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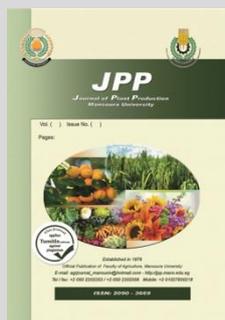
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Morphological and Anatomical Studies on *Physalis peruviana* L. and *Physalis ixocarpa* Brot. Exhornem

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ABSTRACT

This study intends to introduce botanical information about morphological and anatomical characteristics of two species of the genus *Physalis* namely, *Physalis peruviana* L. and *Physalis ixocarpa* Brot. exHornem. The morphological and anatomical attributes of stem, leaf, flower and fruit were studied. Furthermore, scanning electron microscope was used to examine the ultrastructure of trichomes, stomata, pollen grains and seed surface. Results showed that both species are annual herbs with erect, ribbed and solid stems. Leaves are simple and petiolate. Stomata of anisocytic type and various types of trichomes (glandular and non-glandular) were observed. Flowers are yellow, solitary, bell-shaped and bisexual. Pollen grains are monad, tricolporate and lobate. Fruits are fleshy, berry and glabrous included ovoid, flattened and glabrous seeds with lateral hilum. However, the two species varied in a number of morphological characters such as branching, density of trichomes, leaf and pollen grains shape, corolla spots color, and fruit size. These characters have taxonomic importance in distinguishing the genus *Physalis* L. at generic at species rank of the family.

Keywords: Anatomy, Morphology, Scanning electron microscope, *Physalis peruviana* L., *Physalis ixocarpa* Brot. exHornem.

INTRODUCTION

Physalis is a genus of the Solanaceae commonly known as the nightshade family. It was first described by Linnaeus in 1753. It contains about 75 - 90 species which grown in tropical and temperate regions (Zimmer *et al.*, 2019). Most of these species are found in America with only a few in Europe and Asia. A few grown for the edible fruits and also the ornamental fruiting calyx of some species (Hunziker, 2001).

Members of genus *Physalis* are annual or perennial summer-flowering herbs or sometimes slightly woody at base, diffuse or creeping, glabrous or pubescent (Zimmer *et al.*, 2019). Leaves are alternate, often 2 together, simple, mostly angled and distinctly petioled, commonly soft in texture. Flowers are usually on axillary or extra axillary pedicels, mostly blue or yellowish or whitish and not showy, calyx 5-toothed, becoming large and bladder-like and inclosing the globular yellow or greenish often more or less viscid berry, corolla rotate or short campanulate usually with purplish spots in the center, plicate, short tubed and mostly 5-toothed, stamens 5, style slender, the stigma somewhat 2-lobed (Baily, 1969).

Species of *Physalis* are yet confused and taxonomists have yet to completely resolve the taxonomy of the genus. It is well known that morphology and anatomy have great taxonomic importance and participate in differentiating the taxa up to the species level and are consider as a significant source for the elucidation of phylogenetic relationships (Bhat *et al.*, 2018).

Therefore and as the result of the shortage in knowledge about the two species of *Physalis*, the present study intends to introduce detailed morpho-anatomical attributes of two *Physalis* species grown under local conditions; namely, *Physalis peruviana* L. and *Physalis*

ixocarpa Brot. exHornem. Such knowledge would be useful to specialists in various aspects of plant biology.

MATERIALS AND METHODS

Seeds of two *Physalis* species; *Physalis peruviana* L. and *Physalis ixocarpa* Brot.exHornem. were procured from the Vegetables Research Institute, Ministry of Agriculture, Dokki, Giza. Seeds of both species were sown on 15th march 2019 in seedling trays, one seed/hole. The germination substrates consisted of peat-moss and vermiculite (1:1 volume). One month later, seedlings were transplanted to the open field with 60 cm spacing between plants. This trial was carried out at the Faculty of Agriculture, Cairo University, Giza, Egypt.

Samples of developing plants were subjected to the identification. Authentication was carried out utilizing comparison at the Herbarium, Flora &Phyto-Taxonomy Researches, Horticultural Research Institute, ARC, Dokki, Giza, Egypt (CAIM), where plant herbarium specimens were deposited. Likewise, descriptions of studied species mentioned by Bailey (1969), Hesse *et al.* (2009), and Simpson (2010) was consulted. Scientific names of studied species were revised through the Catalogue of Life Annual Checklist (Roskov *et al.*, 2019).

To study the leaf epidermal structure, pollen grain, and seed surface a Scanning Electron Microscopy (SEM) was conducted with different magnifications by JXA-840 A model Electron Probe Microanalyzer – JEOL, Japan at the Central Laboratory, National Research Center (NRC), Dokki, Giza, Egypt.

For anatomical studies, specimens from the internode of the middle portion of the main stem and its corresponding leaf (blade and petiole) were fixed in formalin acetic acid (FAA) solution for two days, and then washed with 50% ethyl

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alcohol, dehydrated with normal butyl alcohol solution series, embedded in paraffin wax. Specimens were sectioned by rotary microtome (20 microns), and stained with crystal violet/erythrosin combination, and finally mounted in Canada balsam (Nassar and El-Sahar, 1998).

RESULTS AND DISCUSSION

The stem

a. Morphology of the stem

The two studied species of *Physalis* are annual herbaceous plants (Fig.1). The stem is erect, ribbed, solid, green in color, and covered with dense trichomes in *P. peruviana* and a few trichomes in *P. ixocarpa*. Branching starts from basal internodes then continues upward in *P. peruviana* whereas being dichotomous in *P. ixocarpa*.

b. Anatomy of the stem

The transverse sections (Fig. 2) reveal that the stem has three ridges. The epidermis is composed of one layer of rectangular cells. Trichomes of glandular and non-glandular types are present. The cortex consists of three layers of collenchyma cells followed by 4-5 layers of thin-walled parenchyma cells. The vascular bundles are arranged in a ring and being separated from one another by the ground tissue. It is obvious that the bundles are relatively different in size; the major bundles are lying on the radii which pass through the stem angles whereas the minor bundles are located in the region between the angles. The pith, which comprises a large portion of the stem, consists of large polygonal parenchyma cells with small intercellular spaces. Worthy to note that, crystals of calcium oxalate are observed in the cortex and pith of *P. peruviana*.

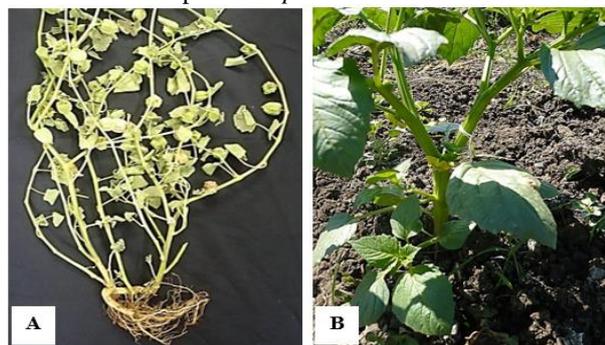


Fig. 1. Photographs showing branching in *Physalis peruviana* L. (A) and *Physalis ixocarpa* Brot. ex Hornem. (B).

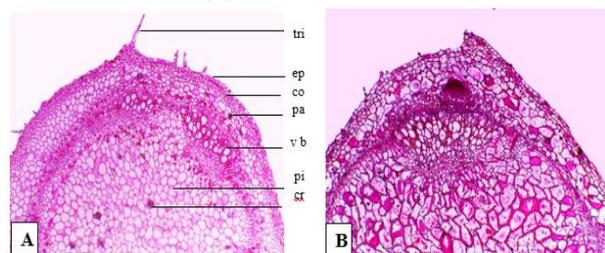


Fig. 2. Transverse sections through the stem of *Physalis peruviana* L. (A) and *Physalis ixocarpa* Brot. ex Hornem. (B). (X 40)

Details: co, collenchyma; cr, crystal; ep, epidermis; pa, parenchyma; pi, pith; tri, trichome and v b, vascular bundle.

The leaf

a. Morphology of the leaf

Mature leaves of *P. peruviana* are simple, petiolate, exstipulate, alternate, opposite in the upper part of the branches, thick, covered with dense trichomes (pubescent),

velvety, dark green in color, 7-11 cm in length, 5.5-7 cm wide, nearly ovate to cordate in shape with reniform or truncate base, acuminate or cuspidate apex, aculeate margin, pinnately netted venation (Fig.3A). This finding is in agreement with those given by Baily (1969) and El-Gohary(1984) who mentioned that leaves are alternate, often opposite or subopposite on short shoots, thicker, soft-pubescent, broad, evenly cordate or truncate at the base, and distinctly petioled.

Leaves of *P. ixocarpa* are simple, petiolate, exstipulate, alternate, thin, slightly pubescent, soft, green in color, 8- 12 cm in length, 4- 5.5 cm wide, trullate in shape with cuneate base, acute apex, aculeate margin, and pinnately netted venation (Fig.3B). This description of leaves is in agreement with those given by Bailey (1969) and Morton (1987) who stated that leaves have pointed apex, wedge-shaped base, variously toothed or notched and long petioled.

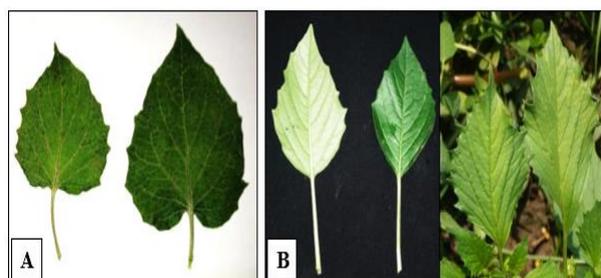


Fig. 3. Photographs showing mature leaves of *Physalis peruviana* L. (A) and *Physalis ixocarpa* Brot. ex Hornem. (B).

b. Leaf epidermal structure using SEM

Scanning Electron micrographs on the abaxial surface of the two species of *Physalis* (Fig.4) show that the epidermal cells have an irregular shapes with wavy margins. Stomata are anisocytic type with raised levels. Trichomes of various types; i.e., non-glandular and glandular are present in the epidermis. The non-glandular trichomes are uniseriate, conical, have a blunt or pointed apex, straight or curved, consisting of 1- 3 cells (Fig. 4 A and B), and their surface is adorned with micropapillae (Fig. 4 G). The glandular trichomes of two types; capitate type with a head of one cell and peltate type with a head of four cells are present in *P. peruviana* (Fig. 4 B and C), while in *P. ixocarpa* are peltate type (Fig. 4 F and H). These results are generally in accordance with that recorded by Dyki *et al.* (1998) on *P. ixocarpa*.

c. Structure of the leaf using a light microscope

a. The leaf blade

Transverse sections of the leaves of the two *Physalis* species (Fig. 5) show that there are two epidermal layers on adaxial and abaxial surfaces of the leaf which are composed of compactly rectangular to barrel cells and covered with thin cuticles. Stomata and trichomes are present on both epidermal cells surfaces. Mesophyll in both species consists of one layer of closely packed elongated palisade tissue followed by 4-5 layers of irregular spongy parenchyma tissue with air spaces. Some cells of spongy tissue situated below palisade tissue contained large druses (calcium oxalate crystals). At the midrib region of both species, the upper and lower epidermis is convex and a bicollateral vascular bundle in an arc shape is embedded in the ground tissue of parenchyma cells with a mass of collenchyma cells underlying the two epidermis. The previously given anatomy of the leaf of the two studied *Physalis* species conforms with those mentioned by El-Gohary (1984) and Dyki *et al.* (1998).

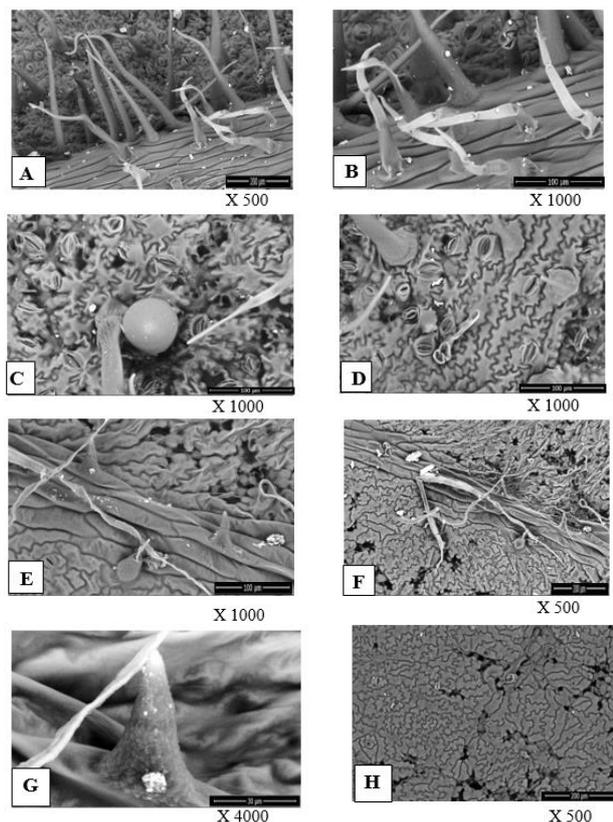


Fig. 4. Scanning electron micrographs of the abaxial surface of leaf blade showing stomata and trichomes types. Details: A, B, C and D: *Physalis peruviana* L., E, F, G and H: *Physalis ixocarpa* Brot. ex Hornem.

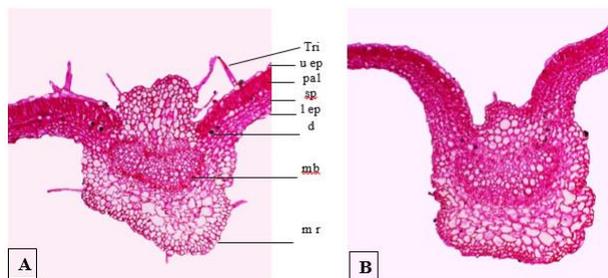


Fig. 5. Transverse sections through the leaf blade of *Physalis peruviana* L. (A) and *Physalis ixocarpa* Brot. ex Hornem. (B). (X 40).

Details: d, druses; lep, lower epidermis; mb, midvein bundle; mr, midrib region; pal, palisade tissue; spo, spongy tissue and u ep, upper epidermis.

b. The petiole

The petiole of the two *Physalis* species as seen in the transverse sections (Fig.6) is convex with two wings at the adaxial surface while the abaxial surface is rounded in *P. peruviana* and somewhat ridged in *P. ixocarpa*. Trichomes are more abundant in *P. Peruviana* while they are rare in *P. ixocarpa*. The petiole of both species is surrounded by a uniseriate epidermis of nearly square to barrel-shaped cells. The ground tissue consists of 2-3 layers of collenchyma cells next to the epidermis, followed by parenchyma tissue which surrounds a median arc-shaped, bicollateral vascular bundle, in addition, there are two accessory small bundles in the ground tissue of the two lateral wings at the adaxial surface. The aforementioned description is in agreement with Bhat *et al.* (2018).

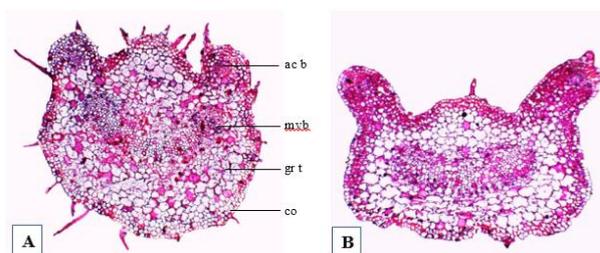


Fig. 6. Transverse sections through the leaf petiole of *Physalis peruviana* L. (A) and *Physalis ixocarpa* Brot. Ex Hornem. (B). (X 40)

Details: acb, accessory bundle; co, collenchyma; gr t, ground tissue and m vb, main vascular bundle.

The flower

Flowers of the two *Physalis* species are solitary, bisexual, hypogynous, and bell-shaped. The calyx is composed of five green sepals. The sepals are hairy in *P. peruviana*, while they are glabrous in *P. ixocarpa*. After maturity, the calyx enlarges to form the green husk which turns to yellow at full fruit maturity, and it surrounds the fruit, while in *P. ixocarpa*, the husk is stripped off at fruit maturity. The corolla is composed of five bright yellow petals with dark purple-brown spots at the base in *P. peruviana*, while spots are dark yellowish-green in *P. ixocarpa* (Fig. 7). Androecium consists of five stamens joined with corolla. The gynoecium have a superior ovary composed of two united carpels. This description is in harmony with those mentioned by Dyki *et al.* (1998), Muniz *et al.* (2014), and Oliveira *et al.* (2016).

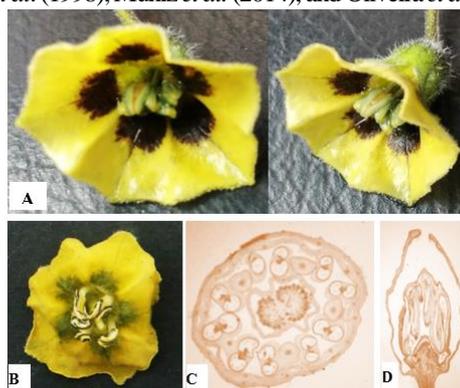


Fig. 7. A- The flower of *Physalis peruviana* L. B- The flower of *P. ixocarpa* Brot. ex Hornem. C- Transverse section of floral bud of *P. ixocarpa* Brot. ex Hornem. D- Longitudinal section of floral bud of *P. ixocarpa* Brot. ex Hornem.

The pollen grain

The pollen grains observed in the two studied species of *Physalis* (Fig. 8) are monad, tricolpate, lobate and semi triangular shaped in *P. peruviana* while prolate (elliptic) in *P. ixocarpa*. Mean polar axis is 31.4µm in *P. peruviana* and 33.7µm in *P. ixocarpa*.

The fruit

Fruits of the two studied *Physalis* species are fleshy, berry, and glabrous. Fruits of *P. peruviana* (Fig. 9A) are ovoid-shaped, completely enveloped in a calyx during its development and ripening, the calyx is green at prematurity stages then turned to yellow at full maturity stage and its contacts with fruits at the base, the color of the fruit is green, yellow or orange depending on maturity stage. The diameter of the fruit ranges from 1.7 to 2.2 cm, and its weight is 3.5 to 5.8 g. Each fruit contains around 200 seeds and the weight of 1000 seeds is 1.0 to 1.25 g. On the other hand, Fruits of *P. ixocarpa* (Fig. 9B) are globose, surrounded with

the calyx till ripening then the calyx is stripped off. Fruits are green or yellow. The fruit diameter ranges from 3.8 to 4.5 cm and its weight is 9.1 to 10 g. Moreover, a number of seeds per fruit ranges from 290 to 370, and the weight of 1000 seeds is 1.25 to 1.54 g. The same morphological description of fruits of the two studied *Physalis* species were reported by Dyki *et al.* (1997), Ostrzycka *et al.* (1998), Muniz *et al.* (2014), Oliveira *et al.* (2016), Barroso *et al.* (2017), Bhat *et al.* (2018), Aluja *et al.* (2019) and Zimmer *et al.* (2019).

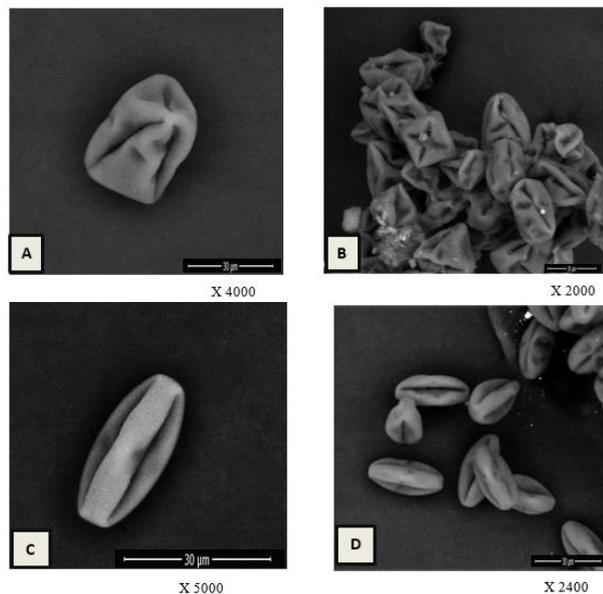


Fig. 8. SEM micrographs of pollen grains.
 Details: A and B. *Physalis peruviana* L. C and D. *Physalis ixocarpa* Brot.ex Hornem.

The seed

Seed characteristics of both *Physalis* species as seen by SEM (Fig. 10) are ovoid, flattened, glabrous, approximately 2.1mm in length and 1.7mm in width. The hilum is conspicuous, lateral in position, and elliptic in shape. The epidermal cells of the seed are irregular reticulate. The anticlinal walls are slightly raised, undulate and thick. The outer periclinal walls are flat with furrows. This description is in agreement with that given by Khafagi *et al.* (2018).

For sake of ease, various differential characters of *Physalis peruviana* L. and *Physalis ixocarpa* Brot.exHornem. previously given are summarized in Table (1) to compare and accurately identify each of the two studied species.



Fig. 9. Photographs showing mature fruits of *Physalis peruviana* L. (A) and *Physalis ixocarpa* Brot. ex Hornem. (B).

Table 1. Morphological comparison between *Physalis peruviana* L. and *Physalis ixocarpa* Brot. exHornem.

Characters	<i>P. peruviana</i>	<i>P. ixocarpa</i>
Habit	Annual, herb, densely pubescent	Annual, herb, slightly pubescent
Branching	Starts from basal internodes then continues upward	Dichotomous
Leaf	Velvety	Soft
-Texture	Dark green	Green
-Color	Nearly ovate to cordate	Trullate
-Shape	Reniform or truncate	Cuneate
-Base	Acuminate or cuspidate	Acute
-Apex	Aculeate	Aculeate
-Margin	Pinnately netted	Pinnately netted
-Venation		
Lower epidermis:	Anisocytic	Anisocytic
-Stomataltype	Raised	Raised
-Stomatal level	Irregular	Irregular
-Sculpture pattern	Non-glandular, capitate and peltate	Non-glandular and peltate
-Trichomes type	Yellow, bell-shaped with five dark purple-brown spots near the base	Yellow, bell-shaped with five dark yellowish-green spots near the base
Corolla		
Pollen grain		
-Unit	Monad	Monad
-Class	Tricolpate	Tricolpate
-Shape	Semi triangular	Prolate (elliptic)
-Outline	Lobate	Lobate
Fruit	Berry, fleshy, glabrous, ovoid, completely enveloped in a calyx, green, yellow or orange, 1.7 - 2.2 cm in diam.	Berry, fleshy, glabrous, globose, completely surrounded with the calyx till ripening then the calyx is stripped off, green or yellow, 3.8 - 4.5 cm in diam.
Seed		
-Shape	Ovoid	Ovoid
-Color	Yellow	Yellow
-Sculpture pattern	Reticulate	Reticulate
-Hilum shape	Elliptic	Elliptic

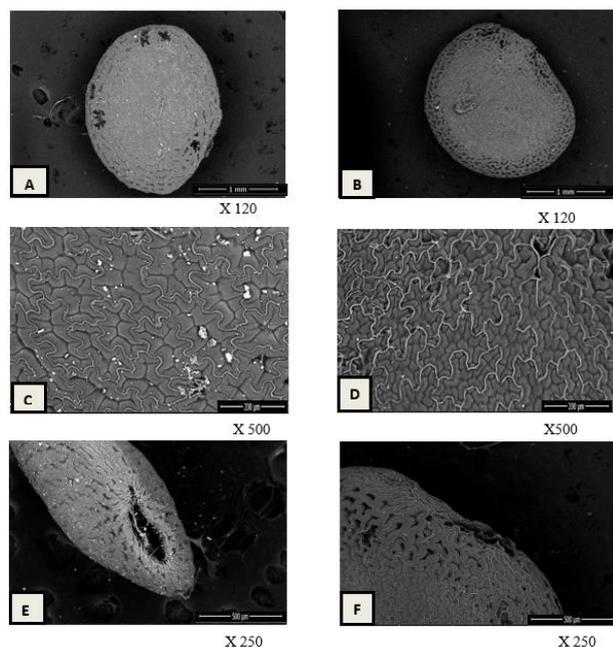


Fig. 10. Scanning electron microscopy micrograph of the two studied species of *Physalis* seed showing seed shape, seed sculpture patterns and hilum shape.

Details: A, C and E: *Physalis peruviana* L. B, D and F: *Physalis ixocarpa* Brot. ex Hornem.

CONCLUSION

Morphological studies on two *Physalis* species; *Physalis peruviana* L. and *Physalis ixocarpa* Brot. ex Hornem. revealed that there is a variation in stem, leaf, flower, and fruit between the two species. While, the two species are similar in stomata type, unit, and class of pollen grain shape and sculpture pattern of seed.

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دراسات مورفولوجية وتشريحية على *Physalis ixocarpa* Brot. ex Hornem. و *Physalis peruviana* L.

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تهدف هذه الدراسة الى تقديم معلومات نباتية حول الصفات المورفولوجية والتشريحية لنوعين من جنس الحرنكش و هما *Physalis peruviana* L. و *Physalis ixocarpa* Brot. ex Hornem. اشتملت الصفات المورفولوجية على الاختلافات في الساق، والورقة، والزهرة و الثمرة. و تمت الدراسة التشريحية على كل من الساق، ونصل و عرق الورقة بواسطة المجهر الضوئي. علاوة على ذلك، تم استخدام المجهر الالكتروني الماسح لفحص التركيب الدقيق للشعيرات، والثغور، وحبوب اللقاح و سطح البذرة. و قد أظهرت النتائج أن كلا النوعين نباتات عشبية حولية ذات سيقان قائمة، مضلعة و مصمتة. الأوراق بسيطة ومعقدة. وقد لوحظت ثغور من نوع anisocytic (الثغور محاط بثلاث خلايا مساعدة)، وأنواع مختلفة من الشعيرات (غدية و غير غدية). الأزهار صفراء، مفردة، جرسية الشكل و ثنائية الجنس. حبوب اللقاح أحادية و ثلاثية الاخداب و مفصصة. الثمار طرية، غنية و ملساء ذات بذور بيضاوية الشكل، مسطحة و ملساء ذات سرة جانبية. و مع ذلك يختلف النوعان في عدد من الصفات المورفولوجية و التي تتمثل في الثغور، وكثافة الشعيرات، وشكل الورقة وحبوب اللقاح، ولون بقع البتلات، وحجم الثمار.

الكلمات الدالة: التركيب التشريحي، الشكل الظاهري، المجهر الالكتروني الماسح، *Physalis peruviana* L.، *Physalis ixocarpa* Brot. ex Hornem.