Acta Societatis Botanicorum Poloniae

DOI: 10.5586/asbp.3490

Publication history
Received: 2015-11-12
Accepted: 2016-02-26
Published: 2016-03-31

Handling editor
Beata Zagórska-Marek, Faculty of Biological Sciences, University of Wrocław, Poland

Authors' contributions
CG, CT: conducting micromorphological observation; LMB: helping to interpret the results and discussion; CG: research designing and writing the manuscript

Funding
Funded by MIUR (Italian Ministry of Education, Universities and Research; Ex60%).

Competing interests
No competing interests have been declared.

Citation
Giuliani C, Tani C, Maleci Bini L. Micromorphology and anatomy of fruits and seeds of bitter melon (*Momordica charantia* L., Cucurbitaceae). Acta Soc Bot Pol. 2016;85(1):3490. http://dx.doi.org/10.5586/asbp.3490

SHORT COMMUNICATION

Micromorphology and anatomy of fruits and seeds of bitter melon (*Momordica charantia* L., Cucurbitaceae)

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Abstract

The aim of this paper is investigating the micromorphological properties of fruits and seeds in the food and medicinal plant *Momordica charantia* L. (Cucurbitaceae). A detailed anatomical description on cross-sections of immature fruits and seeds is reported for the first time.

The fruit is characterized by a thin epicarp, a multi-layered mesocarp and by an inconspicuous endocarp. The seed-coat displays a pattern of organization in five tissues. These endomorphic features were compared and discussed with the results of previous investigations on other representatives of the genus *Momordica*. Since the structure of seed-coat is considered diacritical in the taxonomy of the genus, this report may offer a set of additional character useful for the characterization of the genus.

Keywords

Momordica charantia; Cucurbitaceae; fruit, seed-coat; light microscope; scanning electron microscope; morpho-anatomy; medicinal plant

Introduction

Nowadays about 80% of the world population still uses herbal medicine for primary health problems. In the past two decades, concurrently with the renewed interest in drugs of plant origin as novel sources of bioactive compounds, extensive scientific research have been addressed on the properties of traditional plant drugs [1].

Momordica charantia L., known as bitter melon, is one of such plant. It is a climbing species belonging to the Cucurbitaceae family and growing in tropical areas of Asia, East Africa, Amazon, and the Caribbean [2]. It is an important commercial vegetable crop in many countries with a raining tropical or subtropical climate, and is still used also for medicinal purposes.

The plant was used in Africa and Asia not only for food, but also against several deseases, as anthelmintic, digestive, laxative, and many others [3]. However, the western medicine paid attention to the plant only when the antidiabetic properties of fruits and seeds were experimentally evidenced. Since then, the plant has been the subject of over a hundred scientific articles focusing on its phytochemistry and pharmacology [2].

The plant presents characteristic leaves with serrated margins; probably this feature gave rise to the genus name deriving from the Latin word *momordere* that means "to bite". The flowers are yellow or yellow/orange. The fruit, ellipsoid to oblong in shape, splits on maturity into three valves exposing 5–15 arillate seeds, as it occurred in all of the members of the genus [2]. The fruit mesocarp is soft and spongy with a bitter taste. The nutritional value is scarce, the fruit containing low quantities of carbohydrates and very few proteins, but compared to other Cucurbitaceae has a relatively high nutritional value, due mainly to the iron, phosphorus and ascorbic acid content [2].

Several centuries ago, plants of the genus *Momordica* were cultivated also in Italy, as documented in a picture by an anonymous painter of the fifteenth century representing the *Virgin Mary with the Child and saints* under a pergola of *Momordica* bearing fruits at different ripening stages [4]. Afterwards, the plant cultivation was abandoned, probably as a result of the introduction from the New World of other Cucurbitaceae, more pleasant to taste and more suitable to the Italian climate.

In spite of the extensive literature with phytochemical and pharmacological focus, reports on fruit and seed structure of *Momordica charantia* are lacking. This work aims to highlight the morphoanatomical features of fruits and seeds with a view to focus future studies on their ontogeny. In addition, since several seed-coat features (e.g., number of tissues of the seed-coat, number of cell layers constituting each tissue, etc.) have recognized as valuable diacritical characters in the infrageneric classification of *Momordica* [5], the present report could further enhance the taxonomy of the genus.

Material and methods

Plant material collection and laboratory methods

Momordica charanthia seeds were bought from the online seed trade and used for the morphological investigation. In addition, some seeds were sowed in a soil-based compost in April 2012, afterward the seedlings were planted and cultivated at the Botanical Garden of the University of Florence in May 2012 and the fruits were collected for micromorphological observation at the immature stage in June 2012. Additional observations were also performed on immature fruits on sale at the vegetable markets in Florence, Italy. The micromorphological observations were carried out on immature fruits and on both immature and mature seeds by light microscopy (LM) and scanning electron microscopy (SEM).

LM observations

Frozen fresh material and fixed samples, embedded in historesin, were sectioned and stained with different techniques in order to better describe the layers constituting the seed coat and the pericarp structure. The stainings employed were: toluidine blue as a general tissue staining [6], calcofluor for cellulose [7], Lugol solution for starch [8], periodic acid-Schiff's reagent (PAS) for polysaccharides [9], 1% aniline blue-black in 7% acetic acid for gross protein [10], Sudan Red for total lipids [11], Sudan III, Sudan IV for neutral lipids [6], and ferric trichloride for polyphenols and tannins [12]. Observations were made with a Leitz DM-RB Fluo optic microscope.

SEM observations

Small pieces of seeds were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer at pH 6.8, dehydrated in ethanol in ascending grades up to absolute and then dried using a Critical Point Dryer apparatus. The samples, coated with gold, were observed with a Philips XL-20 SEM.

Results

The fruit has a tuberculate surface presenting numerous swellings; it is emerald-green in color when immature, turning orange-yellow during ripening. At the mature stage it exposes red seeds, provided with an aril, that after maturity exhibit a sculptured surface (Fig. 1a–c).

The pericarp is characterized by five distinct tissues (Fig. 2a–g), as follows:

- Epicarp – the cells are generally isodiametric with those close to the stomata being tangentially-elongated (Fig. 2a,b). The outer periclinal walls are covered by a thin cuticle (Fig. 2c); the underlying two-three layers present occasionally thickened walls.
- Outer mesocarp – this tissue consists of a variable number of layers (5–7) with large isodiametric or radially-elongated cells, apparently devoid of cell sap, and a network of tinny intercellular spaces (Fig. 2a,d).
- Middle mesocarp – the cells are tangentially-elongated and thick-walled, often turgid with a watery cell sap and containing a small amount of starch. Numerous bicollateral vascular bundles occur throughout the middle mesocarp: they are either small and soft, or large and stiff, forming an anastomosing vein-like system arranged in a ring (Fig. 2d–f).
- Inner mesocarp – several layers of thin-walled cells, forming this tissue, present a huge amount of starch in comparison to the preceding layer. The cells are large with wide intercellular spaces. The inner layer bears stomata and delimits a cavity containing the seeds (Fig. 2d,g).
- Endocarp – this tissue, formed by very small, thin-walled and tangentially elongated cells, is thin and translucent. We observed occasionally this inconspicuous tissue.

The seeds are oblong with grooved margins and a sculptured surface (Fig. 1c). The seed-coat may be differentiated into five distinct layers (Fig. 3a–f), that are enclosed by the aril (Fig. 3b,c):

- Epidermis – this tissue consists of a single layer of prismatic palisade cells. They are generally of equal height over the flat surface of the seed, increasing in height at the sculpturations (Fig. 3b,c). The radial walls are often uniformly thickened, nevertheless in some cases they have either straight or branched thickenings running from the inner to the outer tangential walls. The outer walls are thickened. A noteworthy feature is the presence of large starch grains in this layer (Fig. 3c).
- Hypodermis – this tissue is made up of four layers of small, isodiametric, tightly closed sclerenchymatized cells, without intercellular spaces. A huge amount of starch granules is evident (Fig. 3b).
- Sclerenchyma – this layer consists of exceedingly thick-walled cells. The walls are sinuous and the starch content is massive (Fig. 3b).
- Aerenchyma – many cell layers of spongy parenchyma, differing greatly in size and shape, make up this layer. The cells of the outer layers are usually small and frequently sclerenchymatized. Underneath these small cells are one or more layers of either large or small thin-walled cells having very large intercellular spaces (Fig. 3b). The vascular bundles are imbedded in this layer at the flattened surface of the seed.
- Chlorenchyma – a single layer of small, polygonal, and inconspicuous thin-walled parenchyma cells, containing chlorophyll, forms the inner tissue (Fig. 3b,d).

The leaf-like cotyledons have an epidermis of small cells below which, on the inner side, are two sharply defined palisade layers. All of the cells are filled with oil and protein granules (Fig. 3e,f).

Fig. 1 Macrographs of fruit and seeds of *Momordica charantia* L.: immature fruit (**a**); mature fruit exposing arillate red seeds (**b**); mature dry seed (**c**; scale bar = 1 cm).

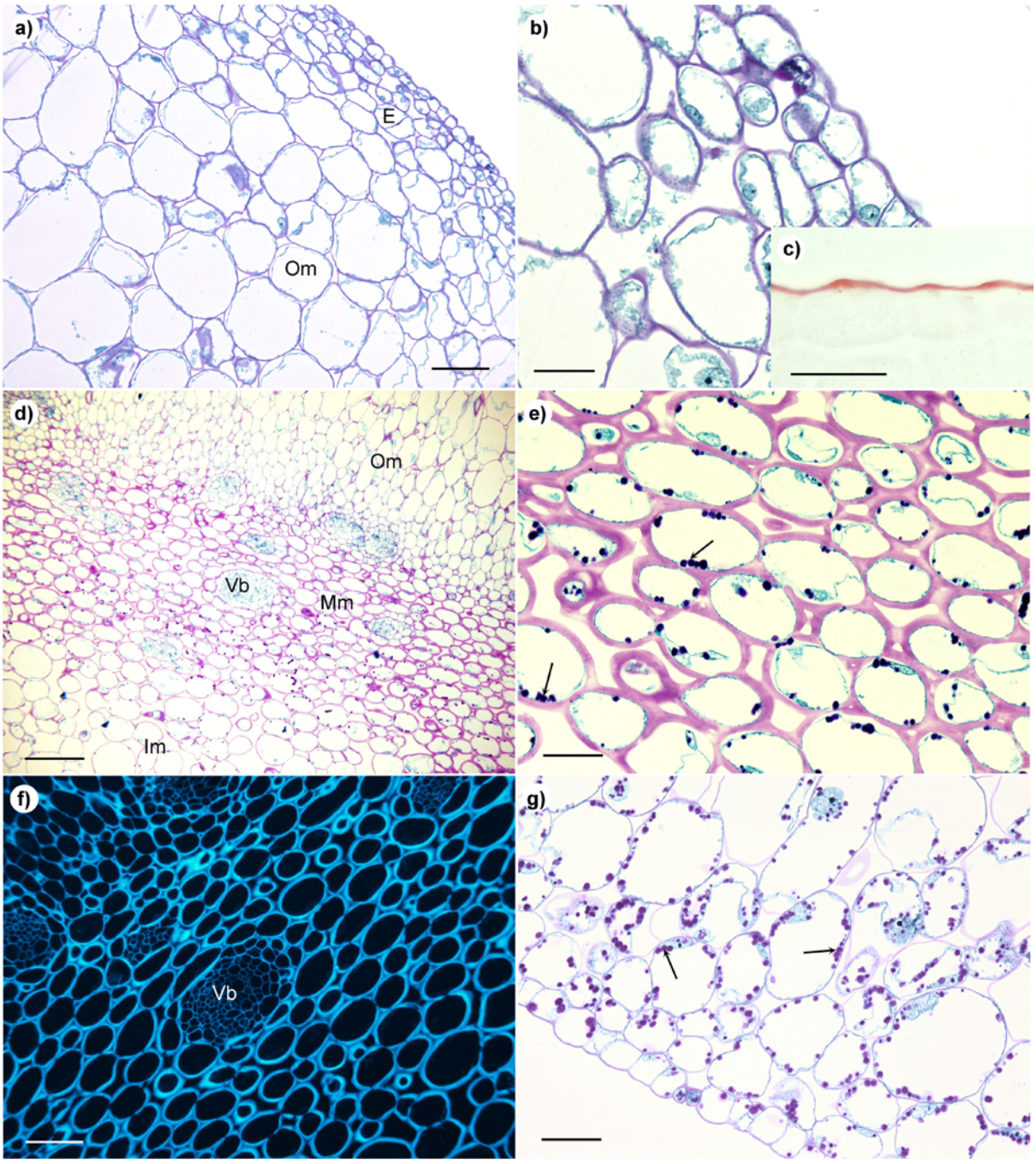

Fig. 2 LM micrographs of cross sections of the immature fruit of *Momordica charantia* L: epicarp and outer mesocarp, PAS reaction + aniline black b (**a,b**); thin cuticular layer covering the epicarp, sudan red (**c**); outer, middle and inner mesocarp, PAS reaction + aniline black b (**d**); middle mesocarp with thick-walled cells containing few starch grains (arrows), PAS reaction + aniline black b (**e**); middle mesocarp, calcofluor (**f**); inner mesocarp with thin-walled cells containing a lot of starch grains (arrows), PAS reaction + aniline black b (**g**). Scale bars = 10 µm (**a,f**); 25 µm (**b,c**); 5 µm (**d,e,g**). E – epicarp; Im – inner mesocarp; Mm – middle mesocarp; Om – outer mesocarp; Vb – vascular bundle.

Discussion

The present work provides novel insights into the micromorphology and anatomy of the fruits and seeds of *M. charanthia*, a species with enormous potential as source of both food and drugs [2].

The structure of fruits and the development of seeds of Cucurbitaceae have been investigated by several authors since long time [13–15].

The distinction of an epicarp and of a multi-layered mesocarp – which may be divided in turn into three different tissues, on the basis of the cell features and cell wall

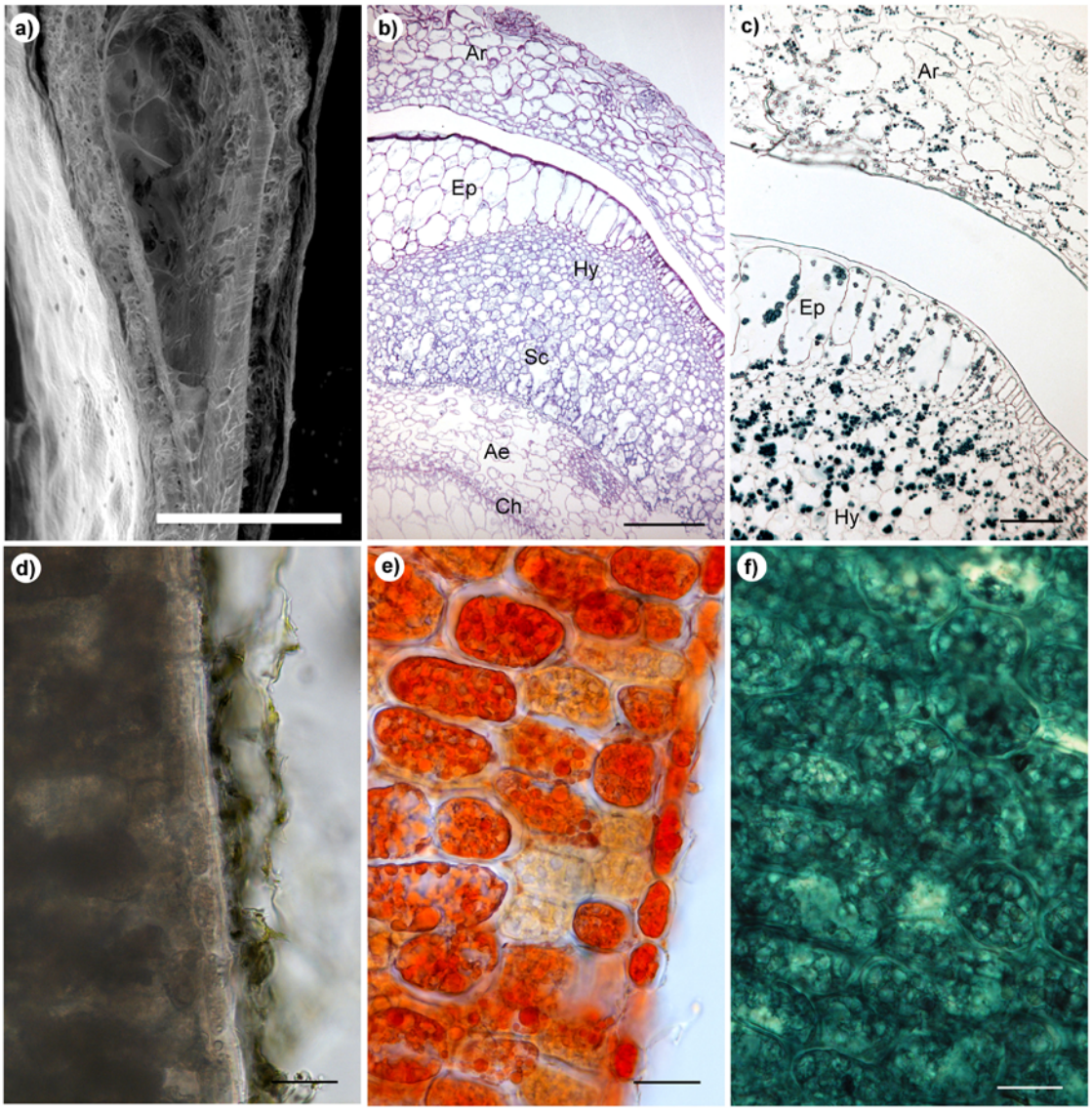

Fig. 3 **a** SEM micrograph of a cross-section of the immature seed of *Momordica charantia* L. **b,c** LM micrographs of cross-sections of immature seeds of *Momordica charantia* L: aryl and seed-coat layers, toluidine blue (**b**); particular of the epidermis and hypodermis of the seed-coat with abundant starch grains, Lugol solution (**c**). **d–f** LM micrographs of cross-sections of mature seeds of *Momordica charantia* L: chlorenchyma, stainless (**d**); cotyledon with abundant lipophilic cellular droplets, sudan red (**e**); cotyledon with copious proteic content, aniline black b (**f**). Scale bars = 1 mm (**a**); 10 µm (**b,c**); 25 µm (**d–f**). Ae – aerenchyma; Ar – aril; Ch – chlorenchyma; Ep – epidermis; Hy – hypodermis; Sc – sclerenchyma.

thickness – is evident. The endocarp is inconspicuous, while in other Cucurbitaceae it remains so firmly attached to the dry seed that some authors defined it as the outer layer of the spermoderm [14].

The exomorphyc and endomorphic features of the seeds of Cucurbitaceae have been critically analyzed and are considered valuable diagnostic characters for tribal or infrageneric classifications or for species identification [15,16]

However, in the genus *Momordica*, the diversity of seed-coat anatomy have not been extensively explored yet. The *Momordica* genus is large, including about 60 species, with only a limited number of species being already investigated. Aguoru and Okoli [5] studied the seed-coat anatomy of five *Momordica* species from Nigeria, including *M. charantia*.

The data are therefore not sufficiently adequate to define the feasible range of variations in the seed-coat structure in this genus. However, within limits of the investigated species, the seed-coat anatomical features proved useful for species identification in *Momordica* from Nigeria [16]. *Momordica charanthia*, *M. multiflora*,

and *M. cabraei* showed five distinct tissues, epidermis, hypodermis, sclerenchyma, aerenchyma, and collenchymatous parenchyma, whereas *M. cissoids* and *M. foetida* showed four distinct layers, lacking aerenchyma. The most striking difference is the number of cellular layers consisting each zone, in particular it has been proposed that the number of layers in the hypodermal tissue could be used to discriminate among these five species.

The seed-coat of the samples we analyzed exhibited a common pattern of organization in comparison to the investigated *Momordica* species. Indeed, our investigation confirmed the occurrence of five distinct tissues in the seed-coat and the presence of four hypodermal layers, thus enhancing the diagnostic value of this character for species identification [5]. However the characterization of the inner layer is not consistent with the previous report since we described a clorenchyma and not a collechymatous parenchyma, in affinity with other members of Cucurbitaceae [15].

Since the structure of fruit and seed coat is considered diacritical in the taxonomy of the genus *Momordica* [2,5], this report offers a set of further characters useful for the taxonomy of the genus, also at with the aim of laying the basis for future ontogenetic investigations.

Acknowledgments

We wish to thank Pietro Di Falco for helping in sample preparation.

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