Memories of the Agricultural and Livestock Area of the VI CCIUTM, Ecuador

Anatomy of the Photosynthetic Cladodes of Prickly Pears (*Opuntia ficus-indica* (L.) Mill.) Plants

Adriana Beatriz Sánchez-Urdaneta[1,2*], Gisela del Carmen Rivero-Maldonado[2], Cecilia Beatriz Peña-Valdivia[3] and Dianelis del Carmen Sánchez-Urdaneta[4]

[1]Research Institute, Faculties of Agronomic Engineering and Health Sciences, Research Group on Sustainable Agriculture and Bioenergy, Universidad Técnica de Manabí, Portoviejo, Manabí, Ecuador; [2]Department of Botany, Faculty of Agronomy, Universidad del Zulia. Maracaibo, Zulia State, Venezuela; [3]Graduate Program in Botany, Colegio de Postgraduados, km 36.5 Mexico-Texcoco Highway. Montecillo, Mexico. 56230; [4]Independent research. Gumilla Foundation, Villa del Rosario, Zulia State, Venezuela.

Abstract | Plants of *Opuntia* have modified stems, which are called cladodes, and are specialized structures able to photosynthesize. This study was conducted in order to compare the anatomy of the cladodes of the fruit-producing cultivars Floreadora, Moradaza, and Solferino of prickly pear (*Opuntia ficus-indica* (L.) Mill.). The thickness of cladodes, cuticle, epidermis, collenchyma, parenchyma cell walls, and xylem walls was measured; the number of cell strata of the epidermis, collenchyma, parenchyma cells, xylem vessels, and calcium oxalate crystals (druse), were counted, as well as the perimeter of parenchyma cells, xylem vessels, and mucilage cells. A completely randomized experimental design with three replications was used. Among the most outstanding results, it was determined that the cv. Solferino presented the greatest thickness of cladodes, cuticle, collenchyma, collenchyma strata, and druses, and the greatest number of parenchyma cells. Such findings may have interpretations for the adaptation of the *Opuntia* plants to certain extreme environments. This is because a thicker cuticle, epidermis, collenchyma, and parenchyma in these plants can improve survival and growth in arid conditions. At the same time, these characteristics can optimize the efficient use of water, while providing greater protection of the inner tissues. All of the above has applicability for the selection and cultivation of promising genotypes.

Received | September 22, 2023; **Accepted** | December 10, 2023; **Published** | December 21, 2023
***Correspondence** | Adriana Beatriz Sánchez-Urdaneta, Research Institute, Faculties of Agronomic Engineering and Health Sciences, Research Group on Sustainable Agriculture and Bioenergy, Universidad Técnica de Manabí, Portoviejo, Manabí, Ecuador. Department of Botany, Faculty of Agronomy, Universidad del Zulia. Maracaibo, Zulia State, Venezuela.
Email: adriana.sanchez@utm.edu.ec
Citation | Sánchez-Urdaneta, A.B., G.C. Rivero-Maldonado, C.B. Peña-Valdivia and D.C. Sánchez-Urdaneta. 2023. Anatomy of the photosynthetic cladodes of prickly pears (*Opuntia ficus-indica* (L.) Mill.) plants. *Sarhad Journal of Agriculture,* 39(Special issue 2): 1-9.
DOI | https://dx.doi.org/10.17582/journal.sja/2023/39/s2.1.9
Keywords | Anatomy, Cladodes, *Opuntia ficus-indica*

Introduction

The family Cactaceae in arid regions of Mexico, Brazil, Argentina, and Bolivia is the main center of diversity, but it is also present in tropical rainforests and temperate zones (Ortega-Baes *et al.*, 2010). According to Korotkova *et al.* (2021), this family has 22,275 scientific names, of which 2,835 are accepted

as correct taxonomic names, grouped into 150 genera, 1,851 species, 91 hybrid species, 458 infraspecific heterotypic taxa (including two hybrids), and 288 autonomous infraspecifics with 16 recognized names. The division into four subfamilies (Pereskioideae, Opuntioideae, Maihuenioideae, and Cactoideae) has been supported by using morphological and molecular characters (Barcenas *et al*., 2011). The subfamily Opuntioideae includes between 220 and 350 species (Griffith and Porter, 2009). The taxonomic placement of species varies partially among authors, a particular case is that of prickly pears (*Opuntia* spp.), as they are commonly known in Mexico, within the family Cactaceae (Lode, 2015).

The goods and services provided by prickly pear plants include "*nopalitos*" (young, tender cladodes) as an edible structure, pads (forage), fruits, soil and water erosion control, climate regulation through carbon sequestration, biodiversity conservation, wildlife habitat, pharmaceutical, and industrial benefits, as well as their aesthetic beauty. A relevant aspect of the physiology of *Opuntia* is the photosynthetic character of its modified stems. In addition to analyzing current trends in research, it is relevant to present new discoveries and plans for future research in all cacti-related areas (FAO, 2018).

Taking into account the current and potential importance of *Opuntia*, several anatomical studies for the Opuntioideae family have been carried out during the last few years. In this regard, Perrotta and Arambarri (2018) studied the anatomy of the cladodes of nine *Opuntia* species from the province of Buenos Aires, Argentina. Among the most relevant applications, they determined that the presence of fibers and a conduit adjacent to the phloem can be useful characters in species identification. However, many aspects require further investigation concerning environmental factors, such as hypodermis, calcium oxalate crystals, and tracheids with bands of secondary thickening.

In order to provide information to be used as a basis for the integral study of this important taxonomic group, the objective of this research was to compare the anatomy of the cladodes of three fruit-producing *O. ficus-indica* cultivars.

Materials and Methods

Plant material

At the San José and Tecamac Experimental Fields, belonging to the Colegio de Postgraduados, State of Mexico, Mexico, 1-year-old cladodes of the fruit-producing cultivars Floreadora, Moradaza, and Solferino, respectively, were collected. The same were placed in the shade for 15 days, previously Bordeaux mixture was applied at their base, then they were planted in pots of 19 L capacity. The soil was prepared with a mixture of leaf soil, black soil, and agrolite. The cladodes were used for the research after rooting. They were established at the San José Experimental Field, the pots were oriented forming rows in a north-south direction, and the cladodes in an east-west direction.

The material was collected from the cladodes, with which permanent sheets were made, using the microtechnique methodology of Johansen (1940), modified by Engleman (Peña-Valdivia and Sanchez-Urdaneta, 2009; Peña-Valdivia *et al*., 2010; Sanchez-Urdaneta and Suarez-Callejas, 2011; Rivero-Maldonado *et al*., 2014, 2020), as described below.

Samples preparation

For fixation and preservation of the samples, parts of the cladode edges were collected and fixed in a solution of formaldehyde, acetic acid, and ethanol (FAA) (5% acetic acid, 10% formaldehyde, 52% ethyl alcohol, 33% water), the ratio of fixative/sample volume was 10:1.

For dehydration and infiltration, the tissues were removed from the FAA, and washed with tap water for 5 minutes. Dehydration was performed with cellosolve (ethylene glycol monoethyl ether; 2-ethoxyethanol), infiltration with xylene, and an automatic tissue changer (Fischer) was used, where they remained for 12 hours at each change. Finally, they were immersed in melted paraffin at 60 °C, two changes for 24 hours, each in an oven.

At inclusion, the tissues were placed in metal boxes and oriented at the bottom according to the median cutting plane to be performed. The boxes were filled with liquid paraffin (60 °C) and allowed to solidify at room temperature. The paraffin blocks were removed from the boxes and adhered to wooden cubes with hot needles. The excess of paraffin around the tissue was removed with a single-edged razor, giving the block a pyramidal shape. Cuts 10 µm thick were made with a rotary microtome (American Optical Company). The medium-sized longitudinal sections were selected under the stereo microscope.

To adhere the sections, hot water containing gelatin was used, and the sections were spread on a microscope slide. Excess adhesive was removed, and the liquid was allowed to drain in a vertical position (two hours minimum). Finally, the microscope slides were left to dry for 24 hours on the plate.

The slides were deparaffinized and hydrated by immersing them in xylene and isopropyl alcohol for 3 min in each liquid. The slides were subsequently placed in saturated safranin: 2 g NaCl (kitchen salt), 0.05 g safranin O, and; 98 mL tap water. The microscope slides remained for 24 h at room temperature, immersed in the safranin contained in a Koplin beaker. The slides were washed by immersing them in Koplin beakers with tap water; three changes of 3 search, to remove excess salts. They were passed quickly (2 to 3 s) through 50%, 70%, and 100% isopropyl alcohol to dehydrate the tissue and preserve the safranin in the cellular parts with an acid reaction. Subsequently, the excess of isopropyl alcohol (100%) was drained off and two drops of fixed green were placed on them: 0.12 g fixed green FCF, 0.12 g; and 95 mL ethanol. The tissue was exposed to this dye for 30 seconds to 1 minute or longer by dripping it onto the slide. The excess of safranin took on a purple hue. The slides were constantly moved so that the fixed green stained homogeneously. When the cell walls were stained green the staining was complete.

The excess of fixed green was removed by rinsing with 100% isopropyl alcohol using a wash bottle, and the residue from the wash was discarded. The slides were passed through 100% xylene (three changes of 3 min each). The slides were mounted with Canada balsam. The mixture was dissolved in sufficient xylene. A drop of resin was placed on the tissue and the coverslip was placed to avoid the formation of air bubbles. All preparations were kept in the oven at 62 °C for one week, or until the resin dried. The excess resin was removed from the slides with a single-edged razor, cleaned with xylene, and washed with soap and water before being observed under a microscope. An optical microscope (LABOMED, CXR3), equipped with a video camera (COLOR CCDCAMERA, Model 2032), was used to evaluate the anatomical characters, observations, and analysis of the slides used. The dimensions and number of the anatomical structures were obtained in the images captured with the video camera, using the Image-Pro Express 5.1 personal computer program.

Data gathered

The variables evaluated were: cuticle thickness (40×), number of epidermis cell layers and their thickness (40×), thickness and number of collenchyma layers (40×), number of calcium oxalate crystals (ocular field, 10×), number, perimeter, and thickness of walls of parenchyma cells (10× and 40×, respectively), number of vessels, perimeter of vessels and thickness of xylem walls (40×), and perimeter of mucilage cells (10×).

Experimental design and statistical analysis

The experimental design used was a totally randomized design with three replicates, one cladode per treatment, each belonging to one plant. Three slides per treatment were evaluated and, in each slide, three sections and three subsamples within each section were reviewed to obtain the evaluated data.

The data obtained were subjected to an analysis of variance, and subsequent multiple comparisons of means with Tukey's test for simple effects, and LSM for interactions, using the SAS statistical package for personal computers (SAS®, 2020, version 15.1).

Results and Discussion

Statistical differences were found for all anatomical variables evaluated for the effect of the prickly pear cultivar. Three prickly pear cultivars were statistically different ($P<0.0001$). Cuticle thickness ranged from 5.74 to 21.82 µm between the cultivars Floreadora and Solferino, respectively (Figure 1A). The cuticle of the cv. Solferino was 1.73 times thicker than that cv. Moradaza, and 3.8 times thicker than that cv. Floreadora (Figures 2B, 2C, 2D, 2E, 3A, 3C, 4A, 4C).

These results differ from those obtained by Perrotta and Arambarri (2018) who determined a thin cuticle of 2 to 3 µm in width in various *Opuntia* species. They obtained values of the outside of the periclinal walls of the epidermis plus the cuticle between 6 to 10 µm wide. The widest values were found in *O. penicilligera* and *O. sulphurea* var. *pampeana*. They evidenced that a broad layer of waxes (epicuticular waxes) with a smooth and shiny, sometimes cracked, appearance is deposited on the cuticle.

According to Niechayev *et al*. (2019), the function of epicuticular waxes has been little studied in CAM species and requires further attention because of their role in survival in hot and dry conditions.

These authors noted that in many Crassulacean acid metabolism (CAM) plants, in addition to a thickened cuticle, as in *Opuntia,* particularly those in semiarid and arid environments, accumulated intracuticular and epicuticular waxes, which provided the main barrier against water loss by transpiration.

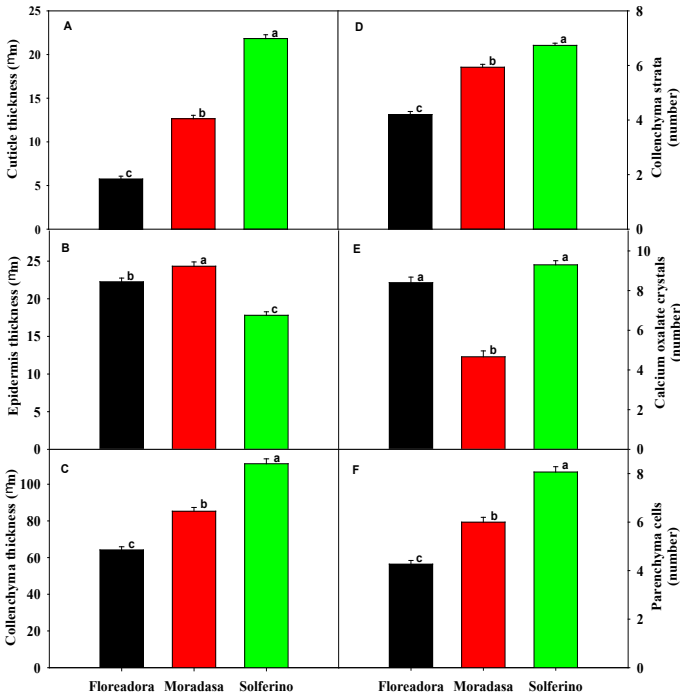

Figure 1: *Effect of prickly pear cultivars on cuticle (A), epidermis (B), and collenchyma thickness (C) and the number of collenchyma strata (D), calcium oxalate cells (E), and parenchyma cells (F) present in 1-year-old cladodes.*

The thickness of the epidermis and collenchyma were statistically significant (P<0.0001) among the three prickly pear cultivars (Figure 1B, C). The epidermis consisted of two or three cell strata, while the collenchyma presented between four and seven cell strata (Figure 5D), with statistical differences (P<0.0001) among the cultivars. Thus, both tissue strata were pluristratified in the three cultivars (Figures 2A, 2B, 2C, 2D,2E, 3A, 3C, 4A, 4C).

In general, the cv. Solferino presented greater cuticle thickness (21.82 µm), collenchymas thickness (111.18 µm), and a greater number of collenchyma strata (6.73 cell strata), and only epidermis thickness lowest value (17.81 µm). This cultivar was followed by the cv. Moradaza, and cv. Floreadora with the lowest values (Figure 1A, 1B, 1C, 1D).

The results obtained were different from the observations of Perrotta and Arambarri (2018) in various species of *Opuntia*, who observed in cladodes cross section, that the epidermis was uniseriate smooth and, with rectangular cells, and in superficial view polygonal, while in the present investigation, the epidermis was formed by two to three cellular strata, being pluristratified. However, these authors evidenced the presence of a hypodermis, from four to seven layers. This presented cellulose in its walls, with irregular thickening, from 13 to 32 µm wide, this tissue was only interrupted by stomatal chambers. In the cross section, hypodermis showed a thickness that varied from 87 µm to 164 µm. The fact that the subepidermal strata observed in this investigation could have corresponded to a hypodermis is not ruled out; to elucidate their nature, ontogenetic studies have been recommended. These results are partially similar in the thickness of epidermal cells, ranged from 17.81 to 23.62 µm, to the study of Perrota and Arambarri (2018) where the size of epidermal cells was less than 50 µm in all species.

These authors observed that the anticlinal patterns of the epidermal cell wall varied from rectilinear to curved; for example, in *O. arechavaletae, O. aurantiaca*, and *O. megapotamica* it was rectilinear to wavy. In that study other species exhibited linear anticlinal cell walls, and others wavy such as *O. penicilligera*, *O. sulphurea* var. *pampeana*, *O. ficus-indica*and *O. ventanensis*; the thickness of the anticlinal cell walls was uniform, which ranged from 2 to 3 µm wide, and the density of epidermal cells showed values below 1000 cells per unit area (cells/mm^2) in *O. aurantiaca*, *O. bonaerensis,* and *O. elata*; while in the other species it ranged from 1007 to 1696 cells/mm^2.

Stomata are a specialized epidermal cell type. According to Niechayev *et al.* (2019), most CAM species showed different stomata on leaves and stems depending on their life form. Characters such as stoma type, spine cells, pubescence of seeds, epidermal pubescence, number of spiral series, and number of stigma nodes have contributed to differentiate between the species closely related to *Opuntia*, however, they have been little used in the taxonomic description of this plant genus (Scheinvar *et al.*, 2015).

The number of cell strata of collenchyma varied from four to seven, being greater in the cv. Solferino. This result is similar to several strata of collenchyma cells in the multistratified hypodermis of *Mammillaria uncinate* (Cactaceae) documented by Loza-Cornejo *et al.* (2017). While in the study conducted by

Jáuregui *et al.* (2017) where the stems of 13 species of Cactaceae in Venezuela, between them *Opuntia caracasana* and *O.* cf. *Bisetosa* were anatomically characterized, a subepidermal hypodermis consisting of 2 to 13 layers of cells could be distinguished, with variation in the degree of thickening of the walls, so it was recognized mainly by the position, size and scarce cellular content. However, the authors did not indicate the nature of these cells, presuming them to be cholenchymal because of the differences in the thickness of their walls.

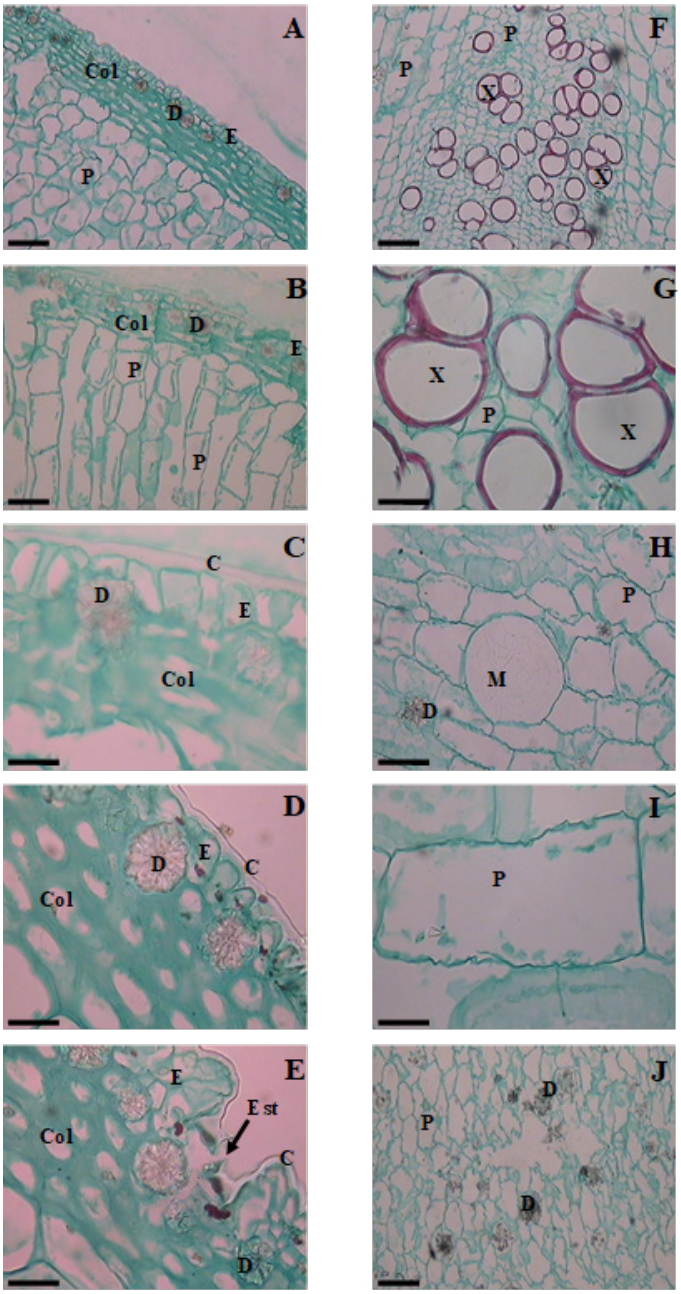

Figure 2: *Longitudinal sections of prickly pear cladodes (Opuntia ficus-indica) of the Floreadora cultivar. C= cuticle (A), E= epidermis (B), Col= collenchyma (C), D= druses (calcium oxalate crystals) (D, J), Est= stoma (E), P= parenchyma (F, G, I), M= mucilage (H), X= xylem (F, G). Bar= A, B, F, and J 40 µm, 10X; C, D, E, G, H, and I 10 µm, 40X.*

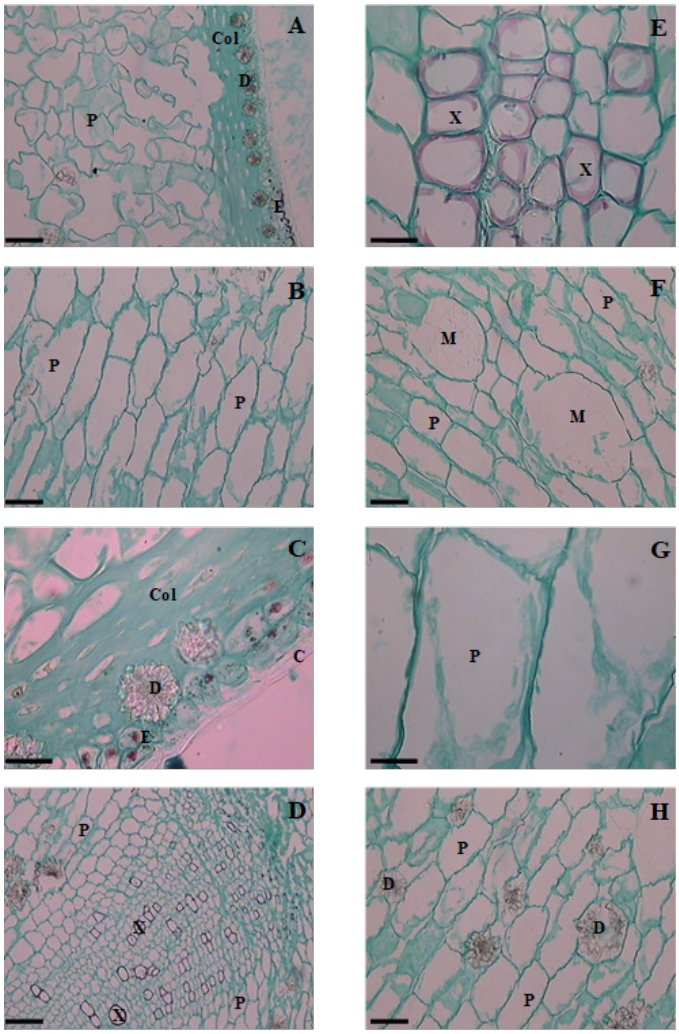

Figure 3: *Longitudinal sections of prickly pear cladodes (Opuntia ficus-indica) of the Moradaza cultivar. C= cuticle (A, C), E= epidermis (A, C), Col= collenchyma (A, C), D= druses (calcium oxalate crystals) (D, H), P= parenchyma (B, D, F, G, H), M= mucilage (F), X= xylem (D, E). Bar= A, B, D, F, and H 40 µm, 10X; C, E, and G 10 µm, 40X.*

Collenchyma is a tissue that provides support, structure, mechanical strength, and flexibility to the petiole, leaf, veins and stems of young plants, allowing them to be easily bent without breaking. They may or may not contain chloroplasts, and therefore perform photosynthesis and store reserve materials. It is located immediately below the epidermis; in addition, it has been seen in the hypodermis of avocado fruit (Carrillo-Lopez and Yahia, 2019). These characteristics would allow the cv. Solferino adaptive advantages in the face of these adverse environmental factors.

There were statistical differences (P<0.0001) between the cultivars Floreadora and Solferino compared to cv. Moradaza (Figure 5E) for the variable number of calcium oxalate crystals. On average, 8.85 calcium oxalate crystals per field (10×) were found in cultivars Floreadora and Solferino, while cv. Moradaza

presented 4.67, representing 1.90 less crystals per field. Calcium oxalate crystals were present immediately after the epidermis, showing a definite pattern (Figures 2A, 2B, 2C, 2D, 2E, 3A, 3C, 4A, 4C). However, the internal parenchyma of the cladode showed abundant calcium oxalate crystals, but without a defined pattern (Figures 2J, 2H, 4H).

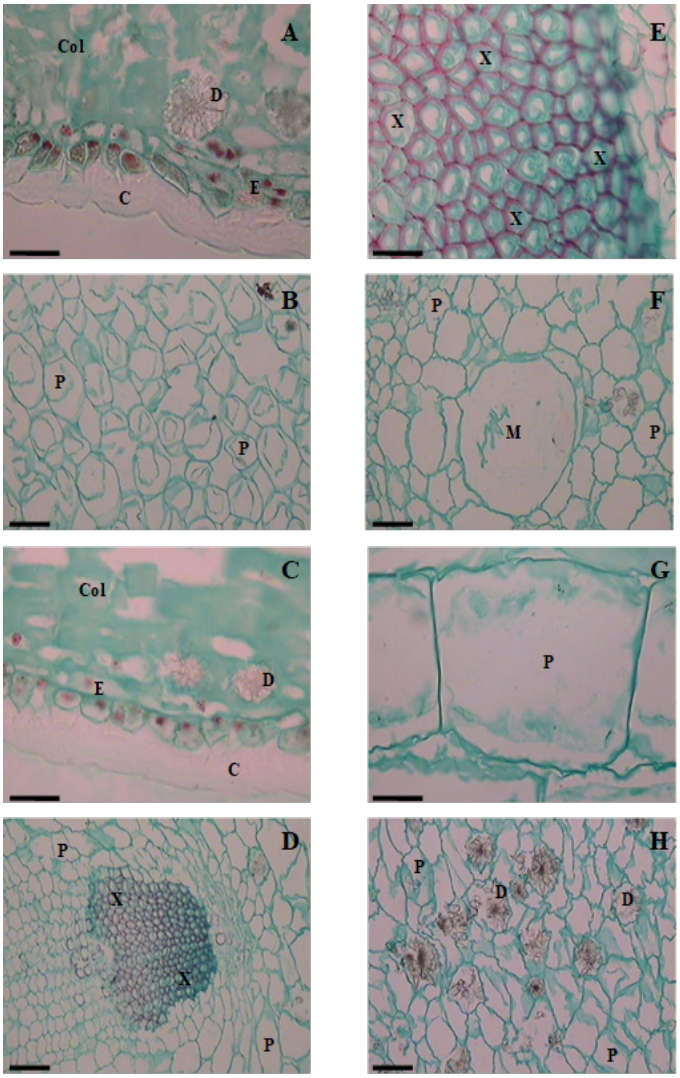

Figure 4: *Longitudinal sections of prickly pear cladodes (Opuntia ficus-indica) of the Solferino cultivar. C= cuticle (A, C), E= epidermis (A, C), Col= collenchyma (A, C), D= druses (calcium oxalate crystals) (C, H), P= parenchyma (B, D, F, G, H), M= mucilage (F), X= xylem (D, E). Bar= A, B, F, and J 40 µm, 10X; C, D, E, G, H, and I 10 µm, 40X.*

These results are similar to those of Perrota and Arambarri (2018); these researchers pointed out that different forms of calcium oxalate crystals could be observed in the cortex and pith. In cross sections, stellate druses with acute sharp points, whose diameter varied from 76 to 126 µm, as well as mucilage deposits (in *O. bonaerensis*, *O. megapotamica*, *O. penicilligera*, and *O. sulphurea* var. *pampeana*) were also observed. Starch was identified as the main reserve compound.

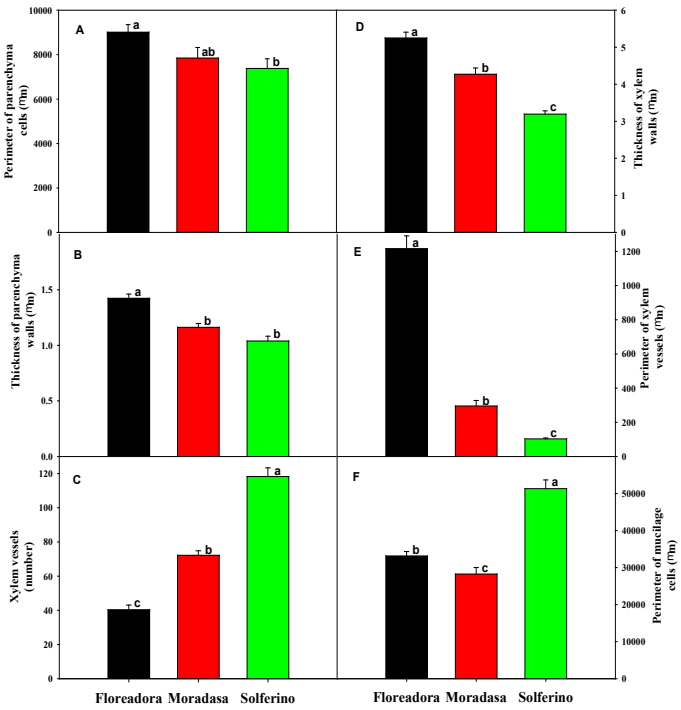

Figure 5: *Effect of prickly pear cultivars on the perimeter of parenchyma cells (A), xylem vessels (E), and mucilage cells (F); the thickness of parenchyma cell walls (B) and xylem cells (D), and the number of xylem vessels (C) present in 1-year-old cladodes.*

In relation to the number of parenchyma cells, statistical differences were found (P<0.0001) due to the effect of the prickly pear cultivars. The cv. Solferino presented the greatest number of parenchyma cells per area (8.07 cells/40000 µm^2), with statistical differences among cultivars. Cultivar Moradaza presented 6.00, and cv. Floreadora 4.27 cells/40000 µm^2, respectively (Figure 5F). These differences were evidenced in Figures 2A, 2B, 2I, 2J, 3A,3B, 3F, 3G, 3H, 3H, 4B, 4F, 4G, 4H. There were also statistical differences (P<0.0209) due to the effect of the cultivar on the parenchyma cell perimeter. Cultivar Floreadora was statistically different from cv. Solferino (Figure 5A). That is, the parenchyma cells of the cv. Floreadora were found to be larger (9009 µm), followed by cv. Moradaza (7845 µm), with no differences between them, and finally cv. Solferino (7379 µm), evidently this variable was closely related, and inversely proportional to the number of parenchyma cells (Figure 2F).

The thickness of parenchyma cell walls proved to be statistically different (P<0.0001) between the cv. Floreadora and cultivars Moradaza and Solferino, with no differences between the latter two (Figure 5B). These differences between cultivars could be appreciated in Figures 2B, 2I, 3B, 3G, 4B, 4G. These figures show the larger size of the parenchyma cells of cv. Floreadora, and the isodiametric shape of the cells

of the cv. Solferino, as well as the thickening of the cell walls of the aforementioned cells. These results were similar to those obtained by Perrota and Arambarri (2018) who observed cell walls with different degrees of cellulose thickening in the cortical parenchyma; these cells showed a greater thickness of their walls, in *O. penicilligera*, *O. sulphurea* var. *pampeana*, and *O. ventanensis*, evidencing the genetic factor. These authors stated that the cortical parenchyma was formed by the outermost photosynthetic cortical cells and innermost cortical storage cells, immediately adjacent to the stele. According to their observations, the chlorenchyma represents 50-70% of the cladode cortex in all species studied. In the superficial view, it is formed by rounded cells leaving small intercellular spaces, and in the lateral view, the shape is rectangular to quadrangular cell outlines, arranged with their major axis perpendicular to the surface.

The innermost cortical parenchyma represented 30 to 50% of the cortex. It is separated from the pith by a ring of vascular tissue. Both inner cortex parenchyma and pith have isodiametric cells with thin primary walls, leaving small intercellular spaces. This parenchyma corresponds to hydrenchyma (water parenchyma), and in relation to that function, they have numerous large, globose mucilage cells.

There was a statistically significant difference ($P<0.0001$) in the number of vessels, wall thickness, and perimeter of xylem vessels of the three cultivars (Figure 5C, 5D, 5E). The number of xylem vessels was greater in the cv. Solferino (118.3 vessels) and lower in cv. Floreadora (40.37) (Figure 5C); and the number of vessels was 2.9 times greater in cv. Solferino. However, the thickness of the xylem vessel walls had an opposite behavior to that described by the number of xylem vessels, which was greater in the cv. Floreadora with thickness of the xylem vessel walls of 5.25 µm, and less in cv. Solferino with 3.19 µm, they were 1.65 times thinner than in cv. Floreadora (Figure 5D).

The perimeter of the vessels had similar behavior to that described by the thickness of the xylem vessel walls (Figure 5E), the same was greater in the cv. Floreadora with values of 1217 µm, and lower in cv. Solferino with 103 µm. That is the xylem vessels of cv. Floreadora were 11.76 times larger than in cv. Solferino (Figures 2F, 2G, 3D, 3E, 4D, 4E).

These results are somewhat different from those of Perrotta and Arambarri (2018); these researchers determined in the *Opuntia* species that in small vascular bundles, the xylem consisted of two to ten narrow vessels. These included helical secondary walls, arranged in a row, interspersed with parenchyma of the xylem. In contrast, in medium-sized bundles, the xylem has a greater number of vessels with helical or pseudohelical secondary cell walls and simple perforation plates. But in general, the xylem of the *Opuntia* species studied (*e.g.*, *O. arechavaletae*) by Perrotta and Arambarri (2018) exhibited a relatively large number of narrow vessels, with helical secondary walls, and parenchyma. These vascular bundles were arranged in a eustele surrounded by differentiated parenchyma of the medullary rays, which appears as a parenchymatous sheath. Differences among the species were that in the xylem of *O. aurantiaca*, *O. bonaerensis, O. elata* (some specimens), *O. ficus-indica, O. megapotamica*, and *O. penicilligera* clusters of libriform fibers were evident. In the xylem *O. ficus-indica*, *O. megapotamica, O. penicilligera, O. sulphurea var. pampeana*, and *O. ventanensis* broad banded tracheids were observed. These special cell types are found between vessels, and around xylem vessels; they have primary cellulosic walls, and internally two to four to six lignified annular rings or secondary wall disks. In *O. megapotamica, O. penicilligera, O. sulphurea* var. *pampeana*, and *O. ventanensis*, they were observed forming large masses in the xylem, extending into the pith, and connecting two or more vascular bundles.

The mucilage cells were statistically different ($P<0.0001$) due to the effect of the prickly pear cultivar. Cultivar Solferino presented the largest mucilage cell size with more than 50 000 µm, with statistical differences among cultivars (Figure 5F). Cultivar Moradaza resulted in having the smallest mucilage cells with values of 28 263 µm; these differences were also noticeable in Figures 2H, 3F, and 4F. There were no statistical differences between cultivars Floreadora and Moradaza compared to cv. Solferino.

In several Cactaceae species such as *M. uncinata,* mucilage cells have been observed in the storage cortex of the stem, which represents mechanisms of adaptation to xeric habitats, since the accumulation of hydrophilic mucilage would facilitate water storage (Loza-Cornejo *et al.*, 2017). As with a greater number of collenchyma strata, the cv. Solferino, when presenting the largest size of mucilage cells, confers

favorable qualities for its survival, adaptation, and evolution in extreme environments.

Conclusions and Recommendations

The Solferino showed the greatest value for the thickness of cuticle, collenchyma, collenchyma strata, calcium oxalate crystals (druses), and the greatest number of parenchyma cells. The thicker cuticle, epidermis, collenchyma, and parenchyma in these plants may improve survival and growth in arid conditions as it increases water use efficiency while providing greater protection of interior tissues. It is probable that with the domestication of prickly pear, some anatomical components were modified, which makes cultivars more sensitive to less favorable environments for growth and development.

Novelty Statement

The anatomical analysis of the *Opuntia* cultivars studied represents an important contribution to the knowledge of this genus of cacti, determining the potential of new genotypes for agricultural production.

Author's Contribution

Adriana Beatriz Sánchez-Urdaneta: Execution of study, collection of data, lab works, and data analysis and interpretation.
Gisela del Carmen Rivero-Maldonado: Helped in data collection and analysis, reviewed literature, and assisted in data interpretation.
Cecilia Beatriz Peña-Valdivia: Planning research, supervision of the study, write up.
Dianelis del Carmen Sánchez-Urdaneta: Helped in data analysis, reviewed literature, and assisted in data interpretation.
All authors review and correct the manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Barcenas, R.T., C. Yesson and J.A. Hawkins. 2011. Molecular systematics of the Cactaceae. Cladistics, 27(5): 470-489. https://doi.org/10.1111/j.1096-0031.2011.00350.x

Carrillo-Lopez, A. and M.Y. Elhadi. 2019. Postharvest physiology and biochemistry of fruits and vegetables. Chapter 6: Morphology and anatomy. pp. 113-130. https://doi.org/10.1016/B978-0-12-813278-4.00006-3

Conde, F.L., 1975. Anatomical comparisons of five species of *Opuntia* (Cactaceae). Ann. Miss. Bot. Garden, 62: 425-473. https://doi.org/10.2307/2395206

Dos Santos Garcia, J., E. Scremin-Dias and P. Soffiarti. 2012. Stem and root anatomy of two species of *Echinopsis* (Trichocereeae: Cactaceae). Rev. Mex. Biodivers., 83(4): 1036-1044. https://doi.org/10.7550/rmb.28124

FAO (United Nations Development Programme Food and Agriculture Organization of the United Nations) 2018. Ecologia del cultivo, manejo y usos del Nopal. P. Inglese, C. Mondragon, A. Nefzaoui y C. Saenz (Eds.). pp. 248.

Griffith, M. and M. Porter. 2009. Phylogeny of opuntioideae (Cactaceae). Int. J. Plant Sci., 170(1): 107-116. https://doi.org/10.1086/593048

Jáuregui, D., M. García and E. Medina. 2017. Caracterización anatómica de porciones juveniles de tallos de Cactaceae presentes en los estados Lara y Falcón (Venezuela). Pittieria, 41: 24-39.

Johansen, D., 1940. Plant microtechnique. McGraw-Hill. New York, USA. pp. 523.

Korotkova, N., D. Aquino, S. Arias, U. Eggli, A. Franck, C. Gómez-Hinostroza, P.C. Guerrero, H.M. Hernández, A. Kohlbecker, M. Köhler, K. Luther, L.C. Majure, A. Müller, D. Metzing, R. Nyffeler, D. Sánchez, B. Schlumpberger and W.G. Berendsohn. 2021. Cactaceae at Caryophyllales.org a dynamic online species-level taxonomic backbone for the family. Willdenowia, 51: 251-270. https://doi.org/10.3372/wi.51.51208

Lode, J., 2015. Taxonomía de las cactáceas. La nueva clasificación de los cactus basada en la investigación molecular y explicada. Vol I y II. 14. 1436 p. ISBN: 978-84-617-2974-6, 978-84-617-3723-9. Ediciones Cactus-Aventuras. Espana.

Loza-Cornejo S., X. Aparicio-Fernandez, R. Patakfalviy and G. Rosas-Saito. 2017. Caracteres anatomicos y fitoquimicos del tallo y raíz de *Mammillaria uncinate* (Cactaceae). Act. Bot. Mex., pp. 120. https://doi.org/10.21829/abm120.2017.1159

Niechayev, N., P. Pereira and J. Cushman. 2019. Understanding trait diversity associated with crassulacean acid metabolism (CAM). Curr. Opin. Plant Biol., 49: 74-85. https://doi.org/10.1016/j.pbi.2019.06.004

Ortega-Baes, P., S. Suhring, J. Sajama, E. Sotola, M. Alonso-Pedano, S. Bravo and H. Godinez-Alvarez. 2010. Diversity and conservation in the cactus family. In: Ramawat, K.G. (Ed.). Desert Plants. Berlin, Heidelberg. Springer. https://doi.org/10.1007/978-3-642-02550-1_8

Peña-Valdivia, C.B. and A.B. Sanchez-Urdaneta. 2009. Effects of substrate water potential in root growth of *Agave salmiana* Otto ex Salm-Dyck seedlings. 2009. Biol. Res., 42: 239-248. https://doi.org/10.4067/S0716-97602009000200013

Peña-Valdivia, C.B., A.B. Sanchez-Urdaneta, J. Meza Rangel, J. JuarezMunoz, R. Garcia-Nava and R. Celis Velazquez. 2010. Anatomical root variations in response to water deficit: Wild and domesticated common bean (*Phaseolus vulgaris* L.). Biol. Res., 43: 417-427. https://doi.org/10.4067/S0716-97602010000400006

Perrota, V. and A. Arambarri. 2018. Cladodes anatomy of *Opuntia* (Cactaceae) from the province of Buenos Aires (Argentina). Bol. Soc. Argent. Bot., 53(3): 345-357. https://doi.org/10.31055/1851.2372.v53.n3.21310

Rivero-Maldonado, G., D. Pacheco, A.B. Sanchez-Urdaneta and M. Quiros. 2014. Comparación anatómica de la lámina foliar entre especies de *Psidium* y *Calycolpus moritzianus* (Myrtaceae). Rev. Fac. Agron. (LUZ), 31(Supl. 1): 173-181.

Rivero-Maldonado, G., D. Pacheco-Rivera, A.B. Sánchez-Urdaneta, G. Sthormes-Mendez, M. Quiros de González and J. Ortega-Alcalá. 2020. Caracteres anatómicos foliares como herramienta para contribuir a la identificación de especies de mirtaceas. 2020. Rev. Fac. Agron. (LUZ), 37 (Supl. 1): 2-8.

Sánchez-Urdaneta, A.B. and E. Suarez-Calleja. 2011. Morfo-anatomía de vitroplantas de zabila (*Aloe vera* L. Burm.f.). Rev. Fac. Agron. (LUZ), 28(Supl. 1): 139-149.

Statistical Analysis System. 2020. SAS STUDIO user's guide: Statistics. Versión 15.1. SAS Institute Inc., Cary, NC. USA. https://bit.ly/394SZIh

Scheinvar, L., G. Olalde-Parray and C. Gallegos-Vázquez. 2015. Unanueva especie del género *Opuntia* (Cactaceae) para el estado de Veracruz, México. Bot. Sci., 93: 33-39. https://doi.org/10.17129/botsci.133