ANATOMICAL INVESTIGATIONS ON *MOMORDICA CHARANTIA* L. PLANTS, NEWLY ACCLIMATED IN ROMANIA

<u>Anca SÂRBU</u>^{1*}, Tatiana Eugenia ȘESAN¹, Teona TĂNASE², Anca Monica PARASCHIV³, Alina Georgiana CÎŞLARIU¹, Florin OANCEA⁴

¹ University of Bucharest, Department of Botany and Microbiology, 1-3 Intrarea Portocalelor, Bucharest – Romania.

 ² Synevo Romania – Central Laboratory, 25 Industriilor Street, Chiajna – Romania.
 ³ University of Bucharest, Botanical Garden "D. Brandza", 32 Şoseaua Cotroceni, Bucharest – Romania.
 ⁴ University of Agronomical Sciences and Veterinary Medicine, Biotechnology Faculty, Doctoral School, 59 Mărăşti Bulevard, Bucharest – Romania.

* Corresponding author. E-mail: anchusa24@yahoo.com

Momordica charantia L. (Cucurbitaceae family), medicinal and nutraceutical plant known from Asia, Abstract: South Africa, South America, the Caribbean region, has been acclimatized in Romania since 1990 until now. This plant, cultivated in the open greenhouses belonging to S.C. HOFIGAL Import Export S.A. has been studied in several projects, among which "Project 160/2014-2017 - MAIA - Multifunctional and innovative products for safe and bioenhanced functional food from newly cultivated plants in Romania", coordinated by ICECHIM/ INCDCP Bucharest. Starting from this project, a documentary study was carried out on the Momordica charantia plants acclimatized in different countries, and a morphoanatomical research was initiated on the specimens grown in Romania, in the greenhouses belonging to S.C. HOFIGAL (Voucher BUC 408946-408950). The anatomical observations concerned the organization of the stem, petiole, and leaf lamina, using sets of cross-sectional and paradermal sections, treated with identification substances (IIK) and differential and successive stains (Iodine Green and Carmine Alum). Structural characteristics have been investigated under optical microscopy and documented through original photographic images and a set of dimensional data, data which are only partially found in the specialised literature. The results of our research are generally within the existent anatomical patterns. However, certain particular aspects have been noticed, regarding the epidermal cells, mechanical tissues, conducting tissues and mesophyll, completing the knowledge regarding the anatomy of acclimatized Momordica charantia plants.

Key words: Cultivated Momordica plants, nutraceutical importance, stem and leaf structure, optical microscopy.

Introduction

Momordica charantia is a tropical and subtropical plant of the *Cucurbitaceae* family, cultivated at large in Asia, Africa, the Caribbean for its edible fruits (bitter cucumber).

The plant is renowned for its many beneficial effects in the fight against diabetes, and its complications (eye diseases, cataract, obesity, hyperglycemia, diabetic foot), for its anticancer / antimutagenic / antitumor, antiviral (among which anti-AIDS), and antibacterial properties. Other important uses are based on its fungicidal effects against phytopathogens and insecticides / larvicides / antipupal effect on plant pests and its beneficial effects against skin conditions, antipsoriasis, anti-wound healing, anti-cardiovascular diseases. The plant also has a hepatoprotective effect, an analgesic effect and is considered a good source of food with a tonic effect on the body [WALTERS & DECKERS, 1988; GROVER & YADAV, 2004; BEHERA & al. 2010, 2011; KUMAR & al. 2010; KUMAR & BHOWMIK, 2010; GUPTA &

al. 2011; MAHMOOD & al. 2012; AGARWAL, 2014; NAGARANI & al. 2014a, b; SAIFI & al. 2014; ANILAKUMAR & al. 2015; TCHEGHEBE & al. 2016; MAHMOUD & al. 2017; DE OLIVEIRA & al. 2018; RAHMAN & al. 2018; ASNA & al. 2020; KOLE & al. 2020; ŞESAN, 2020].

Over time, the plant has been acclimatized in many countries, and since 1990 it has been cultivated in Romania as well. The history of this culture starts with the first seeds brought from Nepal by biologist PhD. Ovidiu BOJOR, mentioned by biologist PhD. STOIANOV (2002), seeds which were cultivated in the open greenhouses of S.C. HOFIGAL Import Export S.A. At the same time, the first cultures of *Momordica charantia* were established at SCDL (Vegetable Growing Research and Development Station) Buzău, within the ASAS (Academy of Agricultural and Forestry Sciences) by VÎNĂTORU (2015) and by VÎNĂTORU & al. (2019).

The technology for cultivating bitter cucumber was disseminated in Romania through a CD created by the company RENTROP & STRATON (CĂPĂŢÂNĂ), and after the 2000s, through various projects, such as those coordinated by: ONISEI (2016-2017), ŞESAN (2017), ŞESAN & al. (2018), VÎNĂTORU & al. (2019), in the south, or CRIŞAN & HĂLMĂJAN (2007) and KESERU & al. (2016), in the western areas of Romania.

Anatomical studies of the vegetative organs have been carried out internationally on *Momordica charantia* acclimatized plants, grown for nutraceutical purposes [AGUORU & OKOLI, 2012; SONKAR & al. 2014; ERŐZ POYRAZ & DERDOVSKI, 2016; SHETHI & al. 2018; SÁ & al. 2018]. In Romania, anatomical research on the vegetative organs of *Momordica charantia* cultivated plants has been carried out by: SĂVULESCU & HOZA (2010), LAGUNOVSCHI-LUCHIAN & al. (2017), LUCHIAN & (IORDACHE) TEODOSIU (2019). All these studies highlighted the existence of a similar organisation plan of the plant, but also the existence of certain particularities supporting their structural variability.

This paper aims to perform the morpho-structural characterization of the local *Momordica charantia* population, cultivated in open greenhouses in the experimental fields of HOFIGAL, Romania. Our results will complete the existing information on the anatomy of the vegetative organs of the *Momordica charantia* plants acclimatized in different countries, including Romania.

This paper is further aimed at creating a reference of structural data and relevant optical microscopy images, to be used in assessing future *Momordica charantia* plants, cultivated in the greenhouses of HOFIGAL, Romania, in several experimental variants, created with the purpose of enhancing their medicinal values.

Materials and methods

Biological material: stems and leaves harvested from the *Momordica charantia* plants (anthesis stage) cultivated in open greenhouses in the experimental fields of S.C. HOFIGAL Export Import S.A., Romania, during 2015-2016 and preserved in 70% alcohol.

Processing methods. For histo-anatomical evaluation, the usual methods used in plant anatomy were used [ŞERBĂNESCU-JITARIU & al. 1983].

The *Momordica charantia* stems and leaves were cross-sectioned manually: in the median area of the stem internode in the middle of the stem, in the median area of the petiole, in the median area of the central leaf segment (Figure 1). The sections were coloured using two differential stains, applied successively (Iodine Green and Carmine Alum) [ŞERBĂNESCU-JITARIU & al. 1983; SÂRBU & al. 2014; SÂRBU & al. 2018], and IIK was

used as a starch recognition substance. For analysing the characteristics of the epidermis, paradermal sections were performed at the established levels of the stems and leaves.

All microscopic slides were analysed under normal and polarized light, using a DOCUVAL optical microscope. Photomicrographs were taken with a digital camera (NIKON D90).

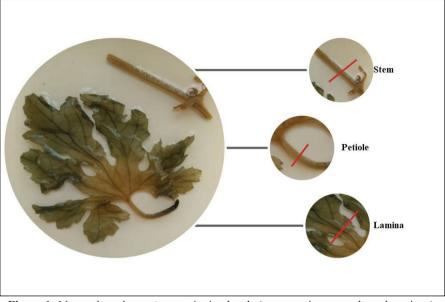


Figure 1. Momordica charantia – sectioning levels (cross sections, paradermal sections).

Results

Stem. *Momordica charantia* is an annual herbaceous plant with an ascending stem (2-3 m), pubescent and provided with tendrils.

In cross section the contour of the stem has 5 obvious angles (diameter 3.8/4.0 mm), corresponding to the morphological appearance of the stem, which longitudinally has 5 ridges and 5 valleculas (Figure 2, Table 1). The epidermis is unilayered, composed of small epidermal cells (in cross section: $10-12 \mu m$ long; $8-10 \mu m$ wide), with the outer tangential wall slightly thickened and covered by a $2.0-2.2 \mu m$ thick cuticle. The epidermis differentiates rare stomata, long uniseriate multicellular non-glandular trichomes (15-20 cells) and short glandular trichomes, which have a single secretory cell apically (Figure 3, Figure 4). Calcium carbonate crystals (double cystoliths) are present in the epidermal cells (Figure 5).

Below the epidermis, a cortical angular collenchyma is present in the area of the ridges, where their apical area includes 8-10 layers of cells and reaches a thickness of 120-150 μ m (Figure 6, Table 1). Otherwise, the cortex is parenchymal, with slight spaces between the cells, composed of 5-6 layers of cells with slightly thickened walls, cells in which starch deposits have been identified (recognition reaction with IIK) (Figure 7).

The central cylinder follows the shape of the stem, also being 5-sided (Figure 2) and slightly oval (2.0 mm length / 2.3 mm width) (Table1). Its outline is marked by a thick (7-10 layers of cells) and continuous sclerenchyma area (Figure 2, Figure 3, Figure 7), in a

pericyclic position. The fundamental parenchyma of the central cylinder contains 10 bicollateral vascular bundles, arranged in two rings: 5 large, central bundles and 5 smaller, peripheral bundles (Table 1). Each bundle has an external phloem area, an intrafascicular vascular meristematic tissue (procambium), a xylem area, and an internal phloem area (Figure 8). The metaxylem has an exarch position, and the protoxylem has an endarch position. In the central cylinder, the xylem is well represented and includes 15 large wooden vessels (diameter $\geq 100 \ \mu$ m) (Table 1). The medullary rays and the medullary parenchyma are parenchymal.

Table 1. Analysed Momordica charantia, stem parameters (cross section through the internode).

Parameters	Measurements/size
Stem diameter	3.8 mm/4.0 mm
Epidermal cell	10.0-12.0 μm length
	8.0-10.0 μm width
CaCO ₃ crystals (cystoliths)	15-30 μm diameter
Cuticle	2.0-2.2 μm thickness
Sclerenchyma	100.0-150.0 µm thickness
Angular collenchyma from the apical area of the ribs	120.0-150.0 µm thickness
Diameter of the central cylinder	2.0 mm/2.3 mm
Conducting bundles/central cylinder	10.0 (5.0 large central bundles,
	5.0 small bundles of ribs)
Xylem vessels (≥ 100 µm diameter)/central cylinder	15.0
Large central conducting bundles	700.0-800.0 μm length
	500.0-600.0 μm width
Small conducting bundles of ribs	400.0-500.0 µm length
	350.0-400.0 µm width

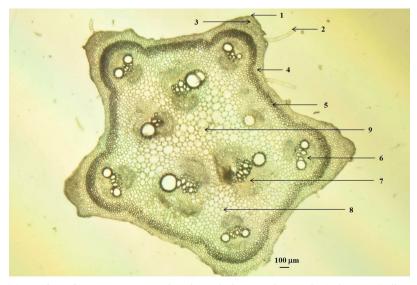


Figure 2. *Momordica charantia*, cross section through the stem internode (colorants: Iodine Green and Carmine Alum): 1 – epidermis, 2 – tector trichome, 3 – collenchyma, 4 – parenchymal cortex, 5 – sclerenchyma, 6 – rib conducting bundle, 7 – central conducting bundle, 8 – parenchymal rays, 9 – parenchymatic pith.

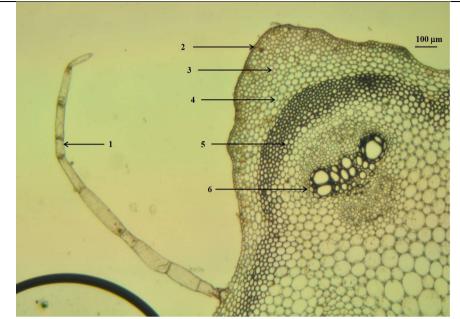


Figure 3. *Momordica charantia*, cross section through the stem internode (colorants: Iodine Green and Carmine Alum): 1 – multicellular tector hair, 2 – epidermis, 3 – angular collenchyma, 4 – parenchymal cortex, 5 – sclerenchyma, 6 – bicollateral vascular bundle from the ridge.

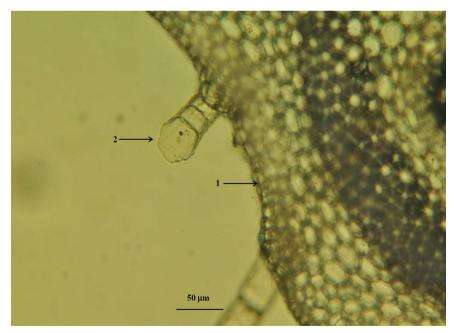


Figure 4. *Momordica charantia*, cross section through the stem internode (colorants: Iodine Green and Carmine Alum): 1 – epidermis, 2 – multicellular glandular hair.

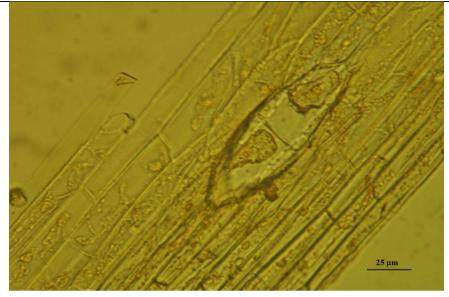


Figure 5. *Momordica charantia*, paradermal section at the level of the stem internode: the epidermis and a double cystolith are observed.

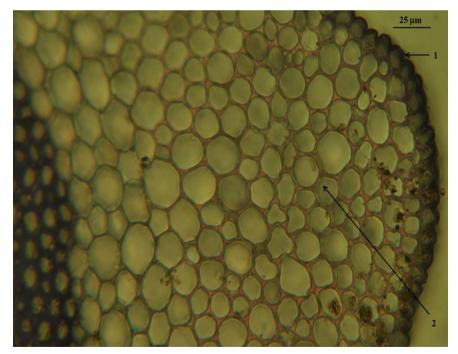


Figure 6. *Momordica charantia*, cross section through the stem internode (colorants: Iodine Green and Carmine Alum): 1 – epidermis, 2 – angular collenchyma from the apical area of the ribs.

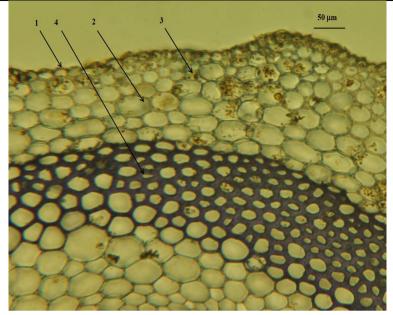


Figure 7. *Momordica charantia*, cross section through the stem internode (colorants: Iodine Green, Carmine Alum, IIK): 1 – epidermis, 2 – cortex, 3 – starch granules, 4 – sclerenchyma.

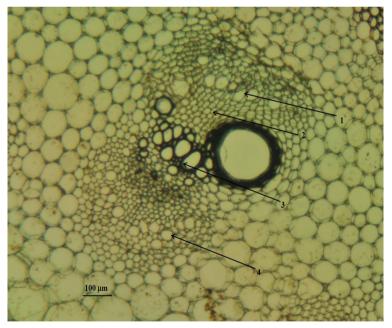


Figure 8. Momordica charantia, cross section through the stem internode with the highlighting of a bicollateral vascular bundle (colorants: Iodine Green and Carmine Alum): 1 – outer phloem, 2 – fascicular procambium, 3 – xylem, 4 – inner phloem.

Petiole. The leaves are alternate, simple, with membranous lamina, palmatilobate, with 5-7 lobes (Figure 1). The petiole and the veins of the lamina are pubescent.

In cross section through its median area, the petiole shows a slightly angled circular outline, modified only by the presence of a shallow adaxial groove, and of the two appendages enclosing it (Figure 9). The diameter of the petiole varies between 2.5 mm - 2.7 mm (Table 2), and its structure is monosymmetric. From a histological point of view, at least four types of tissues are present and characteristic: protective, mechanical, parenchymal, and conducting.

The epidermis is unilayered. The epidermal cells are large, relatively isodiametric cells (16-18 μ m long and 13-15 μ m wide) (Table 2) and are covered by a cuticle (2.2-2.5 μ m thickness), which presents cuticular ridges, more numerous and obvious in the area of the adaxial groove and of the two appendages (Figure 10). The epidermis differentiates rare stomata, uniseriate multicellular non-glandular trichomes and rare long multicellular stalked glandular trichomes (feet composed of 6-7 cells), with multicellular glandular head. Calcium carbonate crystals (cystoliths) are present in the epidermal cells.

Under the epidermis there is a continuous ring of angular collenchyma, with variable thickness on the circumference of the petiole (2-5 layers of cells) and better developed at the level of the adaxial groove and appendages (Figure 11).

The conducting tissues are organized into 11 bundles, of which 7 are located in the meatic fundamental parenchyma and 4 in the two appendages. Five conducting bundles in the fundamental parenchyma are obviously larger and of the bicollateral type (Table 2, Figure 11). In these conducting bundles the xylem is well represented (Figure 11, Figure 12), and at the periphery of the external phloem there are sheaths of amyliferous cells (IIK) (Figure 12).

Parameters	Measurements/size
Petiole diameter	2.5-2.7 mm
Epidermal cells	16.0-18.0 μm length 13.0-15.0 μm width
CaCO ₃ crystals (cystoliths)	20.0-30.0 µm diameter
Cuticle	2.2-2.5 μm thickness
Conducting bundles	11.0
Large conducting bundles from the fundamental parenchyma	5 (400.0-600.0 μm length, 200.0-300.0 μm width)

 Table 2. Analysed Momordica charantia, petiole parametrs (cross section in the median zone).

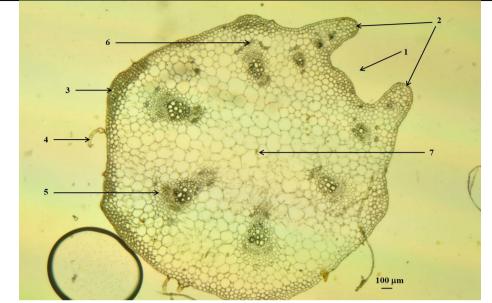


Figure 9. *Momordica charantia*, cross section through the petiole (colorants: Iodine Green and Carmine Alum, IIK): 1 – adaxial groove, 2 – appendages, 3 –angular collenchyma, 4 – multicellular tector trichome, 5 – large bicollateral vascular bundle, 6 – starch cells, 7 – ground parenchyma.

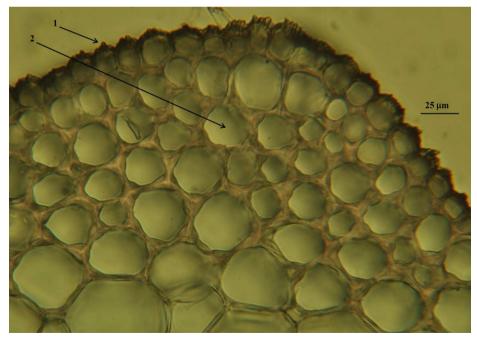


Figure 10. *Momordica charantia*, cross section through the petiole (colorants: Iodine Green and Carmine Alum): 1 – epidermal cells covered by a ridged cuticle, 2 – angular collenchyma (detail).

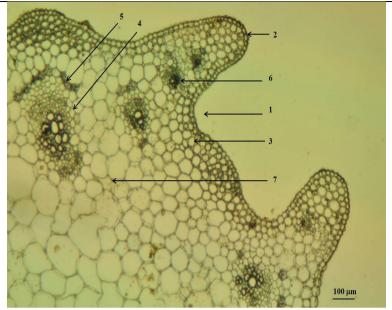


Figure 11. *Momordica charantia*: cross section through the petiole (colorants: Iodine Green and Carmine Alum, IIK): 1 – adaxial groove, 2 – appendage, 3 –angular collenchyma, 4 – large bicollateral vascular bundle, 5 – starch cells, 6 – small vascular bundle, 7 – ground parenchyma.

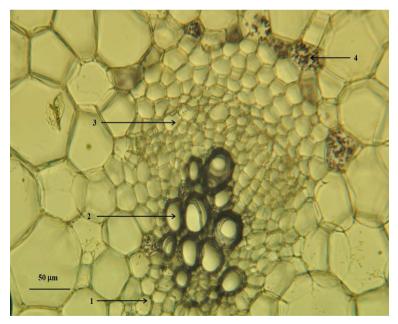


Figure 12. *Momordica charantia*: cross section through the petiole, detail of a bicollateral vascular bundle (colorants: Iodine Green and Carmine Alum, IIK): 1 – inner phloem, 2 – xylem, 3 – outer phloem, 4 – starch cells.

Lamina. The epidermis seen from above highlights the presence of the epidermal cells, arranged in a single layer, of anomocytic stomata, of uniseriate multicellular nonglandular trichomes with walls impregnated with CaCO₃ and of multicellular stalked glandular trichomes, with multicellular glandular head. The lamina is amphistomatic (stomatal index: upper epidermis = 44.79 stomata/mm², lower epidermis = 146.19 stomata/mm²).

Both epidermal layers have heterodiametric epidermal cells, with obviously wavy walls. The cells of the lower epidermis are slightly larger (Figure 13, Figure 14, Table 3). Some of the cells of the lower epidermis have cystoliths (50 μ m / 40 μ m), arranged in groups of 2-3-4 (Figure 15).

In cross section, the shape of the median nervure of the leaf segment is biconvex (1000 μ m / 900 μ m), with both sides prominent: the adaxial side is convex and the abaxial side is obviously convex (Figure 16).

Both epidermis of the nervure show cells that are approximately isodiametric, all walls thin. Multicellular glandular trichomes with multicellular glandular head and uniseriate multicellular non-glandular trichomes of up to $1200 \ \mu m$ in length (Figure 17, Figure 18, Figure 19) are present. The lower epidermis is covered by a cuticle with cuticular ridges (Figure 20).

Adaxially, subepidermally, 4-5 layers of angular collenchyma are identified, and abaxially, above the lower epidermis, a continuous ring of angular collenchyma consisting of 2-3 layers of mechanical cells (Figure 16) is identified.

In the centre of the nervure there is a single bicollateral conducting bundle $(300-350 \ \mu m \text{ diameter})$, located in the meatic fundamental parenchyma. The external phloem cordon is small, and the internal one is voluminous (Figure 21).

The mesophyll shows a dorsiventral organization (Figure 22): one layer of palisade cells (40-50 μ m thickness) and an area of spongy tissue, composed of a layer of heterodiametric collector cells and 5-6 layers of relatively isodiametric cells, with small intercellular spaces between them. The layer of collector cells is better represented in the area where the cells of the lower epidermis contain large cystoliths (40-50 μ m diameter). The spongy parenchyma form ~ 50% of the thickness of the mesophyll and its cells show accumulations of starch. In the mesophyll there are secondary ribs containing collateral conducting bundles, composed of few conducting elements.

Parameters	Measurements/size
Median nervure (cross section)	1000.0 μm length
	900.0 µm width
Conducting bundles (cross section)	300.0-350.0 µm diameter
Lamina (cross section)	180.0-190.0 μm thickness
Cuticle (cross section)	2.0-2.5 μm thickness
Cells of the upper epidermis (paradermal section)	25.0 μm width
	50.0 µm length
Cells of the lower epidermis (paradermal section)	30.0 μm width
	60.0 µm length
Palisade tissue (cross section) – 1 layer	40.0-50.0 μm thickness
Palisade cells (cross section)	40.0-50.0 μm length
	25.0 μm width
Spongy mesophyll (cross section)	90.0-100.0 µm thickness
CaCO ₃ crystals (cystoliths)	50.0 μm/ 40.0 μm diameter
Stomatal index	upper epidermis = 44.79 /mm ²
	lower epidermis = 146.19 /mm ²

Table 3. Analysed Momordica charantia, lamina parameters (cross and paradermal sections).

ANATOMICAL INVESTIGATIONS ON MOMORDICA CHARANTIA L. PLANTS ...

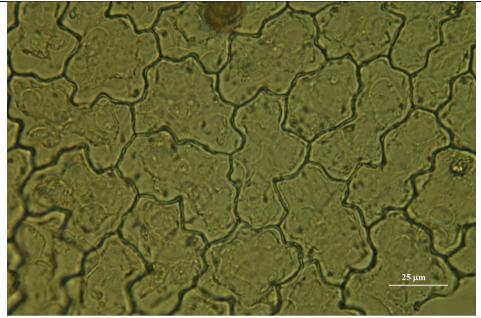


Figure 13. *Momordica charantia*, paradermal section at the lamina level: upper epidermis of the lamina in apical view.

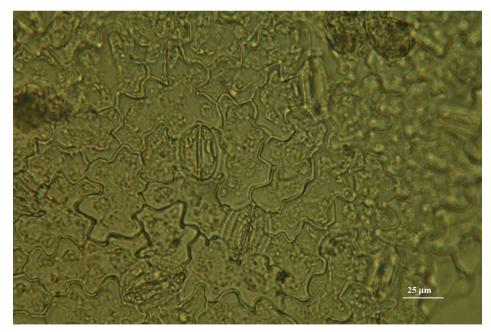


Figure 14. *Momordica charantia*, paradermal section at the lamina level: lower epidermis in apical view.

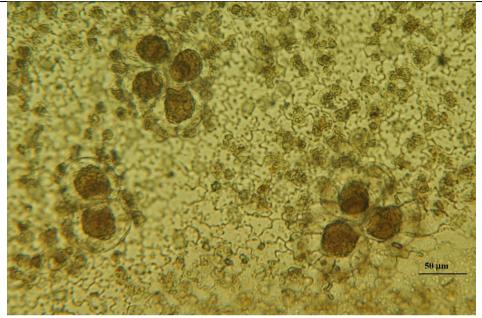


Figure 15. *Momordica charantia*, lower epidermis in apical view: cystoliths arranged in groups of two, three and four respectively are highlighted.

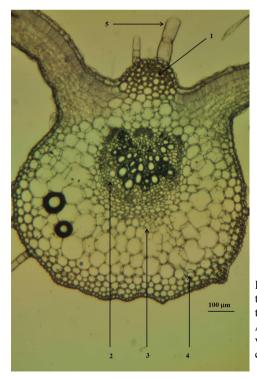


Figure 16. *Momordica charantia*, cross section through the lamina of the leaf segment, at the level of the median ribs (colorants: Iodine Green and Carmine Alum): 1 – adaxial collenchyma, 2 – bicollateral vascular bundles, 3 – parenchyma, 4 – abaxial collenchyma.



Figure 17. *Momordica charantia*, lower epidermis of the median rib of the leaf segment: multicellular tector hairs.



Figure 18. Momordica charantia, multicellular tector hair.



Figure 19. *Momordica charantia*, lower epidermis of the median rib of the leaf segment: multicellular secretory hair.

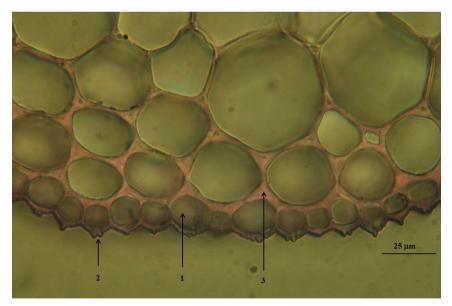


Figure 20. *Momordica charantia*, cross section through the leaf with highlighting of the abaxial face of the median rib (colorants: Iodine Green and Carmine Alum): 1 – epidermal cells, 2 – cuticle with cuticular ridges, 3 – angular collenchyma.

ANATOMICAL INVESTIGATIONS ON MOMORDICA CHARANTIA L. PLANTS ...

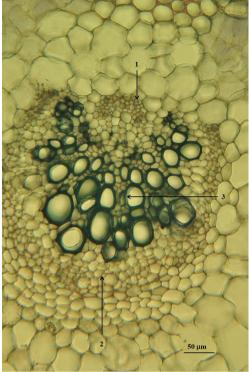


Figure 21. Momordica charantia, cross section through the leaf segment at the level of the median rib, with highlighting the bicollateral vascular bundle (colorants: Iodine Green and Carmine Alum): 1 - outer phloem, 2 - inner phloem, 3 - xylem.

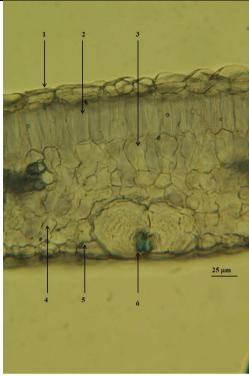


Figure 22. *Momordica charantia*, cross section through the lamina (colorants: Iodine Green and Carmine Alum): 1 – upper epidermis, 2 – palisade tissue, 3 – collecting cells, 4 – spongy tissue, 5 – lower epidermis, 6 – double cystolith.

Discussions

This study provides detailed data regarding the anatomical characteristics of the *Momordica charantia* plants acclimatized in the Hofigal greenhouses, Romania.

The structure of the analysed above-ground vegetative organs corresponds to the descriptions existing in the literature regarding the *Momordica charantia* plants acclimatized in other countries (Bangladesh, Brazil, Nigeria, Turkey etc.), but there is also a series of particular aspects that we point out, aspects related to epidermal cells, mechanical tissues, conducting tissues and mesophyll tissues.

Epidermal cells

Regarding the epidermal cells, our data, supported by the optical microscopy images, show that the cuticle of the epidermal cells of the petiole and of the lamina ribs is thick and shows obvious cuticular ridges. In the specialized literature [SĂVULESCU & HOZA, 2010; SHETHI & al. 2018; ERŐZ POYRAZ & DERDOVSKI, 2016; AGOURU & OKALI, 2012;

SÁ & al. 2018] it is mentioned that the acclimatized *Momordica charantia* plants present a thin and smooth cuticle.

A further aspect refers to the presence of CaCO₃ crystals (cystoliths) in epidermal cells. In the case of the plants analysed in the present study, the presence of cystoliths was identified both in the epidermis of the stem and petiole, and especially in the lower epidermis of the lamina. Publications on this topic show that there are variations regarding this characteristic: cystoliths have not been identified [SĂVULESCU & HOZA, 2010], have been identified in the epidermis of the lamina [GILL & KARATELA, 1982; ERŐZ POYRAZ & DERDOVSKI, 2016; SÁ & al. 2018; SHETHI & al. 2018], and less often in the epidermis of the stem [ERŐZ POYRAZ & DERDOVSKI, 2016]. With regard to the epidermis of the petiole, only the presence of raphides [SHETHI & al. 2018] or druses [SÁ & al. 2018] was signalled.

Mechanical tissues

Mechanical tissues are well represented in the structure of *Momordica charantia* plants analysed in this study, both in the stem (collenchyma 8-10 layers of cells, sclerenchyma 7-10 layers of cells) and in the petiole (collenchyma 3-5 layers of cells). This aspect could also influence, to a certain extent, the vigour of the shape of these plants. For plants acclimatized in other countries, some variations of this characteristic have been reported. In the plants grown in Nigeria [AGOURU & OKALI, 2012], for example, the stem has a collenchyma composed of 4-5 layers of cells and a sclerenchyma composed of 6 layers of cells, while the petiole presents a two-layered collenchyma. The plants cultivated in Brazil [SÁ & al. 2018] have 1-3 layers of collenchyma cells, in the structure of the petiole.

Conducting tissues

Momordica charantia, being part of the *Cucurbitaceae* family, is characterized by the presence of bicollateral conducting bundles. The literature emphasizes this aspect and points out that there are differences regarding the number of conducting bundles in the organs of different acclimatized *Momordica charantia* plants. Our data shows the existence of 10 conducting bundles of different dimensions (5 central and 5 peripheral bundles) in the stem, which was also reported for other cultivated plants [SĂVULESCU & HOZA, 2010; ERŐZ POYRAZ & DERDOVSKI, 2016; SÁ & al. 2018]. However, the plants grown in Bangladesh [SHETHI & al. 2018] only have 8 conducting bundles, 3 central and 5 peripheral bundles.

Regarding the petiole, the numerical variation of the conducting bundles was also observed. Our research has highlighted the presence of 11 conducting bundles, values also confirmed by SĂVULESCU & al. (2010). The research performed by AGOURU & OKALI (2012) mentions 10 conducting bundles, while the data obtained by SHETHI & al. (2018) and SÁ & al. (2018) mention only 7 conducting bundles.

Numerical variations of the conducting bundles were also reported for the median nervure of the leaf segments. Our results, but also certain data from the literature [SĂVULESCU & HOZA, 2010; ERŐZ POYRAZ & DERDOVSKI, 2016; SÁ & al. 2018], attest the presence of a single, large, bicollateral conducting bundle in the nervure. However, for the plants in Bangladesh [SHETHI & al. 2018], three conducting bundles were identified, a large one and two small ones.

Mesophyll tissues

Regarding the mesophyll of the acclimatized *Momordica charantia* plants, some structural variations can also be specified. Our data certify the presence of unilayered palisade tissue in the lamina, similarly with what was reported by SÁ & al. (2018) and SHETHI & al. (2018). The research conducted by SÁVULESCU & HOZA (2010) and ERŐZ POYRAZ & DERDOVSKI (2016) supports both the existence of a uni-bilayered palisade tissue in the structure of the mesophyll. Our observations also highlight the presence in the mesophyll structure of a layer of collector cells. It is located under the palisade tissue of the lamina and is better developed in the area of the mesophyll, where cystoliths form in the cells of the lower epidermis.

Conclusions

Momordica charantia (Cucurbitaceae) is a nutraceutical plant cultivated in many countries around the world, due to its recognized medicinal properties.

The anatomical aspects investigated in the *Momordica charantia* plants cultivated in Romania (the Hofigal greenhouses), have allowed for the characterization of these plants, compared to the plants grown in other countries. Thus, it was noticed that the general organization of the studied vegetative organs is similar, but there are also certain particularities: thick cuticle provided with cuticular ridges, better represented mechanical tissues, the presence of isolated or grouped amyliferous cells, the presence of collector cells located in the mesophyll, the presence of cystoliths of different shapes, sizes, and placements.

In this sense, the following structural aspects are to be highlighted:

- the central cylinder of the stem is an eustel, with 10 bicollateral conducting bundles, disposed as two concentric rings;
- the mechanical tissues are better represented, compared to other *Momordica charantia* plants cultivated; the stem contains collenchyma and sclerenchyma, the leaf only differentiates collenchyma;
- the petiole presents an adaxial groove and two appendages; it has a monosymmetric structure, with separate vascular bundles (bicollateral); in exarh position perifascicular amyliferous cells have been signalled;
- the epidermis of the petiole and of the median nervure of the leaf lobes is covered by a thick cuticle, which forms obvious cuticular ridges;
- the epidermis of the stem, petiole and median nervure of the leaf segments differentiates: long uniseriate multicellular non-glandular trichomes with the wall impregnated with CaCO₃, short glandular trichomes with unicellular gland, long glandular trichomes with multicellular gland;
- the plant forms CaCO₃ crystals (cystoliths): solitary or in groups of two in the epidermis of the stem and petiole and in groups of 2-3-4 in the lower epidermis of the lamina;
- the lamina is amphistomatic, with anomocytic stomata, with 70% more numerous abaxially;
- the palisade tissue is unilayered; the presence of a layer of collector cells, was observed.

In this study, dimensional assessments have been performed for the structures of the analysed organs; these data provide additional information, than are found only sporadically in the literature.

References

- AGARWAL M. 2014. Momordica charantia L.: A potent Medicinal Plant. LAP LAMBERT Academic Publishing, OmniScriptum GmbH & Co. KG, Germany, 75 pp. ISBN 978-3-659-56191-7.
- AGUORU C. U. & OKOLI B. E. 2012. Comparative stem and petiole anatomy of West African species of Momordica L. (Cucurbitaceae). African Journal of Plant Science. 6(15): 403-409. https://doi.org/10.5897/AJPS11.309
- ANILAKUMAR K. R., KUMAR G. P. & ILAIYARAJA N. 2015. Nutritional, pharmacological and medicinal properties of *Momordica charantia*. *International Journal of Nutrition and Food Sciences*. 4(1): 75-83. https://doi.org/10.11648/j.ijnfs.20150401.21
- ASNA A. C., JOSEPH J. & JOSEPH JOHN K. 2020. Chap. 3. Botanical description of bitter gourd, pp. 7-31. In: KOLE C., MATSUMURA H. & BEHERA T. K. (eds.). Compendium of plant genoms, 2020, 135 pp. ISBN 978-3-030-15061-7; https://doi.org/10.1007/978-3-030-15062-4_2
- BEHERA T. K., BEHERA S., BHARATHI L. K., JOHN K. J., SIMON P. W. & STAUB J. E. 2010. Bitter Gourd: Botany, Horticulture, Breeding. *Horticultural Reviews* (ed. JANICK J.). 37: 101-141. https://doi.org/10.1002/9780470543672.ch2
- BEHERA T. K., JOSEPH J. K., BHARATHI L. K. & KARRUPAIYAN R. 2011. Momordica, Netherlands. Springer: 217-246. In: KOLE C. (ed.). Wild Crop Relatives: Genomic and Breeding Resources, 297 pp.; ISBN 978-3-642-21200-0; ISBN 978-3-642-21201-7. https://doi.org/10.1007/978-3-642-21201-7
- CĂPĂŢÎNĂ G. Cultura castravetelui antidiabet. CD, RENTROP & STRATON, ISBN: 978-606-47-0219-7.
- CRIŞAN S. & HĂLMĂGEAN L. 2007. Technological solutions with impact on the yield of *Momordica charantia* L. fruits (Cucurbitaceae) in Arad agro-ecological area. *Bulletin USAMV-CN*. **64**: 6 pp.
- DE OLIVEIRA M. S., DA COSTA W. A., BEZERRA W. F., ARAÚJO M. E., FERREIRA G. C. & DE CARVALHO JUNIOR R. N. 2018. Phytochemical profile and biological activities of *Momordica charantia* L. (Cucurbitaceae): A Review. *African Journal of Biotechnology*. 17(26): 829-846. https://doi.org/10.5897/AJB2017.16374
- ERÖZ POYRAZ I. & DERDOVSKI C. 2016. Morpho-anatomical investigations on Momordica charantia L. (Cucurbitaceae). Anadolu University Journal of Science and Technology – Life Sciences and Biotechnology. 5(1): 23-30. https://doi.org/10.18036/btdc.68664
- GILL L. S. & KARATELA Y. Y. 1982. Épidermal structure and stomatal ontogeny in some Nigerian Cucurbitaceae. Willdenowia. 12(2): 303-310.
- GROVER J. K. & YADAV S. P. 2004. Pharmacological actions and potential uses of Momordica charantia: A review. Journal of Ethnopharmacology. 93(1): 123-132. https://doi.org/10.1016/j.jep.2004.03.035
- GUPTA M., SHARMA S., GAUTA M. A. K. & BHADAURIA R. 2011. Momordica charantia Linn. (Karela): Nature's silent healer. International Journal of Pharmaceutical Sciences Review and Research. 11(1): 32-37, article-007.
- KESERÜ A., BUTA E., BORSAI O., NEGRUŞIER C. & MĂNUŢIU D. 2016. The influence of cultivation method on crop production of bitter gourd (*Momordica charantia* L.). Bulletin UASVM Horticulture. 73(2): 247-248. https://doi.org/10.15835/agrisp.v105i1-2.13051
- KOLE C., MATSUMURA H. & BEHERA T. K. (eds.). 2020. Compendium of plant genomes. Springer Nature Switzerland AG 2020, 13 chapters, 135 pp.; ISBN 978-3-030-15061-7. https://doi.org/10.1007/978-3-030-15062-4
- KUMAR D. S., SHARATHNATH K. V., YOGESWARAN P., HARANI A., SUDHAKAR K., SUDHA P. & BANJI D. 2010. A medicinal potency of *Momordica charantia*. *International Journal of Pharmaceutical Sciences Review and Research*. 1(2): 95-100, article-018.
- KUMAR K. P. S. & BHOWMIK D. 2010. Traditional medicinal uses and therapeutic benefits of Momordica charantia Linn. International Journal of Pharmaceutical Sciences Review and Research. 4(3): 23-28.
- LAGUNOVSCHI-LUCHIAN V., VÎNÂTORU C., ZAMFIR B., BRATU C., TĂPĂLOAGĂ D. & RĂDOI I. 2017. Studies and research regarding acclimatization and breeding of a new vegetable plant, *Momordica charantia* at V.R.D.S. Buzău, România. *The Ecobiotech Journal*. 1(1): 89-91. https://doi.org/10.24190/ISSN2564-615x/2017/01.13
- LUCHIAN V. & (IORDACHE) TEODOSIU G. 2019. Research results regarding the anatomy of some medicinal plants of *Cucurbitaceae*. Scientific Papers. Series B. Horticulture. **63**(1): 635- 641.
- MAHMOOD A., RAJA G. K. R., MAHMOOD T., GULFRAZ M. & KHANUM A. 2012. Isolation and characterization of antimicrobial activity conferring component(s) from seeds of bitter gourd (*Momordica charantia*). Journal of Medicinal Plants Research. 6(4): 566-573. https://doi.org/10.5897/jmpr10.613
- MAHMOUD M. F., EL ASHRY F. E. Z., EL MARAGHY N. N. & FAHMY A. 2017. Studies on the antidiabetic activities of *Momordica charantia* fruit juice in streptozotocin-induced diabetic rats. *Pharmaceutical Biology*. 55(1): 758-765. https://doi.org/10.1080/13880209.2016.1275026

- NAGARANI G., ABIRAMI A. & SIDDHURAJU P. 2014a. Food prospects and nutraceutical attributes of Momordica species: A potential tropical bioresources: A review. Food Science and Human Wellness. 3(3-4): 117-126. https://doi.org/10.1016/j.fshw.2014.07.001
- NAGARANI G., ABIRAMI A. & SIDDHURAJU P. 2014b. A comparative study on antioxidant potentials, inhibitory activities against key enzymes related to metabolic syndrome, and anti-inflammatory activity of leaf extract from different *Momordica* species. *Food Science and Human Wellness.* 3(1): 36-46. https://doi.org/10.1016/j.fshw.2014.02.003
- ONISEI T. 2016-2017. Project NUCLEU "Superior valorification of *Momordica charantia* L. (bitter gourd) for obtaining some food functional ingredients with hypoglycemic effect", AASF, RIB.
- RAHMAN A., SIKTA S. A., SARAH S. H., SHETU H. J. & DASH P. R. 2018. A comprehensive review on Momordica charantia Linn (Karela). International Journal of Botany Studies. 3(1): 85-102.
- SÁ R. D., CADENA M. B., PADILHA R. J. R., ALVES L. C. & RANDAU K. P. 2018. Anatomical study and characterization of metabolites in leaves of *Momordica charantia* L. *Pharmacognosy Journal*. 10(5): 823-826. https://doi.org/10.5530/pj.2018.5.140
- SAIFI A., NAMDEO K. P., CHAUHAN R. & DWIVEDI J. 2014. Evaluation of pharmacognostical, phytochemical and antidiabetic activity fruits of *Momordica charantia* Linn. Asian Journal of Pharmaceutical and Clinical Research. 7(3): 152-156.
- SĂVULESCU E., HOZA G. 2010. Research results regarding the anatomy of Momordica charantia L. species. Lucrări ştiințifice USAMVB. Seria B, Horticultură. 54: 694-700.
- SÂRBU A. (coord.) 2014. Aspecte de citologie și embriologie vegetală (Aspects of cytology and vegetal histology). București: Edit. CERES, 263 pp. ISBN 978-973-40-1065-3.
- SÂRBU A., PARASCHIV M. A., OANČEA F., ŞESAN T. E. 2018. Passiflora caerulea L. treated with Trichoderma plant biostimulants consortium. Morpho-anatomical considerations. Journal of Plant Development. 25(1): 3-14. https://doi.org/10.33628/jpd.2018.25.1.3
- SHETHI K. J., DOTY M. K., LIZA S. J. & RASHID P. 2018. Anatomical study of Momordica charantia L. from Bangladesh. Dhaka University Journal of Biological Sciences. 27(1): 69-74. https://doi.org/10.3329/dujbs.v27i1.46412
- SONKAR S., ARYA V. K., BARANWAL A., SUTAR N. & SUTAR R. 2014. Phytopharmacognostical and anatomical studies *Momordica dioica* Roxb. (*Cucurbitaceae*) leaf. *World Journal of Pharmacy and Pharmaceutical Sciences.* **3**(5): 813-821.
- STOIAN(OV) R. 2002. Castravetele miraculos. Sănătatea plantelor. 48(5): 9.
- ŞERBĂNESCU-JITARIU G., ANDREI M., RĂDULESCU-MITROIU N. & PETRIA E. 1983. Practicum de biologie vegetală. București: Edit. CERES, 296 pp.
- ŞESAN T. E. 2017. PROIECTUL PN-II-PT-PCCA-2013-4-0995-160/2014 (MAIA). [PN-II-PT-PCCA-2013-4-0995-160/2014 (MAIA) Project. Multifunctional and innovative products for safe and bioenhanced functional food from newly cultivated plants in Romania]. Revista HOFIGAL®, Natură şi Sănătate. 54 (decembrie 2016-februarie 2017): 34-35.
- ŞESAN T. E., OANCEA A., TOMA A., SAVIN S., TATIA R., CRĂCIUNESCU O., MOLDOVAN L., OANCEA F., RĂUŢ I., GHIUREA M., VASILESCU G., NEGRU G., BIRA-POPESCU A. F., & POMOHACI C. M. 2018. Action of multifunctional bioproducts on *Momordica charantia* L. Morpho-physiological and phytochemical characteristics. *IX Symposium AGROSYM*, Sarajevo/Jahorina (Bosnia & Herzegovina 3-7.10.2018), Book of Abstracts, Poster 385: p. 650.
- ŞESAN T. E. 2020. Momordica charantia L. new aclimatized plant in Romania. Botanical characters (Review 1). Journal of Plant Development. 27: 83-93. https://doi.org/10.3328/jpd.2020.27.1.83
- TCHEGHEBE O. T., TIMOLÉON M., SEUKEP A. J. & TATONG F. N. 2016. Ethnobotanical uses, phytochemical and pharmacological profiles, and cultural value of *Momordica charantia* Linn.: An overview. *Global Journal of Medicinal Plant Research*. 4(4): 23-39. ISSN- 2074-0883.
- VÎNĂTORU C. 2015. Soiul RODEO, brevet de invenție OSIM nr. 0048 din 23.07.2015.
- VÎNĂTORU C., MUŞAT B. & BRATU C. 2019. Cap. 5.2. Castravete amar (Momordica charantia L.). In: VÎNĂTORU C., MUŞAT B. & BRATU C. Tratat de legumicultură specială. Buzău: Edit. ALPHA MDN, 250-259. ISBN: 9789731394534.
- WALTERS T. W. & DECKERS-WALTERS D. S. 1988. Balsampear (Momordica charantia, Cucurbitaceae). Economic Botany. 42: 286-288.

How to cite this article:

SÂRBU A., ŞESAN T. E., TĂNASE T., PARASCHIV A. M., CÎŞLARIU A. G. & OANCEA F. 2022. Anatomical investigations on *Momordica charantia* L. plants, newly acclimated in Romania. J. Plant Develop. 29: 25-44. https://doi.org/10.47743/jpd.2022.29.1.901