

Anatomical study of different fruit types in Argentine species of *Solanum* subgen. *Leptostemonum* (Solanaceae)

by

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Abstract

Chiarini, F. & G. Barboza. 2007. Anatomical study of different fruit types in Argentine species of *Solanum* subgen. *Leptostemonum* (Solanaceae). *Anales Jard. Bot. Madrid* 64(2): 165-175.

The fruits of 11 species of *Solanum* subgen. *Leptostemonum* were studied. Cross and/or longitudinal microtome sections, stained mostly with astra blue/basic fuchsin, were prepared for microscopic examination. The fruits, notably heterogeneous, were classified into three categories. Three different kinds of cells were found of the epidermis, immediately below which a hypodermis, consisting in any of five types of structures, was always found. The mesocarp presented two histologically differentiated zones, an external one (formed by normal or spongy parenchyma, depending on the species), and an internal one, commonly juicy, and with proliferations among the seeds. This morpho-anatomical information was used to distinguish between non-capsular dehiscent fruits and the berry traditionally described for *Solanum*. The relationship between structure and function, and the probable dispersal syndromes are also discussed.

Keywords: anatomy, Argentina, berry, dispersal syndrome, non-capsular dehiscent fruit, *Solanum*, sect. *Melongena*.

Resumen

Chiarini, F. & G. Barboza. 2007. Estudio anatómico de los diferentes tipos de frutos en las especies argentinas de *Solanum* subgen. *Leptostemonum* (Solanaceae). *Anales Jard. Bot. Madrid* 64(2): 165-175 (en inglés).

Se estudiaron los frutos de 11 especies de *Solanum* subgen. *Leptostemonum*. Para ello, se efectuaron cortes microtómicos longitudinales y/o transversales, teñidos en su mayor parte con azul astral/fucsina, y fueron examinados al microscopio. Los frutos, notablemente heterogéneos, fueron clasificados en tres categorías. Tres diferentes tipos de células fueron encontrados en la epidermis, e inmediatamente por debajo se observó siempre una hipodermis, constituida por uno de cinco tipos de estructuras. El mesocarpio presentó dos zonas histológicamente diferenciadas: una externa (formada por parénquima normal o esponjoso, según la especie) y una interna, comúnmente jugosa y con proliferaciones entre las semillas. Esta información morfo-anatómica fue usada para distinguir entre el fruto dehiscente no capsular y la baya tradicionalmente descrita para *Solanum*. Se discutieron además la relación entre estructura y función y los probables síndromes de dispersión.

Palabras clave: anatomía, Argentina, baya, fruto dehiscente no capsular, síndrome de dispersión, *Solanum*, sect. *Melongena*.

Introduction

Fruits provide the mechanism by which seeds are dispersed. The former also constitute the simplest and most conspicuous trait of Angiosperms. Consequently, the use of fruit types as taxonomic characters has always been fundamental in many plant families. In Solanaceae, for instance, they have already proved to be systematically useful (Bernardello, 1983; Dave, 1986; Filippa & Bernardello, 1992; Barboza & al., 1997; Knapp, 2002a). Different fruit features have shown to be valuable, such as ventilation clefts (Dave

& al., 1979), placentation patterns (Symon, 1984, 1987; Nee, 1986), sclereids (Danert, 1969), stomata and pores (Patel & Dave, 1976), dehiscence (Kaniwsky, 1965; Dyki & al., 1997), and stone cells (Bitter, 1911, 1914; Danert, 1969; Filippa & Bernardello, 1992), among others.

The largest and most diverse genus of Solanaceae (and even one of the largest among Angiosperms) is *Solanum* L., with approximately 1100-1400 species (Nee, 1999; Hunziker, 2001; Bohs, 2005). Almost the third of its species belong to the subgenus *Leptostemonum* (Dunal) Bitter, a group of importance since it

includes food plants (e.g. eggplant, *S. melongena* L.; naranjilla or lulo, *S. quitoense* Lam.; gilo, *S. aethiopicum* L.; cocona or cubiu, *S. sessiliflorum* Dunal) and weeds (e.g. tropical soda apple, *S. viarum* Dunal; silverleaf nightshade, *S. elaeagnifolium* Cav.; sticky nightshade or wild tomato, *S. sisymbriifolium* Lam.; horsenettle, *S. carolinense* L.; buffalo bur, bull thistle or Texas thistle, *S. rostratum* Dunal). Among the sections in which *Leptostemonum* has been divided, sect. *Melongena* (Mill.) Dunal, with 34 species in the New World (Nee, 1999), deserves special attention, because it includes the eggplant, and some of its representatives have been the subject of several studies due to their andromonoecy (Wakhloo, 1975a, b; Dulberger & al., 1981; Solomon, 1986, 1987; Elle, 1999; Connolly & Anderson, 2003). Nevertheless, the boundaries of sect. *Melongena* are unclear, according to recent molecular studies (Bohs, 2005; Levin & al., 2006; Weese & Bohs, 2007). In addition, the fruits of several species assigned by Nee (1999) to this section are different from the traditionally defined berry (Matesevach, 2002; Dottori & Cosa, 2003). In this sense, Knapp (2002a) has discussed the apparent uniformity of fruit type in *Solanum*, suggesting a remarkable range of subtle variation. This author proposed that within the vast subgen. *Leptostemonum*, fruits can be either fleshy berries, hard berries (all without stone cells) or variously modified non-capsular dehiscent fruits. Such classification of fruit types is based mostly on macroscopic features (colour, size, dehiscence), since studies which focus on the anatomical/histological point of view are scarce. For the entire subgenus, only some scattered species have been studied (Miller, 1969; Dave & al., 1979; Dottori, 1998; Dottori & Cosa, 1999, 2003). Thus, a contribution that considers these aspects of *Solanum* subgen. *Leptostemonum* should be welcome.

Taking these facts into account, the present work attempts to assess the anatomical differences among fruit types in Argentine species of *Solanum* subgen. *Leptostemonum* (formerly assigned to sect. *Melongena*) as a contribution towards clarifying the systematic of this group and to understanding the relationship between structure and function.

Materials and methods

Eleven wild species of *Solanum* subgen. *Leptostemonum* were analyzed. All but one species grow in Argentina; some of them are exclusive of this territory, while others are South American or naturalized in other parts of the world. *Solanum marginatum* is native of Africa, but is usually cultivated in many places and grows naturalized in disturbed areas of the Andes

from Colombia to Chile. According to Nee's (1999) *Solanum* classification system (the latest to give formal taxonomic rank to the different species groups) all the considered species belong to sect. *Melongena*. However, the clade system proposed in Levin & al. (2006) is taken into account for the discussion. The following are the voucher data of the studied material:

S. aridum Morong. ARGENTINA. **Córdoba:** Capital, 1-XII-1998, Chiarini 16 (CORD). **Salta:** Capital, 19-I-2002, Barboza 331 (CORD).

S. comptum C.V. Morton. ARGENTINA. **Corrientes:** Capital, near the airport, 13-V-2004, Barboza & al. 999 (CORD). Perichón, 29°24'34"S 58°45'09"W, 13-V-2004, Barboza & al. 1001 (CORD). San Cosme, 27°18'42"S 58°29'22"W, 13-V-2004, Barboza & al. 1005 (CORD).

S. elaeagnifolium Cav. ARGENTINA. **Córdoba:** Sobremonte, San Francisco del Chañar, 9-XII-2001, Chiarini 565 (CORD).

S. euacanthum Phil. ARGENTINA. **Córdoba:** Sobremonte, 29°46'06"S 64°34'03"W, 28-II-2002, Chiarini & al. 560, 563 (CORD). **Neuquén:** Collón Curá, 20-II-2005, Barboza & al. 1181 (CORD).

S. hieronymi Kuntze. ARGENTINA. **La Rioja:** Chilecito, Puesto Las Trancas, 19-II-2003, Barboza & al. 569 (CORD). **Córdoba:** San Javier, Yacanto, 9-I-1996, Cosa 266 (CORD). Rio II, Colazo, 23-VI-1983, Hunziker & al. 3674 (CORD). **San Luis:** Chacabuco, Concarán, 17-II-1989, Hunziker & al. 25332 (CORD).

S. homalospermum Chiarini. ARGENTINA. **Córdoba:** Sobremonte, 29°46'34"S 63°59'59"W, 29-XI-2001, Chiarini 505 (CORD).

S. juvenale Thell. ARGENTINA. **Córdoba:** Capital, 8-XII-2001, Chiarini 504 (CORD). **La Pampa:** Toay, 36°38'51"S 64°22'42"W, 19-II-2005, Barboza & al. 1173 (CORD).

S. marginatum L. f. SPAIN, Canary Islands, 15-VI-2005, Oberiti s.n. (CORD 1040). CHILE. **V Región:** Laguna Verde, 33°06'32"S 71°39'09"W, 8-II-2007, Chiappella & al. 1654 (CORD).

S. mortonii Hunz. ARGENTINA. **Catamarca:** Capayán, 28°41'55"S 66°02'53"W, 23-II-2003, Barboza & al. 633 (CORD); ibidem, 28°42'23"S 66°01'29"W, 23-II-2003, Barboza & al. 639 (CORD); ibidem, 28°34'56"S 65°56'07"W, 23-II-2003, Barboza & al. 644 (CORD).

S. multispinum N.E. Brown. ARGENTINA. **Formosa:** Pilcomayo, Laguna Blanca, 25°07'50"S 58°15'57"W, 14-XII-2002, Barboza & al. 511 (CORD). Route 86, 25°05'49"S 51°18'59"W, 14-XII-2002, Barboza & al. 520 (CORD).

S. sisymbriifolium Lam. ARGENTINA. **Córdoba:** Capital, XII-1998, Chiarini 27 (CORD). **Salta:** Rosario de Lerma, Corralito, 29-XII-1987, Novara 7363 (CORD).

For microscopic examination, whole or cut up ripe fruits were preserved in a formaldehyde-acetic acid-ethanol mixture, then dehydrated in a 50 to 100 % ethanol series, and embedded in Paramat® resin. Cross and/or longitudinal and/or tangential microtome sections 10-12 mm thick were stained mostly with a 1% astra blue solution in a 1% water/basic fuchsin solution in 50° ethanol. Astra blue stains cell wall polysaccharides such as cellulose and pectins, while basic fuchsin shows affinity for lignified, suberized or cutinized walls, i.e., structures embedded in phenolic substances (Kraus & al., 1998). Basic fuchsin also stains chloroplasts and nucleic acids. In some cases, additional sections were stained with a 0.05% cresyl blue solution in water (Pérez & Tomassi, 2002).

The specimens were visualized using a Zeiss Axio-phot microscope and the images were captured with a digital camera assembled to the microscope.

Results

According to Knapp (2002a), who developed a classification that subsumes that of Spjut (1994), the fruits of *Solanum* sect. *Melongena* studied here are berries in a conventional sense, or non-capsular dehiscent fruits. In Spjut's system, and according to our observations, fruits might be classified as follows:

Berry = a simple fruit with an indehiscent pericarp, containing many seeds embedded in a solid fleshy mass, supported by an epicarp that is less than 2 mm thick. E.g.: *S. sisymbriifolium* (Figs. 1 D, 2 E, F).

Foraminicidal capsule (Non-capsular dehiscent fruit according to Knapp, 2002) = Dry or slightly juicy fruit with a thin pericarp, which cracks in an irregular fashion, thus leaving the seeds exposed at the senescent stage. E.g.: *S. homalospermum*, *S. euacanthum* (Fig. 1 A-C).

Carcerulus = Fruit resembling the true berries, but with an aerial space between the seeds and the pericarp when the fruit is completely ripe, as in peppers (*Capiscum*). E.g.: *S. multispinum*.

Details of the anatomy of each species are summarized in Table 1.

Mature fruits are of a single colour. They can be red (e.g. *S. sisymbriifolium*, Fig. 1 D), yellow (*S. multispinum*, *S. juvenale*), black or brownish (*S. mertonii*) or greyish (*S. homalospermum*, *S. euacanthum*, Fig. 1 A-C). The fruiting calyx may be accrescent or not. In the first case, different degrees of accrescence are observed. Calices may enclose the berry almost completely (*S. sisymbriifolium*, Fig. 1D, *S. comptum*) or only half or a third part of the fruit (*S. juvenale*, *S. homalospermum*). Moreover, at maturity, the calyx lobes can split open (*S. sisymbriifolium*, Fig. 1 D) or continue to enclose the berry (*S. comptum*).

The pericarp comprises three clearly distinguishable zones: the exocarp, the mesocarp, and the endocarp.

Exocarp

The cuticle is highly variable and usually thick (especially in *S. sisymbriifolium*), and it can be smooth (e.g. Fig. 2 F), undulate (*S. hieronymi*, Fig. 3 E), or grooved-striate (*S. juvenale*, Fig. 3 B). In all species, cuticular wedges are present among the epidermal cells. Ventilation clefts and stomata are lacking in all cases.

A range of variation was observed on the structures found in the epidermis. At first, a unistrate layer of

small, isodiametric (rectangular or rounded) cells, with dense content and cellulosic walls, was found in the berries (e.g. *S. juvenale*, Fig. 3 B; *S. hieronymi*, Fig. 3 E) and in the fruit of *S. multispinum*. Then, an epidermis formed by elongated or tall cells, more or less lignified, and arranged in one layer, is present in the

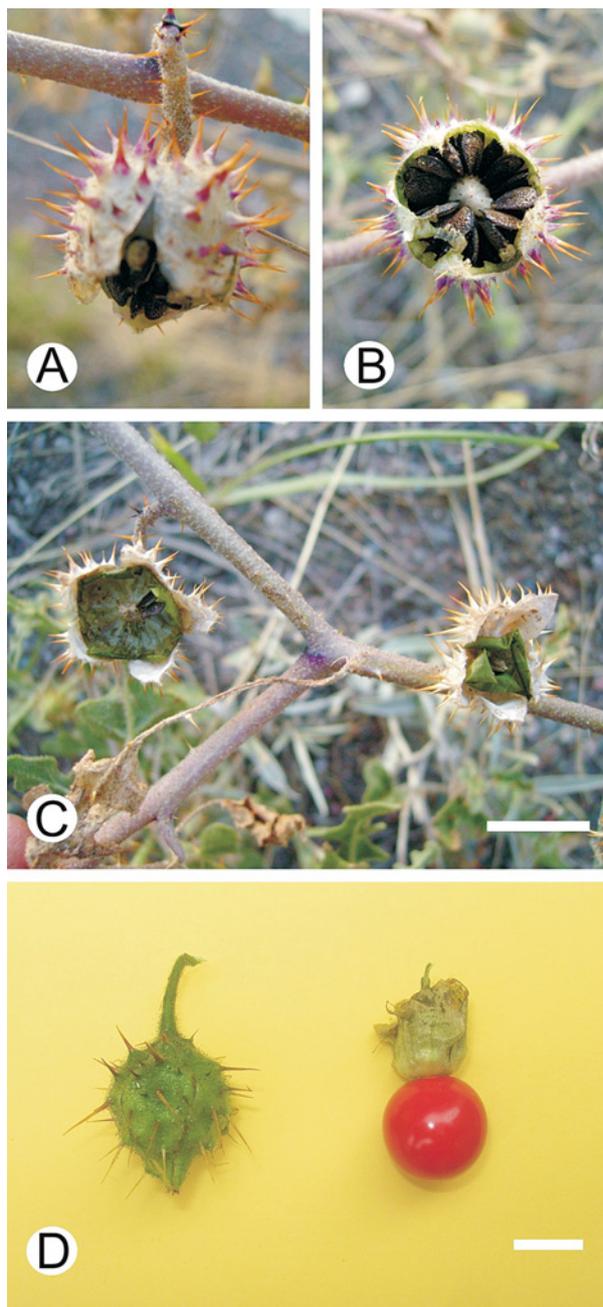


Fig. 1. Macroscopic aspects of two fruit types in *Solanum* sect. *Melongena*: **A-C**, non-capsular dehiscent fruit of *S. euacanthum*; **A**, side view, showing the accrescent calyx and the exposed seeds; **B**, inferior view; **C**, aspect of the fruits after the seeds fall, with the calyx open and the remains of the cracked pericarp; **D**, berry of *S. sisymbriifolium*, immature fruit enclosed by the calyx to the left, mature fruit with calyx withdrawn towards the right. Bar = 1 cm, in all pictures. A, B, C at the same scale.

berries of *S. elaeagnifolium* (Fig. 2 A) and in the non-capsular dehiscent fruits of *S. euacanthum*, (Fig. 3 C). Similar cells were found in *S. marginatum*, but arranged in more than one layer (Fig. 3 F). Finally, in the non-capsular dehiscent fruits of *S. homalospermum* and *S. mertonii*, the epidermis is composed by sclereids, one layer of brachysclereids in the first species (Fig. 2 D) and two layers of macrosclereids in the second (Fig. 2 C).

Immediately below the epidermis, a hypodermis is differentiated, consisting of any of the following kinds of structures: 1) one to several layers of collenchyma (*S. comptum*, *S. multispinum*), 2) one to three layers of radially compressed parenchymatous cells, with dense content, followed by collenchyma (e.g. *S. juvenale*, Fig. 3 B; *S. hieronymi* Fig. 3 E), 3) a well defined layer of fibres with evident pits in both the apical and the basal ends and with a single calcium oxalate crys-

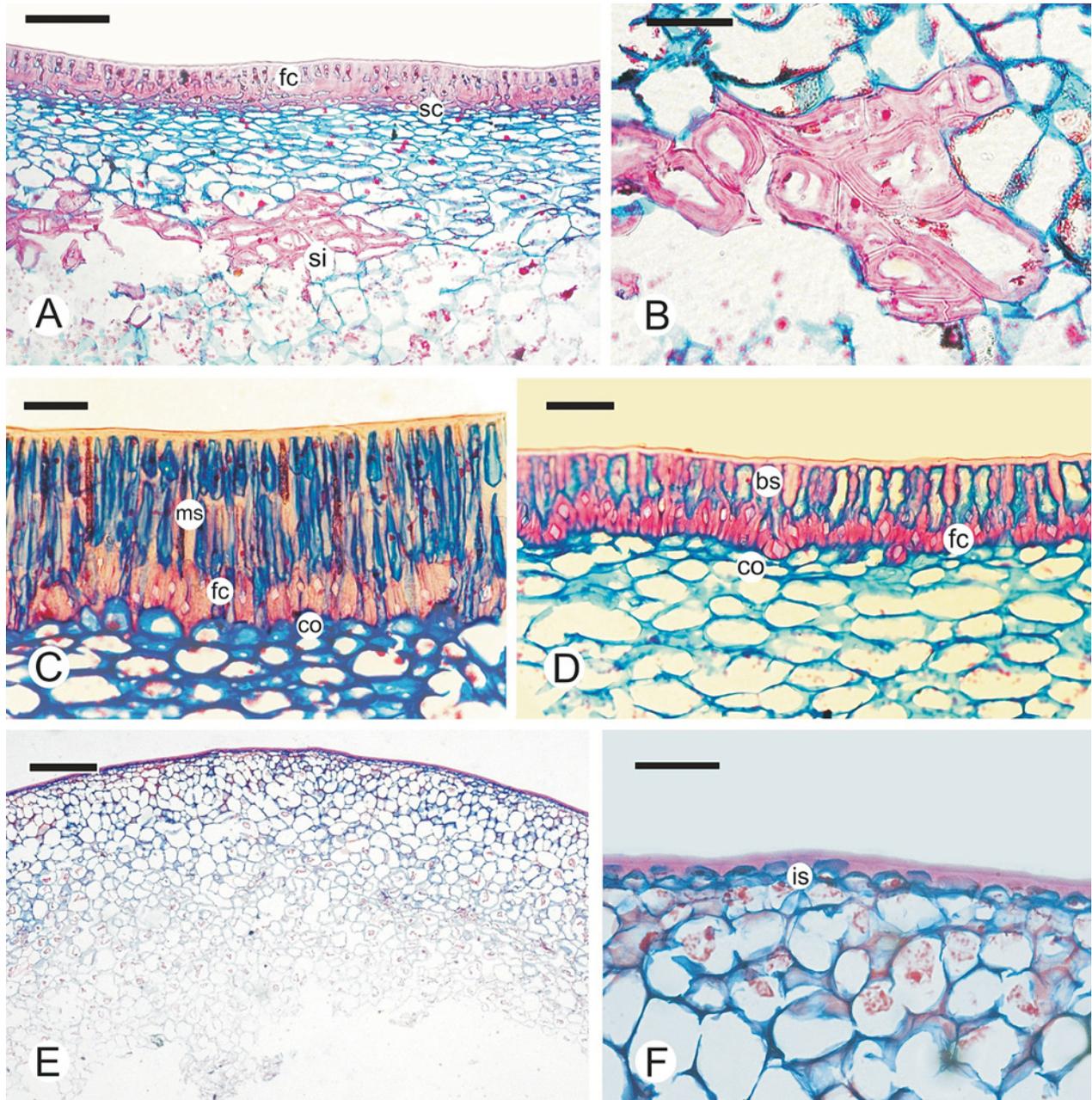


Fig. 2. Photomicrographs of fruit anatomy in *Solanum* sect. *Melongena* species: **A**, exocarp of *S. elaeagnifolium* (Chiarini 565, CORD); **B**, *S. elaeagnifolium*, detail of the sclereids (Chiarini 565, CORD); **C**, exocarp of *S. mertonii* (Barboza & al. 639, CORD); **D**, exocarp of *S. homalospermum* (Chiarini 505, CORD); **E**, pericarp of *S. sisymbriifolium* (Chiarini 27, CORD); **F**, *S. sisymbriifolium*, detail of the epidermis (Chiarini 27, CORD). Abbreviations: bs = brachysclereids; co = collenchyma; fc = fibres containing a crystal; ms = macrosclereids; sc = sclerified collenchyma; si = sclereids islets. Scale bars: A = 80 μ m; B = 15 μ m; C, D, F = 50 μ m; E = 0.5 mm.

tal occupying the whole lumen of each fibre, followed by 1 or 2 true collenchymatous layers (*S. homalospermum*, Fig. 2 D, *S. euacanthum*, Fig. 3 C, *S. mortonii* Fig. 2 C) 4) Several layers of very thickened and lignified cell walls, taller than wide, which could be considered a sclerified collenchyma (E.g. *S. marginatum*, Fig. 3 F). 5) A layer of elongated cells, some of them containing a crystal, followed by sclerified collenchyma (*S. elaeagnifolium*, Fig. 2 A)

The epidermis and the hypodermis constitute the

exocarp in the form of a unit, which generally has layers that gradually decrease their degree of lignification from the outside to the inside of the fruit. Usually, when the fruit is immature, the cell layers located below the epidermis (or below the crystalliferous layer or layer of fibres, when present) have chloroplasts and chromoplasts. When the fruit matures, the chloroplasts disappear and the cells become compressed. A collenchyma is always present, in which the number of layers and the degree of lignification vary according to the species.

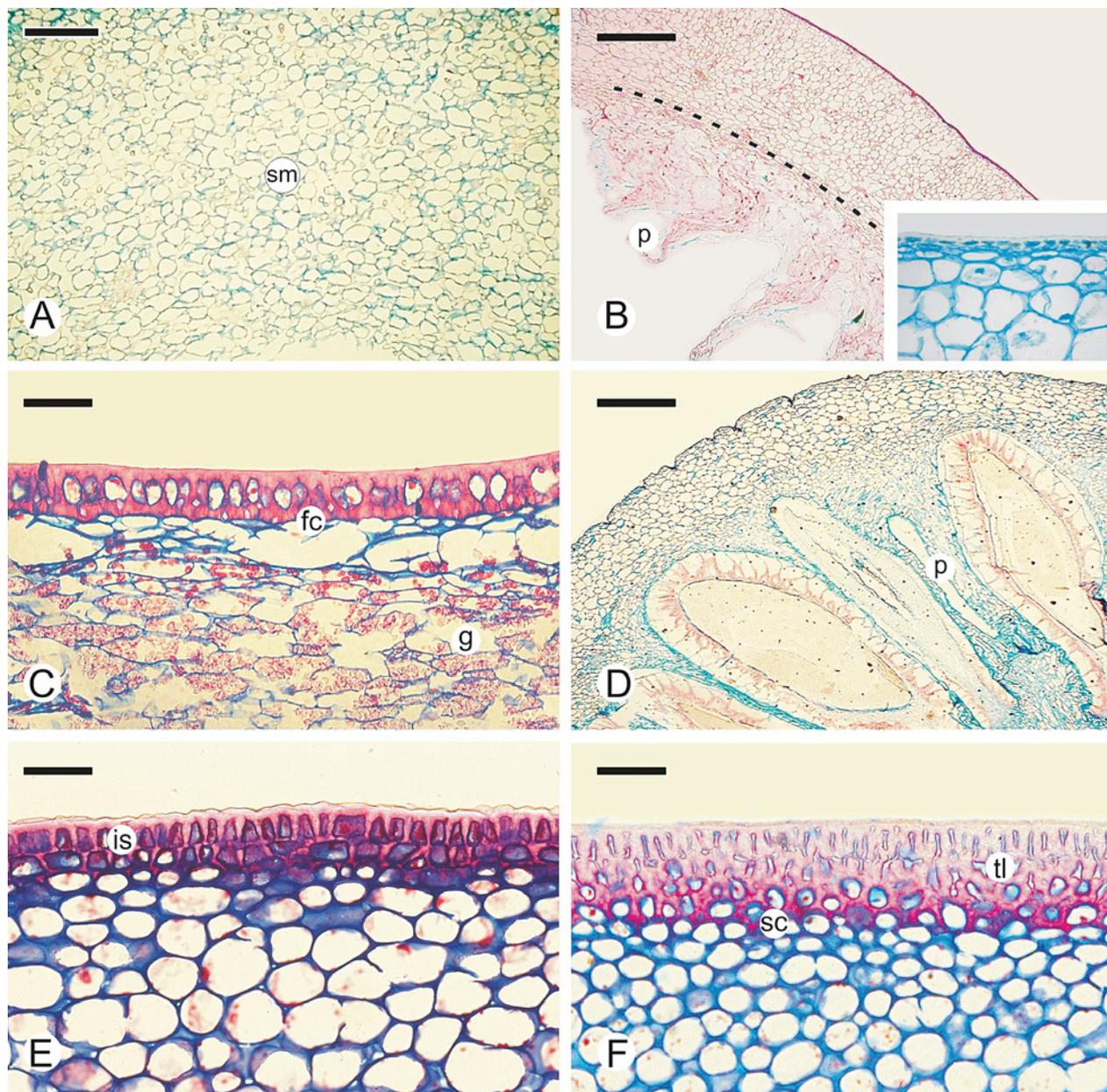


Fig. 3. Photomicrographs of fruit anatomy in *Solanum* sect. *Melongena* species: **A**, spongy mesocarp of *S. multispinum* (Barboza & al. 511, CORD); **B**, pericarp of *S. juvenale* (Chiarini 504, CORD), dotted line showing the division of the two zones of the mesocarp, in the square at the bottom left, a detail of the epidermis; **C**, exocarp of *S. euacanthum* (Chiarini 563, CORD); **D**, pericarp of *S. comptum* (Barboza & al. 999, CORD); **E**, exocarp of *S. hieronymi* (Barboza & al. 569, CORD); **F**, exocarp of *S. marginatum* (Oberti s.n., CORD 1040). Abbreviations: fc = fibres containing a crystal; g = cells filled with grana; is = isodiametric cells; p = proliferations among the seeds; sc = sclerified collenchyma; sm = spongy mesocarp; tl = tall lignified cells. Scale bars: A = 100 μ m; B, D = 1 mm; C, E, F = 50 μ m.

Table 1. Macroscopic and anatomical fruit features of the 11 species of *Solanum* sect. *Melongena* studied.

Species	Fruit type	Fruit colour	Exocarp			Mesocarp	
			Cuticle	Epidermis	Hypodermis	External zone	Internal zone
<i>S. aridum</i>	berry	yellow	grooved-striate	Unistrate, isodiametric cells with dense content	2-3 layers of compressed cells, with dense content	15-20 layers of a normal to somewhat spongy parenchyma	7-10 layers of juicy tissue; proliferations with grana
<i>S. comptum</i>	berry	grey- greenish	smooth	Unistrate, rectangular cells	1-2 layers of collenchyma	10-15 layers of normal, homogeneous parenchyma (Fig. 3D)	Proliferations fusing with the placentas (Fig. 3D)
<i>S. elaeagnifolium</i>	berry	yellow	smooth, with wedges	Unistrate, elongate cells (Fig. 2A)	1 layer of elongate cells, mixed with some cells containing a crystal, followed by a 1-2 layered sclerified collenchyma (Fig. 2A)	8-15 layers of normal to tangentially compressed parenchyma, with sclereids islets (Fig. 2A, B)	5-6 layers of juicy tissue, proliferations with grana
<i>S. euacanthum</i>	non-capsular dehiscent	greyish	smooth	1 layer of round to radially elongate cells	1 layer of fibres that contain a crystal, followed by 1-2 collenchymatous layers (Fig. 3C)	Reduced to 1-2 layers of normal parenchyma (Fig. 3C)	Proliferations with grana (Fig. 3C)
<i>S. hieronymi</i>	berry	yellow	undulate	Unistrate, isodiametric cells with dense content (Fig. 3E)	1-2 layers of radially compressed cells, followed by 2-5 layers of collenchyma (Fig. 3E)	40 layers of normal parenchyma over the veins and 30 underneath	Proliferations with grana among the seeds
<i>S. homalospermum</i>	non-capsular dehiscent	brown black	smooth	Unistrate, brachysclereids (Fig. 2D)	1 layer of fibres with a crystal inside, followed by 1-2 collenchymatous layers (Fig. 2D).	6 layers of a normal to radially compressed parenchyma.	Proliferations with grana among the seeds
<i>S. juvenale</i>	berry	yellow	grooved-striate	Unistrate, isodiametric cells with dense content (Fig. 3B)	2-3 layers of compressed cells, with dense content (Fig. 3B).	15-20 layers of a normal to somewhat spongy parenchyma (Fig. 3B)	7-10 layers of juicy tissue; proliferations with grana (Fig. 3B)
<i>S. marginatum</i>	carcerulus	yellow	smooth	1-2 layers of tall, lignified cells (Fig. 3F)	2-3 tall layers of sclerified collenchyma of tall cells, in transition to normal collenchyma (Fig. 3F)	25 layers of spongy tissue over the veins and 15-20 underneath, with the last layers compressed	Absent
<i>S. mortonii</i>	Non-capsular dehiscent	brown black	smooth	1-2 layers of macrosclereids as long as 4 times wide (Fig. 2C)	1 layer of fibres with a crystal inside, followed by 1-2 collenchymatous layers.	10 layers of normal parenchyma. Small proliferations with grana	Small proliferations with grana
<i>S. multispinum</i>	carcerulus	yellow	undulate	Unistrate, isodiametric cells with dense content	2-3 layers of collenchyma	25 layers of spongy parenchyma over the veins and 15 underneath (fig. 3A)	Tiny proliferations with grana
<i>S. sisymbriifolium</i>	berry	red	smooth	Isodiametric or round cells with dense content (Fig. 2E, F)	2-3 layers of collenchyma (Fig. 2E, F)	Absent	9-12 juicy parenchymatic layers, with the inner cells bigger (Fig. 2E). Proliferation of the placentas

Mesocarp

The number of layers of this structure gives the thickness to the pericarp. The higher the number of mesocarp layers, the thicker the pericarp. Fruits with a thick pericarp have usually more than 10 layers. The mesocarp consists of two zones histologically differentiated: an external one (immediately below the hypodermis), which we identified with astra blue, and an internal one, identified with basic fuchsin.

In the majority of the species, the external zone consists of regular, vacuolated, medium-sized cells with small intercellular spaces. Instead, in *S. marginatum* and especially in *S. multispinum*, the external zone consists of big, very vacuolated or almost empty, loosely connected cells, with large intercellular spaces, forming a spongy parenchymatic tissue, resembling the albedo of the hesperidium (i.e. the white pith of the inner peel of citrus fruits). These cells increase their size towards the endocarp, whose cell walls get loose and undulated. At maturity, the mesocarp is not in direct contact with the seeds.

The internal zone is commonly juicy, and develops proliferations among the seeds. The cells are large, with dense content filled with grana, which disorganize and release their content to the locules and produce a mucilage-like substance that surrounds the seeds in the ripe fruit. It is worth mentioning that in several species this mucilaginous content turns black on contact with the air, perhaps due to its phenolic or saponinic nature.

The thickness of each zone varies notably according to the species. For instance, in *S. juvenale* (Fig. 3 B), the difference between the two zones is remarkable and well-defined, each one having many layers of cells. In other species, the two zones are not so clearly-defined and have fewer layers.

Occasionally, scattered or grouped sclereids are present in the mesocarp of *S. hieronymi* and *S. elaeagnifolium* (Fig. 2 B). Stone cells or sclerosomes, widely present in many sections of *Solanum* and in related genera, are absent in subgenus *Leptostemonum*, or at least in the species analyzed here.

Endocarp

Finally, no specific particularities were observed in the endocarp. This layer, which is very difficult to observe due to its delicate structure, is uniseriate and, as in many Angiosperms (Roth, 1977), lacks stomata in all cases.

Discussion

Anatomical features

All the analyzed structures presented variations, but only some of these variants can be related to a function and can be useful in delimiting fruit types.

The cuticle, for instance, is a variable feature in *Solanum* (Dottori & Cosa, 1999, 2003) and does not seem to be associated to a determined fruit type.

Instead, the epidermis has shown an important diversification. A factor influencing epidermal structure would be the calyx accrescence, since fruits that are almost completely enclosed by it show a simple, thin epidermis, which is the case of *S. sisymbriifolium* (Chaparro, 1989; this paper). Apparently, in these fruits, the protective or mechanical function of a collenchymatous exocarp is strengthened by the enclosing calyx. Another modification related to external factors is the presence of fibres and sclereids in cracking fruits. In this case, there is not a protective calyx and the pericarp is destined to tear, releasing the seeds. All these derived types of epidermal cells (fibres and sclereids) may have originated from the small, isodiametric cells with cellulosic walls.

The presence of a hypodermis, mainly constituted by collenchyma, is constant in all the analyzed species; however, there is a specific variation in the number of layers and the degree of lignification. The collenchymatous hypodermis is common in fruits with a thick outer skin, which is the case of many berries and drupes, such as some species of *Ribes*, *Berberis* and *Paris* (Roth, 1977) and even in berries of some members of Solanaceae (Valencia, 1985; Filippa & Bernardello, 1992). The function of hypodermal cells would be to provide mechanical support or, in some cases, to participate in the dehiscence mechanism (Klemt, 1907; Dyki & al., 1997). The presence of a collenchyma in fruits of Solanaceae and, more precisely, in *Solanum*, has already been noticed (Klemt, 1907; Roth, 1977; Dottori & Cosa, 1999) and the species studied here also fit this pattern.

Some hypodermal cells, whose walls are impregnated with lignin, resemble the outline of a true collenchyma. Layers with such features are called here "sclerified collenchyma". The sclerified collenchyma, may be the structure that make the fruits harder and more resistant to deformation, and perhaps are a defence against phytophage insects. Indeed, fruit features are usually interpreted in relation to vertebrate dispersion and consumption, while the more important insect and microbial attack is neglected (Tewksbury, 2002). Among the species with sclerified collenchyma analyzed here, we observed fruits with no

sign of insect attack (e.g. *S. marginatum*). By contrast, in the case of fruits whose collenchyma consists of no more than three layers, berries show evident harm caused by phytophage insects (e.g. *S. juvenale*).

Regarding the mesocarp, the presence of a spongy parenchyma is very obvious in *S. multispinum*. This tissue, characterised by large intercellular spaces and cells that change their shape from rounded to elliptical, to elongate, and even to stellate, was accurately described in *S. mammosum* (Miller, 1969) with the name of *aerenchyma*. Something similar occurs in the albedo of the orange, where parenchyma cells develop arms in different directions (Roth, 1977). The spongy tissue does not exclusively belong to sect. *Melongenena*, but is also present in several species of sect. *Acanthophora* (Miller, 1969; Nee, 1991; Cipollini & al., 2002; Levin & al., 2005).

Regarding the pulp of the fleshy fruited species, the pattern observed coincides with that which is already known, in which both the placenta and, especially the pericarp, contribute to form the pulp (Garcin, 1888; Murray, 1945). It is the same in the case of *Physalis peruviana* (Valencia, 1985) and other *Solaneae* (Filippa & Bernardello, 1992). Instead, in *Solanum lycopersicum* (sub nom. *Lycopersicon esculentum*) only the placentas are responsible for the formation of the pulp (Murray, 1945; Roth, 1977). The first pattern is the most common and the second one is peculiar to *S. lycopersicum*. The disorganisation of the inner mesocarp and the endocarp noticed in some species, such as *P. peruviana*, occurs also in the fleshy or juicy fruits examined here.

In *S. lycopersicum*, fruit softening is associated with cell disassembly and modifications to the pectin fraction of the cell walls, catalysed by polygalacturonase and pectate lyases (Marín-Rodríguez & al., 2002; Seymour & al., 2002). Pectate lyase sequences have been reported for several species from different families (Medina-Escobar & al., 1997; Marín-Rodríguez & al., 2002). Perhaps different levels of expression of such genes are responsible for the formation of the stiff zone and the juicy zone in the mesocarp of the species here studied.

The fibre layer formed by cells that contain a single prismatic crystal is a type of structure with mineral depositions. It was observed in the hypodermis of *S. euacanthum* (Dottori & Cosa, 2003), and also in *S. homalospermum*, *S. mertonii* and in the species of sect. *Torva* (Chiarini, in prep.). The presence of mineral deposits of calcium oxalate may have evolved as a primary mechanism for controlling the excess of calcium in a great many plants. These deposits would provide multiple benefits to different plant organs, for example, an internal calcium reservoir, or a defence

against herbivores, etc. (Sakai & al., 1972; Thurston, 1976; Franceschi & Horner, 1980; Webb, 1999). Nevertheless, the function of crystals in fruits remains unexplained.

Fruit types and dispersion

Usually, fruits are classified into different dispersal syndromes according to their morphological characters. Van der Pijl's (1982) criterion is usually followed, but direct observation of the dispersion is seldom possible, so the fruits or seeds are assigned to a dispersal syndrome on the basis of speculations, which leads to puzzling discussions, as Levin & al. (2005) pointed out. In this sense, the morpho-anatomical data we provide may clarify some points.

Solanum euacanthum, *S. homalospermum* and *S. mertonii* develop non-capsular dehiscent fruits (sensu Knapp, 2002a). They differ from the traditionally defined berry because they are dry or slightly juicy, they can be easily cracked and have fibres in the hypodermis. In *S. mertonii* and *S. homalospermum* the fibres are combined with sclereids in the epidermis. Surprisingly, this type of pericarp is reminiscent of the *Nicanandra physalodes* pericarp, according to Kaniewsky (1965). This author suggested that the fruit of *N. physalodes* is not a berry, since it is hard and dry, like the fruit of *S. mertonii* and *S. homalospermum*. In these non-capsular dehiscent fruits, changes in temperature and humidity can trigger the rupture of the pericarp, the fibres and the sclereids probably being responsible for such a mechanism. Thus, it is obvious that beyond the external appearance, there are many traits related to the dispersal syndrome. In addition, the colour of the fruits of *S. homalospermum*, *S. mertonii* and *S. euacanthum* is dull and their appearance is unattractive to predators or dispersers.

The berries of *S. aridum*, *S. juvenale*, *S. hieronymi* and *S. comptum* are indehiscent, small to medium sized, yellow when ripe, and a little enclosed by the calyx. The mesocarp has an external and more consistent parenchymatic zone, and an internal one, formed by cells that dissolve, thus releasing its mucilaginous content. Both the mesocarp and the placentas develop projections that surround the seeds. Something similar has been observed in other *Solanum*, such as *S. nigrum*, *S. pseudocapsicum*, *S. lycopersicon*, and in *Physalis* (Garcin, 1888; Murray, 1945). When this sort of fruit matures, it becomes fleshy and pulpy and is eaten either by birds or by terrestrial vertebrates (Edmonds & Chweya, 1997; Knapp 2002b). In addition, the fruits of *S. juvenale* and *S. aridum* would be attractive to consumers, since they have a pleasant odour (Parodi, 1930; our observations).

The fruit of *S. sisymbriifolium* is a particular case. This species, has fruits enclosed by the calyx up to maturity, but the calyx then splits entirely open and shows a red, juicy, indehiscent berry. It is the softest and the juiciest of those studied here. Apparently, the formation of collenchyma in the hypodermis is suppressed, since the calyx develops a protective cover over the fruit. The layers that disorganize are not so much like those of other species (E.g. *S. juvenale*). The placentas contribute much more than those of other species to the formation of the pulp of the ripe fruit (Chaparro, 1989). This fruit would be also a berry, according to previous classifications, but it is clearly different from the berries of other species of the section. This soft, juicy and showy fruit is probably consumed by vertebrates (Von Reis Altschul, 1975). Indeed, brightly coloured fruits would be more attractive to birds (Van der Pijl, 1982; Edmonds & Chweya, 1997).

Beyond the spongy mesocarp, no special features were detected in the fruit of *S. multispinum*. The fact that many species of *Solanum* subgen. *Leptostemonum* have potentially poisonous fruits (Cipollini & Levey, 1997), in addition to the spongy structure, makes dispersion by vertebrates hardly plausible in this case. The function of this spongy tissue has not yet been explained until now, but some authors (Nee, 1979, 1991; Bryson & Byrd, 1994; Levin & al., 2005) have suggested that it might be an adaptation to floating, as the fruits are dispersed by drain water after a rainstorm.

Systematic implications of fruit anatomy

Regarding the fruit, sect. *Melongenina* (as traditionally circumscribed) seems to be a very heterogeneous group, since no single feature is shared by all the species studied here. On the one hand, hypodermal fibres of *S. mortonii*, *S. euacanthum* and *S. homalospermum* are very alike and are not seen in species of other sections (Chiarini, in prep.). On the other hand, the section also includes fleshy-fruited species, such as *S. juvenale*, and species which have spongy fruits, like *S. multispinum* and *S. marginatum*. In agreement with the differences found in fruit type, representatives of section *Melongenina* (sensu Nee, 1999) appear scattered in the molecular studies of Bohs (2005), Levin & al. (2006) and Weese & Bohs (2007) and would not form a natural group. Although these phylogenetic analyses do not include all the species examined here, they provide an interesting subject for discussion. For instance, *S. aridum* (sub. nom. *S. conditum*) and *S. carolinense* are placed in the same clade (the “Carolinense clade”), and both species develop yellow, odorous, mammal-syndrome fruits. Nevertheless, *S. hieronymi* and

S. comptum, with a similar fruit type, appear separated from each other, *S. hieronymi* being closer to *S. elaeagnifolium*. Interestingly, *S. sisymbriifolium*, with juicy berries, seems to be more related to *S. rostratum* (a species of the “Androcercas clade”), which has dehiscent fruits (Whalen, 1979). *Solanum multispinum* and *S. marginatum*, both with spongy fruits, are distantly related: the former species is isolated among the New World clades, while the latter species is placed within the African clade.

At the same time, Cipollini & al. (2002), in a study that remarks phytochemical aspects, state that there is no significant correlation among the fruit types they distinguished and the phylogenetic lineages in *Solanum*. For these authors, fruit typology may be due to physiological constraints, holding an independent evolution of the different dispersal syndromes. Regarding morpho-anatomy, our data lead to similar conclusions.

It is very important to compare morpho-anatomical information to a phylogenetic background, as a means to arrive at safer conclusions. For instance, some authors suggested that stone cells or accretions of sclerenchyma in the mesocarp of some *Solanum* and *Lycianthes* species may be remnants of a stony endocarp or rudiments of a drupaceous fruit (Bitter, 1911, 1914; Danert, 1969). To test which kind of fruit is ancestral and which is derived within the family Solanaceae, Knapp (2002a) mapped fruit characters onto a molecular tree framework provided by previous works. As a result, the berry appears as a synapomorphy of the large and derived “Solanoideae clade”. Thus, sclereidial islets, as we found in *S. elaeagnifolium* and *S. hieronymi*, would be secondarily derived characters that would have been either lost or gained several times along the different clades’ evolution.

If the capsule is considered ancestral and berry derived (Knapp, 2002a), the non-capsular, dehiscent, or “capsule-like” fruits probably represent a secondary derivation to a capsule from a berry-fruited ancestor. It is noticeable that all these dry and variously dehiscent fruits derived from fleshy fruits are found in species occurring in arid habitats (Matesevach, 2002), which suggests that environmental factors have been important in the evolution of the fruits of *Solanum*. There is a parallelism with species from other parts of the world (Symon, 1979, 1984; Whalen, 1979, 1984; Lester & Symon, 1989; Knapp, 2002a) which have similar fruits. In some species of *Solanum* section *Androcercas*, the fruit becomes a cup-like structure containing loose seeds, and their release is mediated by wind or rain shaking (Whalen, 1979; Symon, 1987). Among the species studied here, a similar means of

dispersal is evident in *S. euacanthum*. Nevertheless, the morphological analysis revealed important histological differences, such as the absence of sclereids or fibres in the epidermis of sect. *Androceras* species (Chiarini, unpublished data).

In short, we can certainly hold the existence of a biological relationship between histology and the dispersal syndrome. As Knapp (2002a) pointed out, fruit features are not uniform in *Solanum*, and we provide anatomical information to support the recognition of at least three different fruit types. Finally, we propose that a significant morphological variation is not associated with significant DNA sequences changes. Fruit traits seem to respond quickly to selection constraints on the dispersal syndromes. Our study shows that some species, closely related as regards molecular phylogenies (Bohs, 2005; Levin & al., 2006; Weese & Bohs, 2007), differ notably regarding fruit traits.

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