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SEM characterization of olive (*Olea europaea* L.) fruit epicuticular waxes and epicarp

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ABSTRACT

Fruit cuticle micromorphology of several cultivars of *Olea europaea* L. was described with the aim to individualize features of the epicarp and epicuticular waxes useful to technological processings. All olive cvs. analyzed in this paper showed only epicuticular waxes arranged in crystalloid structures (granules, platelets, plates and rodlets). The epicarp aspects examined in this work were: the entire thickness of epicarp from surface to the base of epidermal cell, the height of the epidermal cell, the thickness of the cuticular flange that is wedged between epidermal cells and the thickness of the cuticular membrane, the continuous layer on the top of the epidermal cell. The cuticular membrane was the most important parameter, being the only barrier up the epidermal cells. *Gentile di Chieti* and *Dritta* cvs. had the thickest cuticular membrane (17.04 and 16.05 μm for green olives, respectively), *Castiglionese*, *Carboncella* and *Kalamata* cvs. had a medium cuticular membrane while the cuticular membrane of *Intosso* cv. was very thin (8.46 μm for green olives). The epicarp thickness decreased during ripening of fruits in all examined cultivars. Multivariate approaches were also used to identify similarities and differences between cultivars.

1. Introduction

Olive (*Olea europaea* L.) fruit is a drupe constituted by three distinct anatomical parts: epicarp (skin or peel or epidermis), mesocarp (pulp or flesh) and woody endocarp (stone or pit), enclosing the olive kernel (or seed). All three parts influence the quality of the end product. The epicarp is a protective tissue that accounts for about 1–3% of the drupe weight. The surface of olive fruits is covered by a thin extracellular layer, the cuticle. It mainly consists of an insoluble polymer and lipids. The polymer is a polyester matrix of long chain hydroxy-fatty acids, called cutin. The lipids represent a complex mixture of aliphatic and cyclic components usually called waxes. These compounds are embedded in the polymer matrix (intracuticular waxes) and also move onto the surface (epicuticular waxes) where they are often found forming complex three-dimensional structures. The skin itself is covered by a layer of wax, representing 45–70% of the skin weight (Bianchi, 2003).

Waxes are an essential structural element of the surface and of fundamental functional and ecological importance for the interaction between plants and their environment (Barthlott et al., 1998). While cutin and intracuticular waxes mainly function as a barrier against water loss, epicuticular waxes serve different purposes such as water-repellence and protection from pathogenic microorganisms and insect attacks (Jeffree, 1986; Martin and Juniper, 1970). It is commonly accepted that the resistance of most plants to biotic or abiotic attacks is fundamentally due to a mechanical barrier and a defense by chemical substances. Secondary plant metabolites that are thought to play a role in the resistance against insects and fungal pathogens, have been found (Ludwig-Müller et al., 1997). There is also evidence for the involvement of structure and physical form and hydrophobic constituents of the wax fractions in the resistance of the plants to fungal diseases (Garcia et al., 1997). Epicuticular waxes have been described with some chemical composition data for the leaf surfaces of the olive tree (Bianchi et al., 1993), and a study on the susceptibility of different olive varieties to the fly *Bactrocera oleae* reporting various physical and chemical factors influencing the attack of the fly, is appeared (Nellenschwander et al., 1985). Scanning Electron Microscopy was used to compare the ultrastructure of epicuticular waxes of leaves of susceptible (*Bella di Cerignola* and *Carolea* cvs.), moderately resistant (Leccino cv.), and resistant (*Cipressino* cv.) olive varieties to the fungal disease

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caused by *Spilocaea oleagina* (Marsilio et al., 1997). The significance lies in the fact, that the cutin and embedded waxes are almost impermeable to water.

Studies of the microstructural characteristics and physical properties of surfaces have also been made in different agricultural products with the purpose of establishing parameters of quality and their relation with the sensorial properties. For apples, the superficial characteristics were investigated and their three-dimensional structure was determined with confocal laser scanning microscopy (CLSM), conventional light microscopy (LM), and environmental scanning electron microscopy (ESEM) (Veraverbeke et al., 2003). Studies in coffee (Cardona et al., 2008) and peach (Yang et al., 2005) have been reported using the atomic force microscopy (AFM) to characterize the roughness of the fruit epicarp and the cells that compose it in different states of development. The different qualities of fruits were studied by obtaining microstructural properties with scanning electron microscopy (SEM) and relating them to texture profiles (Allan-Wojtas et al., 2003; Lanza and Di Serio, 2011; Lanza, 2012; Martens and Thybo, 2000).

The objective of this study was to characterize, by the SEM techniques, the epicarp of several olive cultivars, analyzing the micro-changes that occur during ripening from unripe to ripe stage with the aim to individualize features of their epicarp and epicuticular waxes useful to technological processings. Multivariate approaches are also used to identify similarities and differences between cultivars.

2. Materials and methods

2.1. Plant material for epicuticular waxes analysis

Drupes of *O. europaea* L. cultivars *Gentile di Chieti*, *Carboncella*, *Dritta*, *Castiglionese*, *Intosso*, *Kalamata*, *Cucco*, *Cassanese* and *Ascolana tenera* were harvested at the green ripening stage. The green olives were harvested during the ripening period, prior to the varaison and when they had reached normal size. The colour of the fruit may be from green to straw yellow. The fruits were collected between the months of September and October in the Catalogue Field of olive varieties of CRA-OLI Città Sant'Angelo (Italy) (latitude: 42°31′8″76 N; longitude: 14°3′36″36 E; height: 317 m a.s.l.), using a cutting tool to remove them by the peduncle, they were allowed to fall into a sterilized receptacle that contained cotton in the bottom to avoid alterations in their microstructure. The fruits were transported in the Laboratory of Microscopy of CRA-OLI where the epicarp of olive fruits were processed for scanning electron microscopy.

To investigate the ultrastructure of epicuticular waxes, the olive skin samples (approx. 5.0 mm × 5.0 mm sizes) were cut from the fruit with a blade, delicately placed on aluminium stubs covered with the bi-adhesive tape, placed with the surface to observe oriented upwards in the oven at 30 °C for 3–5 h and then coated with gold (25 nm thick) in a Balzers Union SCD 040 Sputter Coater (Balzers, Wiesbaden, France) (Lanza et al., 2007). Representative areas were examined with a Scanning Electron Microscope (Philips XL 20) at 20 kV.

2.2. Plant material for epicarp characterization

Drupes of *O. europaea* L. cultivars *Gentile di Chieti*, *Carboncella*, *Dritta*, *Castiglionese*, *Intosso* and *Kalamata* were harvested at green, cherry and black ripening stage. The green olives were harvested during the ripening period, prior to the varaison and when they had reached normal size. The colour of the fruit may be from green to straw yellow. The cherry olives were obtained from rose, wine-rose or brown-coloured fruits harvested when turning colour, before the stage of complete ripeness was attained. The black olives were obtained from fruits harvested when fully ripe or slightly before full ripeness was reached; they may, according to production region and time of harvesting, be reddish black, violet black, deep violet, greenish black or deep chestnut not only on the skin but also through the flesh.

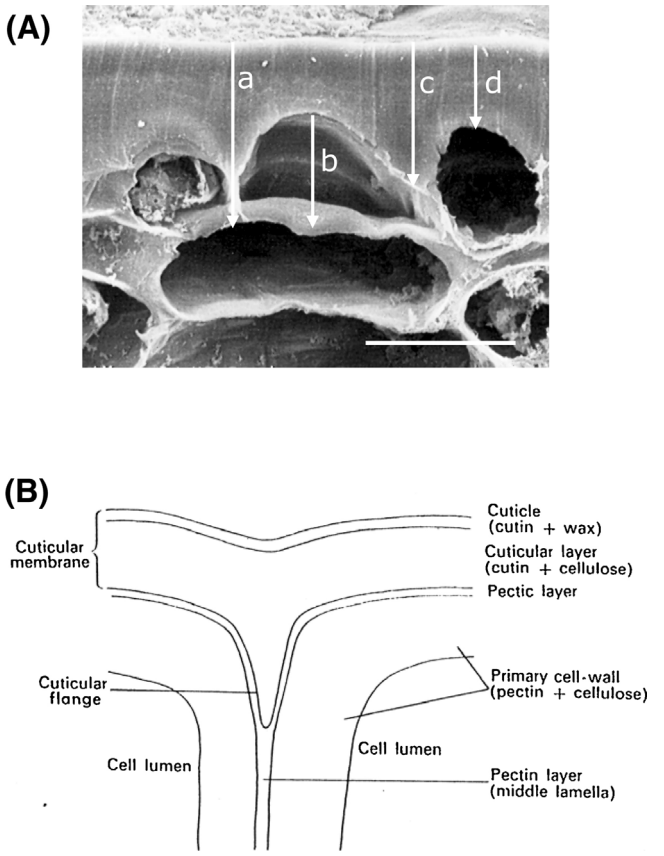

Fig. 1. (A) Epicarp aspects analyzed: (a) epicarp layer; (b) epidermal cell; (c) cuticular flange and (d) cuticular membrane. Bar = 20 μm. (B) Scheme of plant cuticle from Stace (1963).

To investigate epidermal olive tissues, tissue blocks (approx. 3.0 mm × 1.5 mm × 1.5 mm sizes) were cut revealing the epicarp in longitudinal view. The samples were fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). Each sample was rinsed 3 times in cacodylate buffer and slowly transferred through a short alcohol series (70°, 95° and 100°), before critical-point dried in supercritical liquid carbon dioxide. To avoid alteration of the wax crystals, no standard dehydratation procedures were applied to the specimens. The dry tissues were then mounted on aluminium stubs and coated with gold (25 nm thick) in a Balzers Union SCD 040 Sputter Coater (Balzers, Wiesbaden, Germany). Representative specimens were examined with a Philips XL 20 Scanning Electron Microscope equipped with Scandium Image Analyzer (Olympus) to obtain measurements of the different tissues.

The epicarp aspects examined in this work are the followings and are explained in Fig. 1:

a) the entire thickness of epicarp from surface to the base of epidermal cell,
b) the height of the epidermal cell,
c) the thickness of the cuticular flange that is wedged between epidermal cells,
d) the thickness of the cuticular membrane, the continuous layer on the top of the epidermal cell.