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Structure and development of the elaiosome in *Myrtus communis* L. (Myrtaceae) seeds

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Abstract

Myrtus communis L. (Myrtaceae) is a fleshy-fruited shrub occurring in the Mediterranean area, whose seeds are snail shaped, with a very thick coat and a central elaiosome. This structure is a fleshy and edible appendage responsible of seed dispersal by ants, a phenomenon known as myrmecochory. The elaiosome develops very early from the external integument cells near the funicular and micropylar area, through cell enlargement and cell divisions. At the latest stages of development, some internal integument cells participate to its formation so that myrtle elaiosome can be classified among those originating from both epidermal and inner tissues.

The low content of lipids, starch and proteins revealed by histochemical and biochemical analyses on *M. communis* elaiosomes suggests that the myrtle is a plant with a multiple pattern of seed dispersal. Myrmecochory seems to play a secondary role.

Keywords: *Myrtus*; Elaiosome; Ants; Myrmecochory; Lipids; Proteins; Starch

Introduction

The term elaiosome was introduced for the first time by Sernander (1906) to indicate all fleshy and edible appendages of disseminules dispersed by ants. Elaiosomes contain reserves, usually lipids but sometimes also proteins or starch that seem to play no role in germination but may be designed to attract and reward ants (van der Pijl, 1982). Elaiosome does not refer to a precise anatomical structure and can develop from different tissues. In fact, it may develop only from the outermost layer of the organ on which it forms, through cell enlargment and/or periclinal divisions, or development can involve inner layers as well (Werker, 1997). Moreover, not only seeds but also fruits may have elaiosomes.

Seed dispersal by ants is known as "myrmecochory", which is a widespread phenomenon involving many taxa. Myrmecochorous plants are found in most part of the world in temperate forests, in the tropics and in xerophytic communities of the Mediterranean countries, in the Californian chaparral, in Australia and in South Africa (Werker, 1997).

Aronne and Wilcock (1994) found elaiosomes in seeds of *Myrtus communis* L., a fleshy-fruited shrub common in the Mediterranean woodlands, maquis and garrigue. *M. communis* is an erect, much-branched shrub, up to 5 m. Its leaves are up to 5 cm, ovate-lanceolate, acute, entire, coriaceous, punctate, very aromatic when crushed. Flowers are up to 3 cm in diameter, sweet-scented. Pedicels are long, slender, with 2 small,

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caducous bracteoles. Petals are suborbicular and white. Fruits are berries, 7–10 × 6–8 mm, broadly ellipsoid to subglobose, usually blue-black when ripe (Campbell, 1968). Seeds are snail shaped, with a very thick seed coat and a central elaiosome. These evergreen plants are very aromatic because of the high essential oil content in the leaves, flowers and fruits glands. It is reported that fruits are bird-dispersed (Herrera, 1984), but more recently Aronne and Wilcock (1994) showed seed adaptation to ant dispersal and revealed the presence of unsaturated fatty acids in the elaiosome that play a role of ant attractant.

The aim of this paper is to describe the anatomy and ontogeny of the elaiosome of *M. communis* and to show by histochemical and biochemical tests the presence of lipids, starch and proteins in the elaiosome.

Materials and methods

M. communis seeds were collected in November 2002 and March 2003 from hills of Santa Maria in Castello, Vecchiano (Pisa, Italy; 43°47′N, 10°13′E) at 86 m above sea level. These two different periods of harvesting were chosen because fruits usually ripen in mid-November, but ripe fruits, if not dispersed, remain attached to the pedicel until the end of February and are therefore winter-persistent.

Plant voucher specimens are deposited in PI (Pisa Herbarium).

Light microscopy

Anatomy and ontogeny studies were carried out on ovules and seeds at different stages of development, from flower buds up to mature fruits.

Sections (25 μm) of fresh materials were cut using a Leitz 1720 cryostat at −14 to −16 °C. Other sections (3 μm) were cut using a Leica 2055 microtome, after fixing the material in FAA (Sass, 1958) and embedding it in LR White acrylic resin (SIGMA).

The sections were submitted to the following histochemical tests: Toluidine Blue (TBO) (O'Brien and McCully, 1981) as a generic dye for DNA, cytoplasm and some cell wall components; PAS (O'Brien and McCully, 1981) for non-cellulosic polysaccharides and starch; Red Nile (Fowler and Greenspan, 1985) for lipids detected by induction of fluorescence.

Fluorescence microscopy

A Leica DM LB fluorescence microscope with group A filter (515–560 nm) was used.

Determination of protein content

The protein content was determined by Bensadoun and Weinstein (1976) technique, which is a modified Lowry protein assay so that protein can be assayed in the presence of interfering chemicals. The only difference is the first step precipitation of proteins with Na-deoxycholate (2%) and trichloroacetic acid (6%) so as to separate the proteins from interfering material.

Elaiosomes were removed with a razor blade from the seeds collected in November 2002 and March 2003. They were air-dried and their weight was 360 and 473 mg, respectively. Ultraviolet spectra of the elaiosome extracts were obtained using a LKB spectrophotometer.

Results

Ontogeny of the elaiosome

The elaiosome becomes hard after several days of exposure to air. It is white in colour differing from the rest of the seed, which is generally dark (Fig. 1).

Ontogeny studies on *M. communis* ovules showed that the elaiosome begins to develope very early from the external integument cells near the funicular and micropylar area. It fully develops through cell enlargment and cell divisions when the seed is mature. At the latest stages of development, some internal integument cells participate to its formation. At maturity, the elaiosome occupies the central groove and the micropylar area of the seed.

At the earliest stages of ovule ontogeny, the external integument, which is formed by two or three layers of cells, and the two-layer internal integument, enveloping the nucellus, become evident (Fig. 2A). Near the funicular area some cells of the external integument begin to divide and to increase in size (Fig. 2B). The

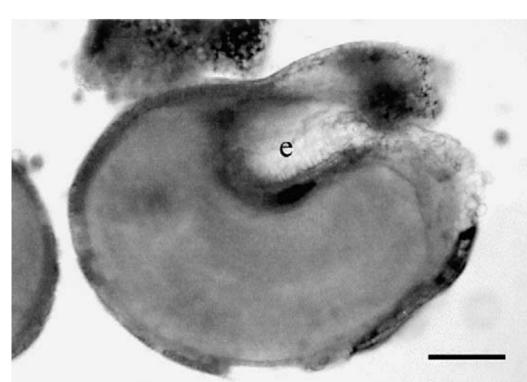

Fig. 1. *Myrtus communis* seed. Scale bar = 1 mm; e = elaiosome.