

COMPARATIVE MORPHOMETRIC AND ANATOMICAL ASPECTS OF THREE *CITRUS* L. SPECIES (RUTACEAE) FRUITS

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ABSTRACT

The purpose of this study is to present the morphometric and anatomical features of three species of *Citrus* L. fruits: *Citrus reticulata* Blanco, *Citrus* × *sinensis* Osbeck and *Citrus margarita* Lour. Morphometrically were determined the length, width, area and volume for 10 fruits of each species. Anatomically, were analyzed the exocarp and mesocarp tissues, including the secretory cavities and endocarp components, respectively the number of carpels (segments) and the length of the juice vesicles. The studied species fruits disclose both similarities and differences concerning their morphometric and anatomical features.

KEY WORDS: anatomy, morphometry, exocarp, mesocarp, *Citrus* species

INTRODUCTION

Citrus is a group of flowering plants belonging to Rutaceae family. The Latin word *Citrus* was borrowed from ancient Greek "kedros", but the Romans applied the word to several different trees with fragrant foliage or wood (compare the completely unrelated cedars (Andrews, 1961; Scora, 1975). The origin of *Citrus* flowering plants, belonging to Rutaceae family, due to the most recent research, is in Australia, New Caledonia and New Guinea (Calabrese, 2002; Liu & Tanumihardjo, 2005), whereas other researchers consider that the origin is in the part of S-E Asia, Myanmar and in a province of China, Yunnan (Gmitter & Hu Xulan, 1990; Rainer, 1975). Concerning the *Citrus* genus taxonomy, it is complex and no one can say precisely the right number of species due to the fact that many known species of them are clonally-propagated hybrids, evidenced by genetic research which showed that even some wild, true-breeding species are of hybrid origin (Nicolosi *et al.* 2000). Cultivated *Citrus* may be derived from as few as four ancestral species. Natural and cultivated origin hybrids include commercially important fruits such as the orange, grapefruit, lemon, some limes and some tangerines.

Citrus reticulata Blanco (syn. *C. nobilis* Andrews, *Sinocitrus reticulata* (Blanco) Tseng.) is originating from India, Pakistan, Vietnam and east of China and has been brought in Occident by Arabians. It is an evergreen shrub that reaches heights of up to 5 m. It has thorny branches, rounded and symmetrical crown with lanceolate green leaves, white perfumed flowers. The fruits - mandarin - are small, spherical, red - orange, sweet and cold resistant (Web 18).

Orange was considered traditionally as a species of the genus *Citrus* - *Citrus sinensis*. Recent research has shown that most of these trees are actually hybrids produced by *Citrus maximum* Merr. (pomelo), *Citrus reticulata* Blanco (tangerine) and *Citrus medica* L. and it

correct scientific name is *Citrus × sinensis* Osbeck. Old name without specification of hybrid is still widely used. *Citrus × sinensis* Osbeck is a medium-sized tree, but under optimal conditions can reach even 13 meters high. The crown is compact, large, round or pyramidal. The large branches have sometimes thorns up to 10 cm in length. The leaves are oval (7-10 cm long) with straight or slightly crenate margins. The glabrous petiole is not winged. The fruits are round, big with an orange peel that can have red - orange, yellow - orange, green-yellow (rarer) color and with a present or absent navel (rudiment of fruit located in the abaxial part of the fruit) (Web 19).

Citrus margarita Lour. is popularly known as the Fortunela or Kumquat (syn. *Citrus japonica* var. *margarita* (Lourie) Guillaumin and *Fortunella margarita* (Lourie.) Swing. The plant is originated in the Far East (China and Japan). It is a small persistent shrub, branched with thorny leaves at the base, reaching up to 80 cm in the natural and 1.50 m pot. The leaves are 10-11 cm long, are coriaceous, oval, shiny green and with visible ribs. The oval flowers are small, 1 cm long, white and fragrant. They bloom in spring and summer (van Wyk, 2005). Fruits of *Citrus margarita* are small, oval, dark yellow-orange, acid and can be consumed nuts. If the fruit is left on the branches one month before consuming them will be tastier and less acidic (Morton, 1987). Recent research has suggested that the closely related genus *Fortunella* (kumquats), and perhaps also *Poncirus* and the Australian *Microcitrus* and *Eremocitrus*, should be included in *Citrus* genus. In fact, most botanists now classify *Microcitrus* and *Eremocitrus* as part of the genus *Citrus* (Nicolosi *et al.*, 2000; Freitas de Araújo *et al.*, 2003).

In literature there are relatively many studies relating the species belonging to the genus *Citrus*, starting in the 19th century. Mostly researches followed the cytological aspects (Bacchi, 1944; Banerji, 1954; Chen, 1944, Mohesh *et al.*, 2005, Nakamura, 1942; Randhawa & Choudhury, 1960; Yarnell, 1940), embryological aspects (El-Tomi, 1954; Furusato, 1951; Gurgel & Sobrinho, 1951; Johri & Ahuja, 1956; Pieringer & Edwards, 1965) and genetical aspects (Koltunow *et al.*, 1996; Rao *et al.*, 1992; Reforgiato *et al.*, 2005; Sharma & Bal, 1957) etc. Ample references on the structure of the vegetative organs, especially those concerning the secretory cavities and juice vesicles of some *Citrus* species are found in some ample studies of Bain (1958), Brent *et al.* (1990); Davis (1932); Kahn (2013); Turner *et al.* (1998) and Matas *et al.* (2010). Succinct references are in general studies concerning the angiosperms anatomy (Esau, 1960; Metcalfe & Chalk 1950). The morphometric data of these fruits almost lack. In Romanian literature there are few data on the structure of some vegetative organs of *Citrus* species most of them being mentioned in some lectures and manuals of Anatomy and Morphology of Plants (Buia & Péterfi, 1965; Tarnavschi *et al.*, 1974; Toma & Gostin, 2000; Ianovici, 2010).

The purpose of this study is to analyze the morphometric and anatomical features of three species of *Citrus* fruit pericarp: *Citrus reticulata* Blanco, *Citrus × sinensis* Osbeck and *Citrus margarita* Lour., and to highlight the similarities and differences between them, contributing to the knowledge of this group of plants in general and particularly of their hesperidium - a modified berry.

MATERIALS AND METHODS

For the morphometric aspects, the measurements were carried out on 10 fruits of each studied species, as follows: the fruit length (L), the width (W), the surface (S) and volume (V),

were used conventional mathematical formulas (for sphere: $S = 4\pi r^2$, $V = 4/3\pi r^3$ and for ellipsoid: $S = \pi ab$, $V = 4/3\pi abc$ where $c = \sqrt{a^2 - b^2}$).

For the anatomical aspects, small pieces of the fruit pericarp (exocarp and mesocarp) of the three species fruit were fixed in FAA (formalin: glacial acetic acid: alcohol 5:5:90). The cross sections of the pericarp were performed by free hand made technique (Bercu & Jianu, 2003). The samples were stained with alum-carmin and iodine green. Anatomical observations and micrographs were performed with a BIOROM-T bright field microscope, equipped with a TOPICA 6001A video camera.

RESULTS AND DISCUSSIONS

Morphometric aspects. The mandarin and orange fruits are almost spherical in shape. It has been determined the length, width, surface and volume (Table 1) as follow:

- The mandarin fruits length and width (diameter) is 3.80 – 6.00 cm;
- The mandarin fruits radius is 1.90–3.00cm;
- The mandarin fruits area is between 4534.16–11304.00 mm²;
- The mandarin fruits volume is between 28716.34–44855.94 mm³.
- The orange fruits length is 40 – 7.40 cm;
- The orange fruits width is 2.70 – 3.70 cm;
- The orange fruits area is between 9156.24 – 17194.64 mm²;
- The orange fruits volume is between 82406.16 – 21067.22 mm³.

The Kumquat fruits are ellipsoid-like in shape. It has been determined the length, width, surface and volume (Table 2) as follow:

- The oval Kumquat fruits length is 1.50 – 3.00 cm;
- The oval Kumquat fruits width is between 0.50 – 1.40 cm;
- The oval Kumquat fruits area between 117.75 – 659.4 mm²;
- The oval Kumquat fruits volume is between 875.46 – 4722.80 mm³.

Anatomical aspects. All studied modified berry fruits, in the cross-section, consist of exocarp (flavedo), mesocarp (albedo) and endocarp, separated into segments filled with juice vesicles which contain the juice of the fruit. The exocarp of all three species fruits consists of epidermis, a parenchymatous zone treated with a hypodermis and parts of the secretory cavities which penetrate deep into mesocarp (albedo). The outer most external part of the fruit – flavedo– is composed of epidermis –a single-layered tabular cells - covered by a thick cuticle that covers the surface and intrudes part way between the anticlinal walls of cells as Matas *et al* (2010) observed for different species of *Citrus* fruit, and stomata as well.

The epidermis is followed by a multilayered parenchyma with slightly thickened cells walls: 5-8 layers of cell for *C. reticulata*, 5-6 for *C. × sinensis* and 4-5 for *C. margarita*. Flavedocells consists carotenoids with chromoplasts, which give the characteristic color of the mature fruit, orange for *C. reticulata*, *C. margarita* and yellow-orange for *C. reticulata* (Fig 1, A, C, D; Fig. 2, A-C).

The mesocarp (albedo) is a tissue characterized by several layers of large oblong-ovate parenchyma cells and numerous intercellular air spaces and a characteristic white appearance for all three studied species. Within this parenchymatous tissue occur crystal idioblasts and secretion cavities secreting essential oils. As Turner *et al*, 1998 reported for *C. limon* “the

secretory cavities develop schizogenously and their epithelial remain living long after maturation” and this can be extended to other *Citrus* species as well. The white spongy albedo is rich in pectin (Turner, 1998).

TABLE 1. The morphometric measurements of *Citrus reticulata*, *Citrus × sinensis* and *Citrus margarita* fruits.

The species	No. of fruct	The length and width (cm)	Radius (cm)	Aria (mm ²)	The volume (mm ³)
<i>Citrus reticulata</i> Blanco	1.	3.80	1.90	4535.16	28716.34
	2.	4.00	2.00	5024.00	33493.33
	3.	4.20	2.10	5538.96	38722.72
	4.	4.40	2.20	6079.04	44855.94
	5.	4.60	2.30	6644.24	50939.17
	6.	4.80	2.40	7234.56	57876.48
	7.	5.00	2.50	7850.00	65416.66
	8.	5.40	2.70	9156.24	82406.16
	9.	5.60	2.80	9847.04	91905.70
	10.	6.00	3.00	11304.00	113040.00
<i>Citrus × sinensis</i> Osbeck	1.	5.40	2.70	9156.24	82406.16
	2.	5.60	2.80	9847.04	91905.70
	3.	5.80	2.90	10562.96	102108.61
	4.	6.00	3.00	11304.00	113.040.00
	5.	6.20	3.10	12070.16	124724.98
	6.	6.40	3.20	12861.44	137188.69
	7.	6.60	3.30	13677.84	150456.24
	8.	6.80	3.40	14519.36	164522.74
	9.	7.00	3.50	15386.00	179503.33
	10.	7.40	3.70	17194.64	212067.22
<i>Citrus margarita</i> Lour.	1.	1.50	0.50	117.75	875.46
	2.	1.90	0.90	268.47	1085.97
	3.	2.10	0.90	296.73	2134.40
	4.	2.40	1.10	414.48	2640.52
	5.	2.60	1.20	486.84	3257.43
	6.	2.70	1.20	508.68	4184.19
	7.	2.80	1.20	527.52	5058.54
	8.	2.80	1.30	571.48	3944.75
	9.	3.00	1.30	612.30	6094.10
	10.	3.00	1.40	695.40	4722.80

In our findings all internal secretion cavities, for the three studied species, are lysigenous cavities, formed by the dissolution of the central secreting cells walls such as Mansfield reported, since 1916 (Fig. 1, B, D; 2, A).

The secretory cavities have a circular shape in *C. reticulata*, circular and oval in *C. margarita*, whereas in *Citrus × sinensis* they are oval in shape. All secretory cavities are coated with a layer of secretory cells with thin tangentially elongated cellulose walls, penetrating the mesocarp (Fig. 1, B, C; Fig. 2, A, C). Their sizes are between 330.99-633.33 µm in length and 280 – 426.66 µm width for *C. reticulata*, 281.25-404.76 in lengths and 190.47-323.80 width for *C. × sinensis*. *Citrus margarita* secretion cavities are 88.87-332.32 µm in length and 112.21-222.32 µm width (Table 2).

The exocarp color is orange for *Citrus reticulata* and *C. × sinensis* and yellow-orange for *C. margarita* (Table 2).

The endocarp is made up of a number of carpels and consists of numerous oil cells of different size for all studied species (Table 2; Fig. 3). However, *Citrus reticulata* and *C. margarita* possess 8-10 carpels and the most numerous carpels belong to *C. × sinensis* (10-15 carpels) (Table 2).

TABLE 2. The exocarp color, the endocarp segments number and sizes of secretory cavities and juice vesicles of the studied species

The species	No. of fruct	The exocarp color	No. of carpels	The secretory cavities sizes (μm)		The endocarp oil cells length (mm)	
				L	l	first row	second row
<i>Citrus reticulata</i> Blanco	1	orange	8	340.00	333.33	2-3	3-4
	2	orange	10	433.33	286.66	2-4	5-6
	3	orange	9	453.33	300.00	2-4	4-6
	4	orange	9	520.00	280.00	2-3	4-5
	5	orange	10	633.33	420.00	3-4	5-6
	6	orange	8	633.33	426.66	2-3	3-5
	7	orange	8	373.33	313.33	2-3	4-6
	8	orange	9	533.33	386.66	2-4	4-6
	9	orange	10	376.33	333.33	3-4	4-6
	10	orange	10	533.33	400.00	3-4	4-6
<i>Citrus × sinensis</i>	1	orange	12	281.25	255.00	4-5	5-6
	2	orange	14	376.19	323.80	5-6	6-8
	3	orange	10	323.80	275.19	4-5	5-7
	4	orange	11	404.76	285.71	4-6	6-8
	5	orange	13	395.23	309.52	4-6	6-8
	6	orange	15	309.52	190.47	5-6	7-8
	7	orange	12	285.71	238.09	5-6	6-7
	8	orange	10	357.07	238.09	4-6	6-8
	9	orange	13	342.79	276.13	4-6	7-8
	10	orange	12	323.74	219.00	5-6	6-8
<i>Citrus margarita</i>	1	yellow-orange	10	222.22	138.88	1-2	2
	2	yellow-orange	8	188.88	111.11	1	1-2
	3	yellow-orange	9	333.33	222.22	1	2
	4	yellow-orange	9	277.77	200.00	1-2	2
	5	yellow-orange	8	222.22	166.66	1	1-2
	6	yellow-orange	10	300.00	161.11	1	2
	7	yellow-orange	9	244.44	155.55	1	2
	8	yellow-orange	8	216.66	112.22	1	2
	9	yellow-orange	10	288.88	188.88	1-2	2
	10	yellow-orange	10	266.66	166.66	1	2

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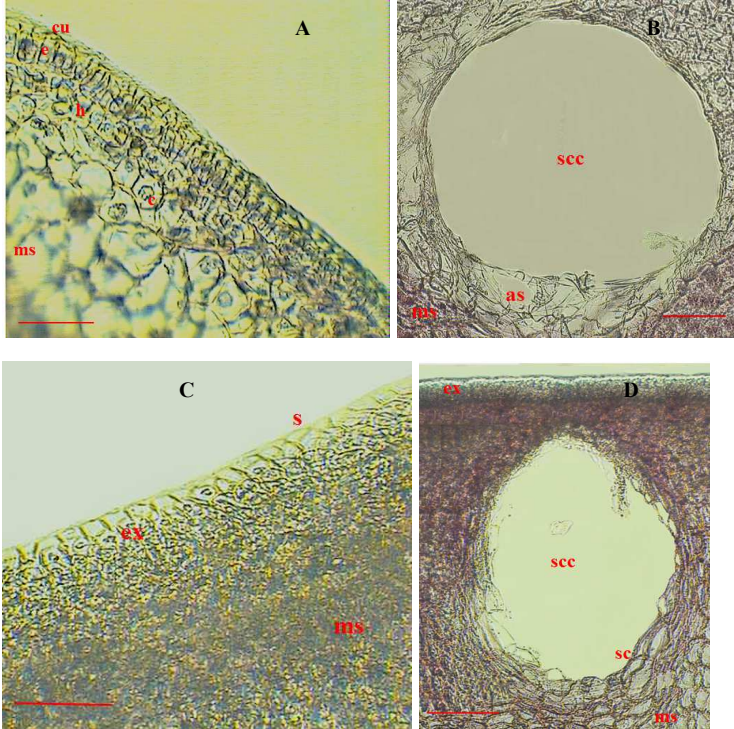


FIG. 1. Cross section of exocarp and mesocarp of *Citrusreticulata* (A, B), *Citrus×sinensis*(C, D): as- air space, e- epidermis, ex- exocarp, h- hypodermis, ms- mesocarp, s- stoma, sc- secretory cells, scc- secretory cavity. Scale bar 100 μ .

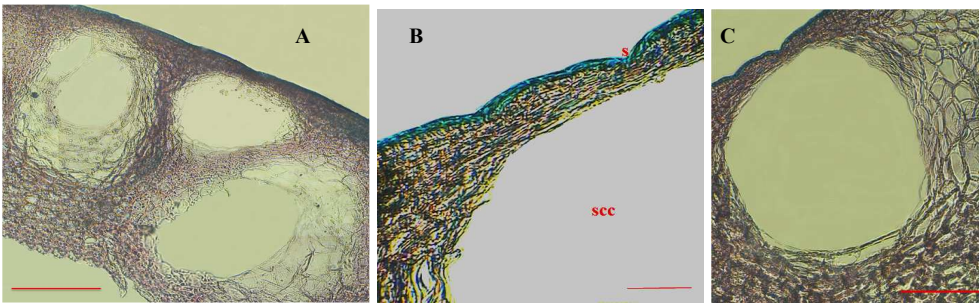


FIG. 2. Cross section of exocarp and mesocarp of *Citrus margarita* (A-C): s- stoma, scc- secretory cavity. Scale bar 100 μ .

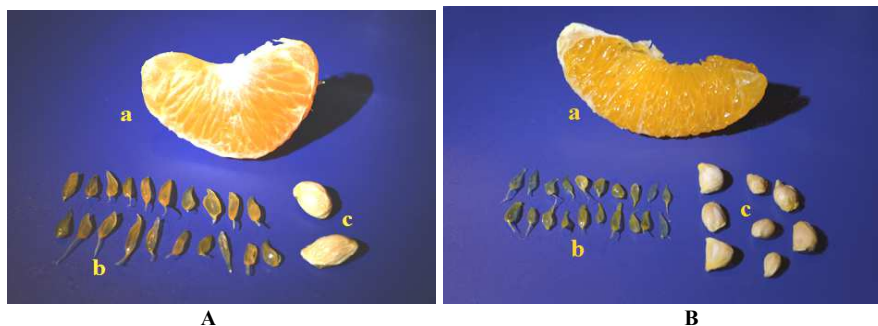


FIG. 3. Natural view of a carpel (a) with 20 oil vesicles (b) and two seeds (c) of *Citrus reticulata* (A), *Citrus* × *sinensis* (B).

CONCLUSIONS

Concerning the morphometric aspects of the studied species fruits, they have distinctive features in terms of length, width, area and volume. The largerfruits in length and width possess *Citrus* × *sinensis* and *Citrus reticulata*, followed by *Citrus margarita*. The largest area and volume have the fruits of *Citrus* × *sinensis* and *Citrus reticulata* and the lowest *Citrus margarita* fruits.

Anatomically, the investigated species fruits present constant anatomical characters, such as the epidermis which consists of flattened cells covered by a thick cuticle, the presence of stomata and the mesocarp which has large parenchyma cells with air spaces, the presence of the oil sacssecretory cavities, extended from the exocarp to mesocarp. The secreting cells – oil vesicles - are filled with flavored essential oil. The exocarp of all studied *Citrus* species fruits, contain pigments that give the distinctive color of each fruit.

Differences appear concerning the epidermal cells cuticle of *C. margarita* which is thicker in comparison with the rest of *Citrus* species, the number of hypodermal layers, which are more numerous in *Citrus reticulata*, followed by *C. × sinensis* and *C. margarita*. Paradoxically, the numerous secretory cavities have the small fruits of *C. margarita* than the rest of studied species fruits. Concerning the length of the endocarp secreting cells, *C. sinensis* and *C. reticulata* are longer than those of *C. margarita*.

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