social and cultural importance. This is widespread throughout the whole Mediterranean basin region in the form of wild or cultivated population (Lakusic *et al.* 2007, Perez-Lopez *et al.* 2010, Rkhis *et al.* 2011, Koubouris *et al.* 2012, Chiappetta and Muzzalupo 2012). The olive tree is an important biological source of pharmaceutical raw material and food products. Leaf is pubescence and acts as a defensive barrier against biotic and/or abiotic stresses (Karabourniotis *et al.* 1998, Giorio *et al.* 1999). A dense indumentum provides protection against insects and pathogens. It may also reflect radiant energy received by the leaf or help reduce water losses due to transpiration. Hair layers can also absorb ultraviolet-β region of the spectrum, protecting the leaves against ultraviolet-β radiation damage (Karabourniotis *et al.* 1998).

Pollen grain characterization has been utilized with success in taxonomy of both living and fossil species (De Leonardis *et al.* 1995). Taxonomists and paleobotanists have recognized the importance of pollen development and morphology in clarifying the classification of plants. A number of specific pollen characteristics have been cited as useful for differentiation between closely related plant groups (Javady and Arzani 2001).

Materials and Methods

Plant materials of *O. europa* were collected from the College of Sciences campus (Erbil). For preparation of the tissue sections used plastic and paraffin methods. The glutaraldehyde (2.5%) fixed samples were post fixed in 1% osmium tetroxide, dehydrated and cleared in acetone and embedded in araldite mixture. Half micrometer thick sections were stained by 1% toludine blue in 1% borax (Ruzin 1999). Pieces of sample have been fixed in modified aceto-alcohol for 24 hrs. After that the samples were dehydrated using ascending concentrations of ethyl alcohol (85, 90, 95 and 100%) for 1 - 4 hrs for each concentration, then the samples were placed in 3:1, 1:1, 1:3 absolute alcohol and xylene and then xylene for 0.5 – 1.0 hr for each concentration for clearing processes. Then the samples were embedded in a mixture of xylene and paraffin (1/3 xylene + 2/3 paraffin) in the oven (60°C) for 30 min. Tissues were then transferred to fresh molten paraffin and kept at 60°C for 24 - 48 hrs. Paraffin blocks were made and sectioned with the thickness of 8 µm

*Author for correspondence: <chnar_najm@yahoo.com>.

using a rotary microtome (Bright, MIC). The sections were stained in safranin (1%) and fast green (0.1%). Finally the sections were mounted by DPX (Destrin Plastisizer Xylene).

Pollens were collected from the open flowers or mature flower buds. The pollen grains were prepared for light microscope (Erdtman 1952). The samples were prepared for Scanning Electron Microscope (SEM), washed three times with phosphate buffer solution (PBS) and then dehydrated in an alcohol series of 50%, 70%, 80%, 85%, 90% and 95% and three changes in 100% followed by three changes in 100% acetone 30 min. for each, then coated with gold in a sputter coater and observed under the Scanning Electron Microscope (SEM). Coated samples were viewed under the SEM (XL 30, Philips) operated in 10 to 15 Kv at various magnifications to obtain the best images. Normally, 500 to 10,000 µm magnifications were used for pollen morphological studies. The most typical pollen grains for each species were photographed with a Sony Video Printer or saved as a tiff image in compact discs.

Results and Discussion

Transverse sections (TS) of stem showed circular outline and multiserrate epidermis. Collenchyma cells are present in the cortex (lacunar collenchyma). Secretory canals are present. Fibrous layer occurs near the primary phloem and next to the secondary phloem tissue (Fig. 1).

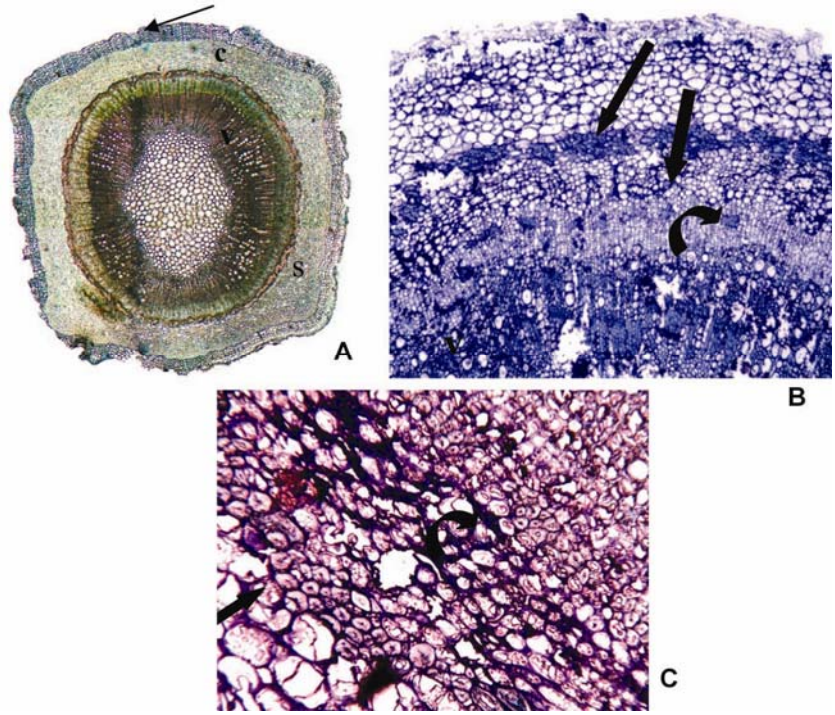

Fig. 1. TS of *Olea europaea* stem: (A, B, C) showing multiserrate epidermis (small black arrow), phloem fiber (large black arrow), stone cells (arch black arrow), c: collenchymas cells, s: secretory canals, v: vascular bundle. A = 4X, B = 10X, C = 40X.

The outline of the petiole in TS is circular and peltate trichomes are present, fibrous layer occurs next to the phloem and xylem tissue. Collenchyma cells are present in the cortex (lacunar collenchyma). Secretory canals are present. The midrib vascular tissue system is closed and a

fibrous layer occurs next to the phloem tissue. Vascular bundle is surrounded by bundle sheath. Secretory cells are present in the cortex. Collenchyma cells are present in the cortex beneath the epidermis. Trichomes are peltate. The lamina consists of two layers of cells; druses and trichosclereids are present in the spongy mesophyll layer. Trichomes are peltate and composed of stalk and flattened head. The shape of the margin is straight, pointing slightly downwards and rounded pointing downwards (Fig. 2). The adaxial anticlinal walls are straight. The abaxial anticlinal walls are also straight and stomata are anomocytic (Fig. 3).

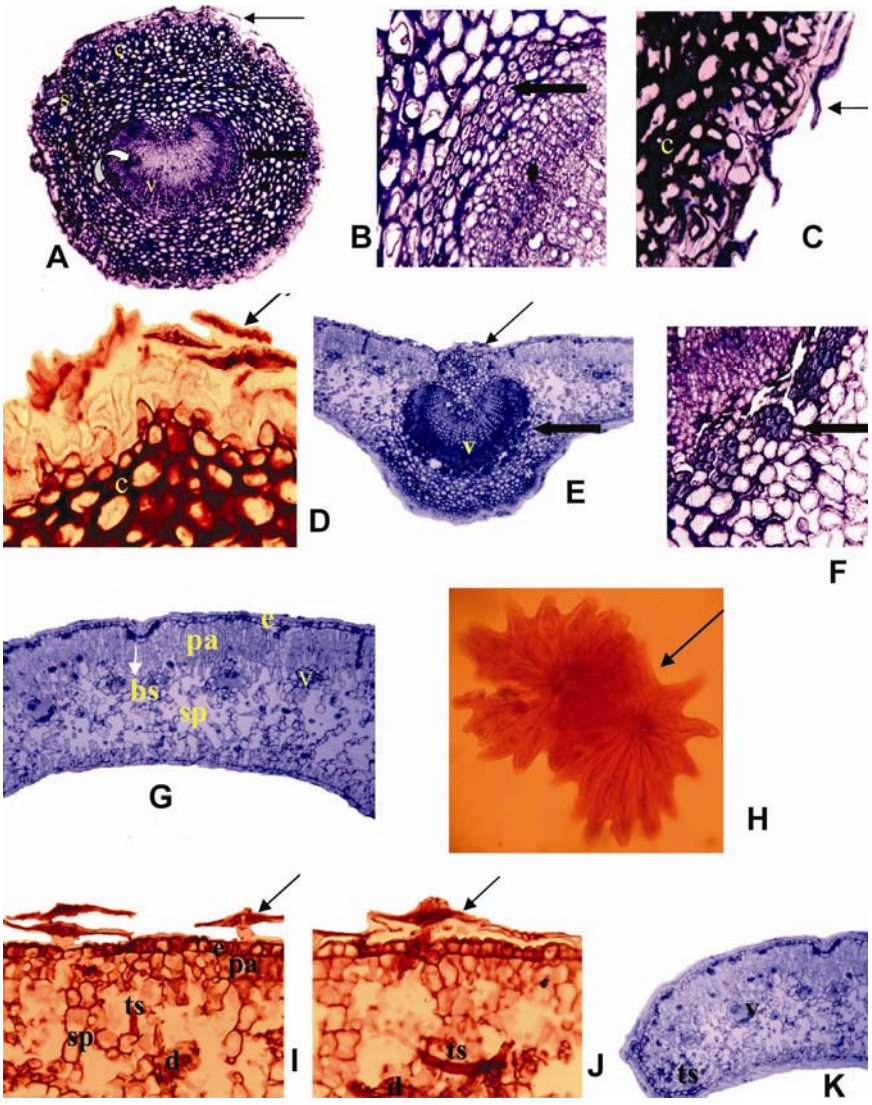

Fig. 2. A, B, C, D. TS of petiole; E, F. TS of midrib; G, I, J. TS of lamina, H. peltate trichomes; K. TS of margin, trichomes (small black arrow), phloem fiber (large black arrow), xylem fiber (arch white arrow), c: collenchymas cells, s: secretory cells, v: vascular bundle, e: epidermis, pa: palisade layer, sp: spongy layer, d: druses, ts: trichosclereid, bs: bundle sheath. A, E, G = 4X, H,K = 10X, C, D, F, I, J = 40X.

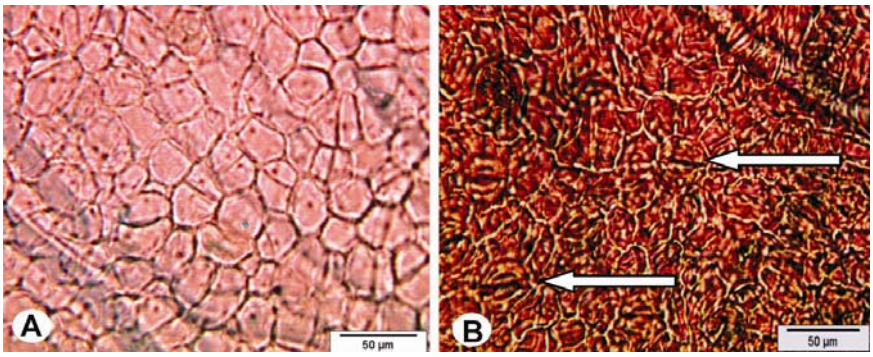

Fig. 3. *Olea europaea* leaf: A. abaxial surface, B. adaxial surface, showing the stomata and the cuticle layer, stomata (large white arrow). A,B = 40X.

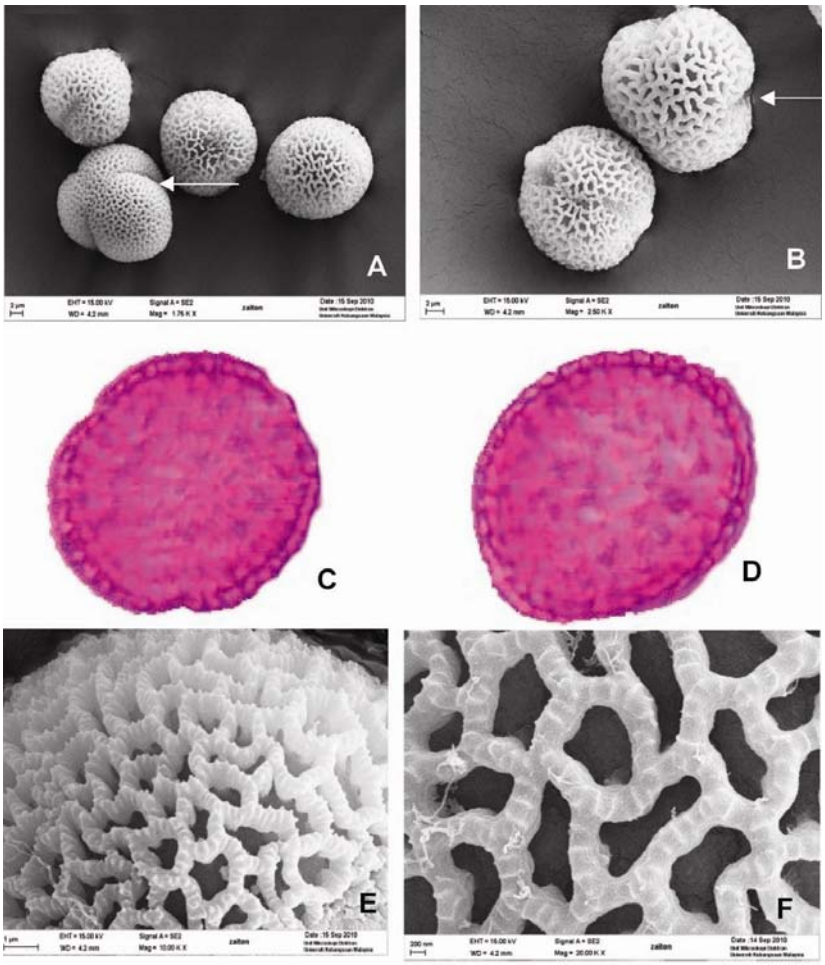

Fig. 4. Pollen grains of *Olea europaea*. A, B. equatorial and polar view (SEM), C, D. equatorial and polar view (LM), E,F. ornamentation (SEM), colpus (small white arrow). C,D = 40X.

Mechanical collenchyma tissues in the stem and petiole were observed. The vascular bundle in the stem and petiole are closed, a fibrous layer occurs in the stem near and next to the phloem tissue and occurs in the petiole near the phloem and next to the xylem. Fibers and stone cells were present in the secondary phloem of the stem in *Olea* species (Ayoub and Qrunfleh 2006). Secretory cells are present in both stem and petiole and peltate trichomes present in the petiole. The present study further revealed that the midribs by the outline of the adaxial surface which was slightly humped by circular shape surface of the abaxial and the presence of collenchyma in both epidermis layers and present trichomes. However, the present study showed that the margin was straight and slightly downwards with rounded tip. The druses calcium oxalate (druses) crystals and trichoseclereids are present in the mesophyll layer. The palisade cells of leaf arranged radially a large, thick walled and vascular bundles sheath or leaf veins sheath as in *Oligomerus subulata*, *Diplotaxis tenuifolia* (Metcalfe and Chalk 1950), Oleaceae and Papaveraceae (Metcalfe and Chalk 1950, Crookston and Moss 1970). For microscopic identification of olive leaves, the natures of the leaf surface are most important things to consider, abaxial surface consists of a dense indumentums that provides protection against insects and pathogens and may reflect radiant energy received by the leaf or help reduce transpiratory water losses (Karabourniotis *et al.* 1998, Palliotti *et al.* 1994, Lakusic *et al.* 2007).

The study of pollen grains using scan electron microscope and light microscope showed *O. europaea* pollen grains were of tricolpate. The shape of pollen grains in equatorial view is subprolate and circular. The ornamentation is reticulum cristatum (Fig. 4). Javady and Arzani (2001) and Koubouris *et al.* (2012) studied the *O. europaea* cultivars and reported that the differences in exine patterns of the olive cultivars included differences in the dimension and shape.

The anatomical evaluation of *O. europaea* showed the druses and secretory canals. The anticlinal walls were straight. The structure of the stomatal apparatus was present on the abaxial epidermis which are anomocyte type and trichomes were present. Pollen grains were tricolporate prolate-spheroidal. Exine ornamentation was reticulum cristatum.

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