

MORPHOMETRIC AND ANATOMICAL COMPARATIVE FEATURES OF CITRUS LIMON (L.) BURM., CITRUS MAXIMA (BURM.) MERR. AND CITRUS PARADISI MACFAD. FRUITS

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ABSTRACT

The purpose of this study is to present morphometric and anatomical features of the three species of Citrus L. fruit: Citrus limon (L.) Burm., Citrus maxima (Burm.) Merr. and Citrus paradisi Macfad. 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.

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). 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 and 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.

Lemon - Citrus limon (L.) Burm. (syn. C. medica L., C. limonum Risso, Limon vulgaris Mill.) is one of the most popular citrus plant because of its fruits active principles. Citrus limon is considered an evergreen shrub, native to Southeast Asia (China and North verses South India). It can reach 5 to 10 m height. Lime entered Europe during ancient Rome (web 4). The alternate elliptic in shape leaves are dark green at maturity and are 6.25-11.25 cm in length, with a winged petiole. Slightly fragrant flowers can be solitary, the armpit two or more leaves. The flowers are white on the upper surface of the petals and purple on the lower one. The fruit is yellow but the color may vary from light green to bright yellow, sometimes it can be variegated. The fruit matures in late autumn in the northern hemisphere. Some fruits are seedless, but most have a few seeds (Morton, 1987).

Citrus maxima (Burm.) Merr. (syn. C. grandis (L.) Osbeck, C. decumana Murr. non L., Aurantium maximum Burma) –common known as pomelo or pummelo- was often considered a variety of grapefruit, but actually different varieties of pomelo derive

from grapefruit and different varieties of oranges. It is a tree that reaches 5-10 m (maximum 15 m) high, with low and scattered branches, the younger possessing thorns. The petiolate leaves, up to 7 cm long, are ovate to elliptic in shape, dark green with short hairs on them. They are 5-10 cm long and 2-5 cm width, rounded to sub cordate base, slightly crenate margins, and an acute apex. The flowers have five creamy white petals covered with short hairs, with numerous stamens. In contrast to grapefruit where the flowers grow in clusters, the pomelo flowers and fruits born single. The pear-shaped green fruits are large (20-30 cm long) and ripen to yellow, orange, or red (Bailey and Bailey, 1976; Morton, 1987; van Wyk, 2005).

Citrus x paradisi Macfad. (syn. *Citrus paradisi* Macfad., *Citrus decumana* var. *paradisi* Nichols, *C. decumana* var. *racemosa* Roem., *C. racemosa* Maro), has received a scientific name *Citrus paradisi* only around half XIX century. Until then it was thought that is only another variety of pomelo - fruit of orange whose accidental crossing it results. It began to be widely cultivated in the early XX century. It is a tree, native to Asia, growing up to 10m, whose branches are devoid of thorns. The winged petiolate leaves are similar to those of *Citrus maxima*, lamina being shorter and narrower with a ciliate mid vein. Flowers are white growing in clusters of 2 to 20 flowers. The yellow or pink color fruits are almost spherical in shape and smaller than those of pomelo (Dianxiang et al., 2008).

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, Gogoi et al, 2005, Nakamura, 1942; Randhawa and Choudhury, 1960; Yarnell, 1940), embryological aspects (El-Tomi, 1954; Furusato, 1951; Gurgel and Sobrinho, 1951; Johri and Ahuja, 1956; Pieringer and Edwards, 1965) and genetical aspects (Koltunow et al., 1996; Rao et al., 1992; Reforgiato et al., 2005; Sharma and 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); Batanouny, 1992; Davis (1932); Kahn (2013); Turner et al. (1998); Matas et al. (2010), and succinct references are in general studies concerning the angiosperms anatomy (Esau, 1960; Metcalfe and Chalk 1950). The morphometric data of this fruits almost lack. In Romania literature there are few data on the structure of *Citrus* fruit species anatomy (Bercu, 2015; Tarnavschi et al., 1974) most of them referring to some vegetative organs being, mentioned in some lectures and manuals of Anatomy and Morphology of Plants Anatomy (Buia and Péterfi, 1965; Niculescu, 2009).

The purpose of this study is to analyze the morphometric and anatomical features of this three species of *Citrus* fruits pericarp 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.

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

For the anatomical aspects, small pieces of the exocarp and mesocarp of the three species fruit were fixed in FAA (formalin: glacial acetic acid: alcohol 5:5:90). Cross sections of the leaf were performed by free hand made technique (Bercu and 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 DISCUSSION

Morphometric aspects. The lemon fruit is a typical ellipsoid in shape with two nipple-shaped protuberances at its ends. The lemons fruits are ellipsoid like in shape those of pomelo an oblong ellipsoid, whereas grapefruits are almost spherical in shape. It has been determined the length, width, surface and volume (Table 1) as follow:

The lemon fruits length is 7.00 – 9.20 cm

The lemon fruits width is 6.60 – 4.40 cm

The lemon fruits aria is 2417.8 - 4945.50 mm²

The lemon fruits volume is 87530.64 – 152150.20 mm³

The pomelo fruits length is 11 - 15 cm

The pomelo fruits width is 7 – 12.20 cm

The pomelo fruits aria is 6264.30- 14365.50mm²

The pomelo fruits volume is 467421.18 – 1432958.62 mm³

The grapefruit fruits length (diameter) is n 6.40 – 9.20cm

The grapefruitfruits radius is 3.20 – 4.60cm

The grapefruitfruits aria is 12861.44 – 26576.96 mm²

The grapefruit fruits volume is 137188,69 – 407513.38 mm³

Table 1.

The morphometric measurements of the studied Citrus species fruits

The species	No. of fruct	The length (cm)	The width/radius (cm)	Aria (mm ²)	The volume (mm ³)
<i>Citrus limon</i>	1.	7.00	4.40	2417.8	87530.64
	2.	7.20	4.80	2712.96	72788.71
	3.	8.00	5.20	3265.60	124092.80
	4.	8.20	5.80	3733.46	108195.67
	5.	8.20	6.40	4119.68	105587.39
	6.	8.40	6.00	3956.40	116278.59
	7.	9.00	6.60	4662.90	142638.11
	8.	9.00	7.00	4945.50	139858.74
	9.	9.20	5.40	3899.88	145348.52
	10.	9.20	6.20	4477.64	152150.20
<i>Citrus maxima</i>	1.	11.00	7.40	6389.90	467421,18
	2.	11.4	7.00	6264.30	474896,58
	3.	11.6	7.20	6556.32	505754,52
	4.	12.00	7.80	7347.60	586338,48
	5.	12.40	8.20	7981.88	658185,82
	6.	12.80	9.20	9244.16	786862.89
	7.	13.20	9.00	9325.80	818618.72
	8.	13.60	10.00	10676.00	965537.44
	9.	14.20	11.40	12707.58	1199976.77
	10.	15.00	12.20	14365.5	1432958.62
<i>Citrus paradisi</i>	1.	6.4	3.20	12861.44	137188.69
	2.	6,8	3.40	14519.36	164557.74
	3.	7.00	3.50	15386.00	179503.33
	4.	7.20	3.60	16277.76	195333.12
	5.	7.40	3.70	17194.64	212067.22
	6.	7.80	3.90	19103.76	248348.88
	7.	8.00	4.00	20096.00	267946.66
	8.	8.20	4.10	21113.36	137188.69
	9.	8.40	4.20	22155.84	310181.76
	10.	9.20	4.60	26576.96	407513.38

Table 2

The exocarp color, the secretory cavities length and width, number of carpels and juice vesicles size of the studied speciesfruits

Species	No. of fruct	The exocarp color	The secretory cavities sizes length and width (�m)		No. of carpels	The 20 cells length of an endocarp carpel (mm)	
			L	I		first row	second row
<i>Citrus limon</i>	1.	yellow	343.54	242.90	8	3-4	5-6
	2.	yellow	247.75	199.17	10	4-6	6-8
	3.	yellow	276.90	218.61	9	4-5	6-8
	4.	yellow	267.19	218.61	8	3-5	5-7
	5.	yellow	412.93	281.76	9	3-5	6-8
	6.	yellow	340.06	242.90	10	4-6	6-8
	7.	yellow	281.16	228.32	10	4-6	6-8
	8.	yellow	388.64	242.90	8	4-5	5-7
	9.	yellow	301.19	252.61	8	3-4	5-6
	10.	yellow	342.85	240.00	9	4-5	6-8
<i>Citrus maxima</i>	1	yellowish-green	17.72	130.30	13	6-8	8-10
	2	yellowish-green	151.51	145.45	14	5-7	8-12
	3	yellowish-green	169.69	136.36	12	5-7	8-10
	4	yellowish-green	166.66	121.21	15	6-8	9-12
	5	yellowish-green	145.45	115.15	15	6-8	9-12
	6	yellowish-green	169.69	115.14	14	5-7	8-12
	7	yellowish-green	169.69	127.26	12	5-7	8-10
	8	yellowish-green	151.50	115.14	13	6-8	8-10
	9	yellowish-green	157.56	133.32	14	6-8	9-12
	10	yellowish-green	175.74	127.26	12	5-7	8-10
<i>Citrus ×paradisii</i>	1	yellow-red	281.25	225.00	13	6-8	9-12
	2	yellow-red	281.23	212.50	12	6-8	8-10
	3	yellow-red	287.50	250.00	13	7-8	8-11
	4	yellow-red	281.25	243.75	14	6-9	10-12
	5	yellow-red	243.75	215.62	14	6-8	8-11
	6	yellow-red	225.00	171.87	12	5-7	7-9
	7	yellow-red	231.20	193.75	13	5-8	8-12
	8	yellow-red	265.62	212.50	14	6-8	9-12
	9	yellow-red	187.50	181.25	12	5-8	8-10
	10	yellow-red	203.12	193.75	13	5-7	8-12

II. Anatomical aspects. All studied species fruit is a hesperidium with a rind comprising several distinct tissues. Externally, in cross-section, it consists of exocarp (flavedo) and mesocarp (albedo). The exocarp consists of epidermis, formed by a single continuous layer of more or less conical cells for *Citrus limon*, slightly radially elongated for *C. maxima* and tabular for *C. x paradisi*, covered on the outer periclinal wall by a hydrophilic cuticle. Such as Matas et al (2010) reported for *C. clementina*, the cuticle intrudes part way between the anticlinal walls of cells in our findings for *C. limon* and *C. maxima*. The epidermis is interrupted by the presence of stomata. Just below the epidermis a many-layered spongy tissue, with hypodermal value (albedo), is present: 6-7 layers of cells for *C. limon*, 8-12 layers of cells for *C. maxima* and 7-8 layers of cells for *C. x paradisi* (Fig. 1, A; 2, A, C). Within this parenchymatous tissue occur crystal idioblasts and several secretory cavities, appearing as a large hollow space, surrounded by flattened essential oil gland cells (Fig. 1, B, 2, B, D). The whitespongy of albedo is rich in pectin.

The development of the secretory cavities of *Citrus* species remains controversial and as well the relation between the development of secretory cavities and the essential oil product which is also unknown. Some authors consider that the secretory cavities develop by lysing or gelling cell walls (Bavaru and Bercu, 2002; Fahn (1935); Metcalfe and Chalk, 1950; Tarnavschiet *al.*, 1974). Recent studies of Turner *et al.*, (1998) and She-Jiang Liang *et al.* (2006) show for *C. limon* and *Citrus medica* L. var. *sarcodactylis* that the secretory cavities developed schizogenously and their epithelial remain living long after maturation and this can be extended to other *Citrus* species.

The secretory cavities of *C. limon* and *C. x paradisi* have a circular shape whereas *Citrus maxima* is oval-shaped. All secretory cavities are coated with a layer of secretory cells with thin tangentially elongated cellulose walls, penetrating the mesocarp (Fig. 1, B; Fig. 2, B, D). Their sizes are between 247.75- 412.93 μm length and 199.17- 281.76 μm width for *C. limon*, 145.42 – 175.74 μm length and 115.14 – 145.25 width for *C. maxima*. The length of *Citrus x paradisi* secretory cavities is 187.50 – 287.50 μm and 143.75 – 250.00 μm width (Table 2). They are numerous in *C. limon* and *C. x paradisi* flavedo and smaller in *C. maxima*. Flavedo cells consist of carotenoids with chromoplasts, which give the characteristic color of the mature fruit yellow for *C. limon reticulata*, yellowish-green for *C. maxima* and yellow red for *C. x paradisi* (Table 2).

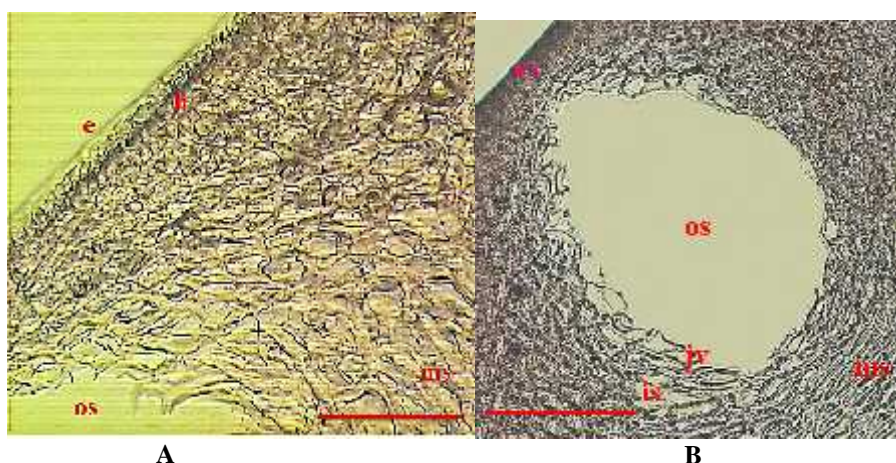


Fig. 1. Cross section of exocarp and mesocarp with oil sacs of *Citrus limon* (A, B): e- epidermis, ex- exocarp, h- hypodermis, is- intercellular space, jv- juice vesicle, ms- mesocarp, os- oil sac. Scale bar 150 μm .

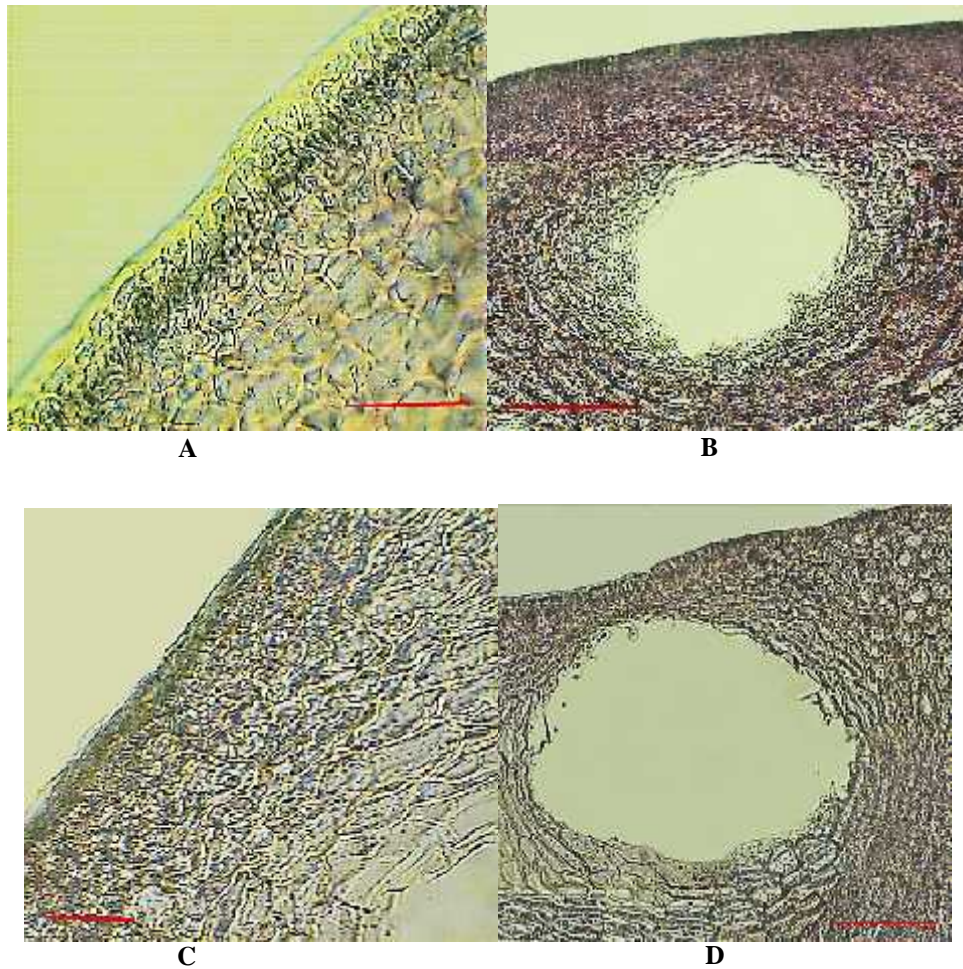


Fig. 2. Cross section of exocarp and mesocarp and oil sacs of *Citrus maxima* (A, B) and *Citrus x paradisi* (C, D). Scale bar A- 150; B-D-100 μ m.

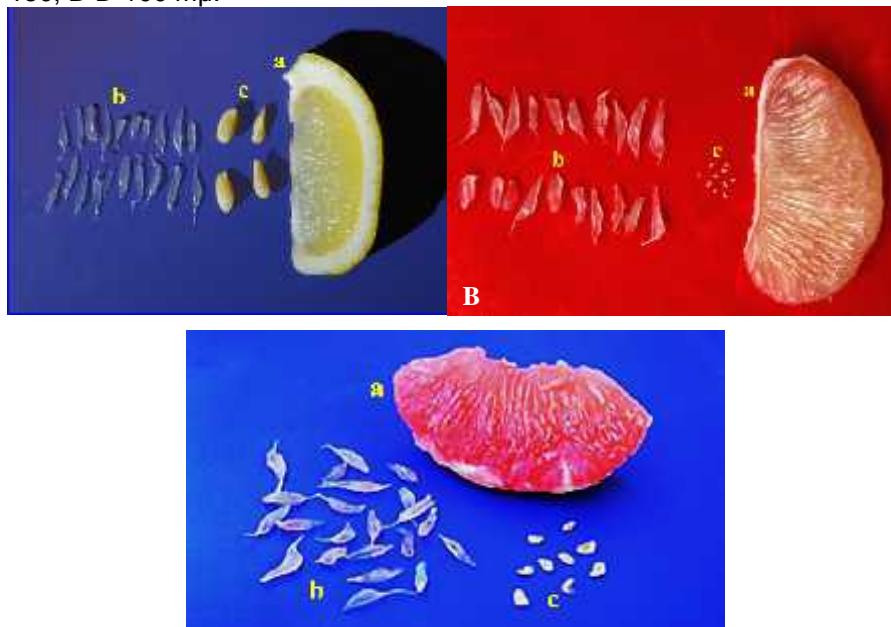


Fig. 3. Natural view of a carpel (a) with 20 juice vesicles (b) and seeds (c) of *Citrus limon* (A), *Citrus maxima* (B) and *Citrus x paradisi* (C).

Endocarp (or pulp) is the membranous content of the fruit's and appear segmented (divided) into a different number of carpels: 8-10 carpels for *Citrus limon* fruits, 12-15 for *C. maxima* and 12-14 carpels for *C. x paradisi* (Table 2). Each carpel is composed of juice

vesicles (juice sacs) - secreting spindle like cells with thin membranes, containing essential oil. According to K. Esau (1960), the juice sacs originate as multicellular hairs in which the interior of the enlarged distal part breaks down and fills with liquid. The juice vesical length is 5-6 mm for *C. limon*, 4-12 mm for *C. maxima* and 5-10 mm for *C. xparadisii* fruits (Fig. 3; Table 2). Such as Brent et al. (1990) and Cameron et al. (1964) reported, the color of the pulp is variable, depending on the species and the ripening stage. Usually, it has the color of the outer peel.

CONCLUSIONS

Concerning the morphometric aspects of the studied species fruits, they have distinctive features in terms of length, width, area and volume. The larger fruits in length and width are those of *Citrus maxima*, followed by *C. xparadisii* and *C. limon*. The largest area and volume have the fruits of *Citrus maxima*, followed by *Citrus xparadisii* and *C. limon*.

Anatomically, the investigated species fruits present constant anatomical characters, such as the epidermis which consists of a single-layered cells covered by a thick cuticle, the presence of stomata and mesocarp which has large parenchyma cells with airspaces, the presence of the secretory cavities, extended from the exocarp to mesocarp. The juice 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 conformation, the cuticle thickness, (thicker for *C. maxima* in comparison with the rest of *Citrus* species), the number of hypodermal layers, which are more numerous in *C. maxima*, followed by *Citrus xparadisii* and *C. limon*. Paradoxically, the larger fruit of *C. maxima* have a smaller number and size of secretory cavities than the rest of species. Concerning the number of carpels and the length of the endocarp juice vesicals, *Citrus maxima* and *C. xparadisii* are numerous and longer than those of *C. limon*.

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