Seed Characterization and Scanning Electron Microscope (SEM) Morphology of the Testa of Three Groups of Argentine Opuntia ficus-indica (Cactaceae)

C. Degano[1] , M. E. Alonso, J. Ochoa, and A. Catan

Facultad de Agronomia y Agroindustrias
Universidad Nacional de Santiago del Estero
Av. Belgrano (s) 1912, CP 4200
Santiago del Estero, Argentina
claudeg@unse.edu.ar

ABSTRACT

The external morphology of the seeds and the SEM structure of the epicuticular waxes of seeds of three groups of *O. ficus-indica* naturalized to Argentina, i.e., a spiny red-fruited variety, a spiny orange fruited variety and a spineless yellow-fruited variety, were studied. The seeds of the three groups are not differentiated easily with the naked eye. However, hairs can be observed on the surface of the seeds of the red-fruited group with the aid of a stereomicroscope. All three types of *O. ficus-indica* have a hilum in the apical or subapical position, but only the red and orange types have an aril. Different kinds of epicuticular waxes and wax granules were observed on the seeds of these *Opuntias* with SEM techniques. However, the differences in epicuticular wax characteristics were not sufficient to characterize them according to species.

Keywords: seed, Opuntia ficus-indica, scanning electron microscopy.

INTRODUCTION

Fruits of *Opuntia ficus-indica* are a valuable food in semiarid areas. In Argentina, the three kinds of fruits that are preferred in the establishment of new plantations are clones with: spines and red fruits, spines and orange fruits, and spineless yellow fruits. There is not complete agreement in the taxonomy of these clones, as some botanists place these fruit clones as different species while others consider them only as biological forms or varieties (Bravo-Hollis, 1978). For example, Britton and Rose (1919-1923) uses presence or absence of spines to separate *O. ficus- indica* from O. streptacantha.

Morphological characters of ovules and seeds and their ultrastructural characteristics (Table 1) often exhibit large variations in the surface of seeds. These seed surface characteristics are often of valuable assistance in delimiting generic and taxonomic relationships (Maiti et al., 1994; Engelman, 1960). Characters most frequently used for taxonomic purposes in cactus seeds are seed surface ultrastructure, seed shape, and presence or absence of a shiny surface (Hoffman, 1989; Engelman, 1960).

[1]Corresponding author

The aim of this work was to assess the external morphology of these three groups of cactus seeds, and the ultrastructure of the seed testa for their possible use as plant taxonomic characters.

Table 1. Summary of Seed Morphological Characteristics in Three *Opuntia ficus-indica* Groups

Seed Character	Opuntia ficus-indica spiny plant, red fruit	Opuntia ficus-indica spiny plant, orange fruit	Opuntia ficus-indica spineless plant, yellow fruit
Seed length	4.65 ± 0.43	4.64 ± 0.43	5.18 ± 0.52
Hilum	Eliptic with no elevated outgrowths	Subapical and slightly deep	Apical and laterally extended
Micropyle	Apical	Subapical	Apical and covered by hilum
Aril	Small	Ribbon like	Absent
Hairs	Two types	Absent	Absent
Warty granules diameter (µm)	Abundant 38.8 µm	Abundant 47.5 µm	Few 28 µm
Epicuticular waxes	Plates	Aggregate coat of randomly oriented fibers	Plates with rough surface.

MATERIALS AND METHODS

Red fruits of spiny cactus, orange fruits of spiny cactus, and yellow fruits of spineless *O. ficus-indica* were obtained from a commercial plantation located 18 km west of the capital city of Santiago del Estero, (lat. 27°45² S. long. 64°20² W.). The seeds were separated from the pulp, washed with warm water, and fixed in FAA (Johansen, 1940). The seeds were examined and photographed with a stereomicroscope. For the scanning electron microscope (SEM) study, the seeds were fixed in glutaraldehyde, dehydrated through an alcohol-acetone series, dried in a critical-point drying apparatus, mounted on stubs in different positions and coated with gold in a sputter coater. The specimens were observed and photographed with a Joel SEM at the Electron Microscopy Laboratory of the North West of Argentina, National Council of Scientific Investigations and Technology, National University of Tucuman (LAMENOA-CONICET-UNT). The following seed characters were measured on 20 representative seeds: length of the main axis, seed form, shine of the testa, position of the micropyle and hilum, and presence or absence of an aril. The epicuticular waxes were classified according to Metcalfe and Chalk (1979).

RESULTS

The red cactus seeds were 4.65 ±0.3 mm (n=20) long in the main axis. Their shape was spheric to reniform, with both faces convex, and the testa was rough and shiny (Figures 1 and 2). The hilum was eliptic and had no elevated outgrowths. The micropyle region was apical in position with a small aril (Figure 2). In the SEM, two kinds of hairs were observed: unicellular striped hairs and multicellular hairs with a long unicellular stalk with a head of four to five short cells (Figure 3). The cuticle was present in the form of a reticulum of sharp ridges, with epicuticular waxes occurring in plates and warty granules (38.8 Fm in diameter) in the micropilar region (Figures 3, 4, and 5).

The orange cactus seeds were 4.64 ±0.43 mm long and circular to reniform in shape with a conical apex (Figure 6). The testa was rough and shiny while the hilum was in a subapical slight depression. The micropyle was near the apex and had a ribbon-like aril that extended to the lateral region. Through the SEM, the epicuticular wax appeared as an aggregate coat of randomly oriented threads (Figure 7) with abundant warty granules (47.5 Fm in diameter) that were in the micropilar region (Figures 8 and 9).

The yellow seeds from the spineless fruits were 5.18 ±0.52 mm long, were circular to reniform in shape, and had a pointed funicular end. The testa was rough and shiny. The hilum was apical in position and was laterally extended to cover the hilum (Figures 10, 11, and 12). No aril was observed. Through the SEM, the epicuticular wax appeared as plates with a rough surface (Figure 12) and with a few warty granules (28 Fm in diameter) in the micropilar region (Figures13 and 14).

DISCUSSION

The seeds of the three groups of *O. ficus-indica* are not easily differentiated with the naked eye. Distinguishing characters observed with a stereomicroscope are microscopic hairs on the seed surface of the variety with red fruits and the presence of an aril in the red and orange varieties. All three groups had a hilum in the apical or subapical position. We feel these differences are too small to justify dividing any of these varieties into species.

Under the SEM, the seeds of the varieties with three fruit colors can be differentiated. While all three varieties have epicuticular wax in plate form, there is significant variability in the wax plate form of all three varieties. For example, the seeds of the red fruit have the epicuticular wax present in the form of stripes with multicellular hairs, while the wax of the yellow forms has a rough surface and the orange-fruit varieties have randomly oriented threads. All three varieties have wax in the form of warty granules.

Mixed epicuticular wax also occurs in the families Caprifoliaceae, Fabaceae, and different genera of Eucalpytus. *Opuntia quimilo*, a cactus native to Argentina, has wax plates with an elaborate network of minute flakes (Metcalfe and Chalk, 1979). Weisser (1973) and Maiti et al. (1994) found differences among genera of the Cactaceae, but significant differences that would warrant placing these groups into different species were not found.

The warty granules observed on the seed surfaces have been described by Scheinvar (1985) as drusas. However, we disagree with this designation, as Esau (1993) states that drusas are formed from intracellular products and not epicuticular products.

The differences we have found in epicuticular wax morphology, and in the sizes of the wax granules are additional useful characters for plant taxonomy (Metcalfe and Chalk, 1979). While these differences help to separate these Opuntia clones with different color fruits into different biological forms, we agree with Bravo-Hollis (1978) that the differences are not significantly great to justify placing them into different species.

REFERENCES

Bravo-Hollis, H. 1978. Las Cactaceas de Mexico. Vol 1. Univ. Nac. Autonoma de Mexico, Mexico.

Britton, N. L. and J. N. Rose. 1919-1923. The Cactaceae. 4 vols. Carnegie Institute of Washington Publ. 248 pp.

Engleman, M. E. 1960. Ovule and seed development in certain cacti. American Journal of Botany, 47:460-467.

Esau, K. 1993. Anatomia de las plantas con semillas. 2nd Edition, Ed. Hemisferio. Sur. Bs As. 512 pp.

Hoffman J. Adriana E. 1989. Cactaceas. Flora Silvestre de Chile. Ed. Fundacion Claudio Gay. 272 pp.

Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill. New York. 523 pp.

Maiti, R. K., J. L. Hernandez-Pineiro, and M. Valdez-Marroquin. 1994. Seed ultrastructure and germination of some species of Cactaceae. Fiton 55:97-105.

Metcalfe, C. R. and L. Chalk. 1979. Anatomy of the Dicotyledons. Part VII, Epicuticular wax and its morphology. pp. 158-160. Vol 1, 2nd Ed. Clarendon Press, Oxford.

Scheinvar, L. 1985. Flora Ilustrada Catarinense. Parte I: As Plantas: Cactaceas. pp. 59-88. Santa Catarina, Brasil.

Weisser, P. 1973. Morfologia externa de las semillas de algunas cactaceas chilenas y su utilidad en taxonomia. Anales de Museo de Historia Natural de Valparaiso. No. 6.

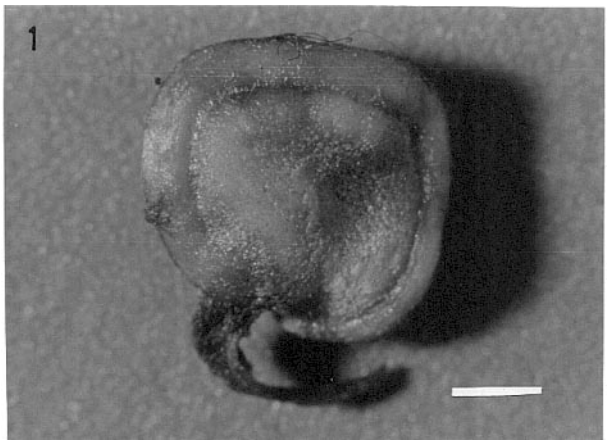

Figure 1. Spheric Seed of Red Fruits of Opuntia ficus-indica
with Convex Face
Bar = 1 mm

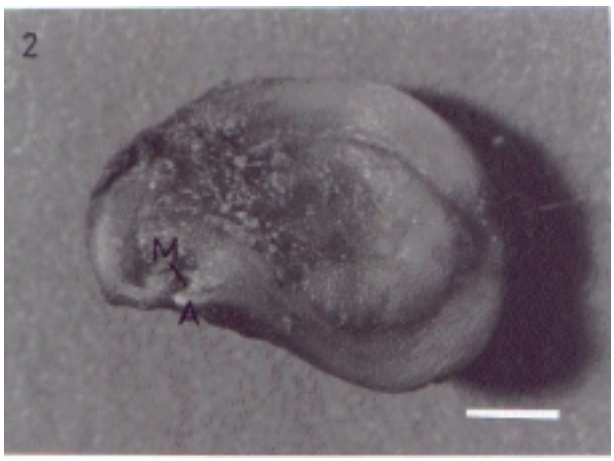

Figure 2. Seed of Red-fruited Opuntia-ficus indica
Micropyle (M) and little aril (A) can be seen.
Bar = 1 mm

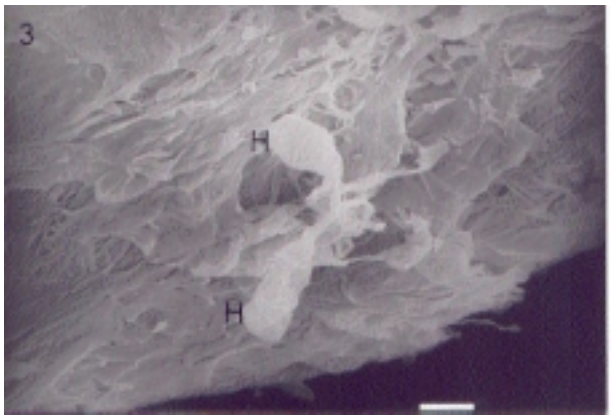

Figure 3. SEM Micrograph of Red-fruited O. ficus-indica Seed
Multicellular hairs (H) and the rough surface can be seen.
Bar = 100 Fm

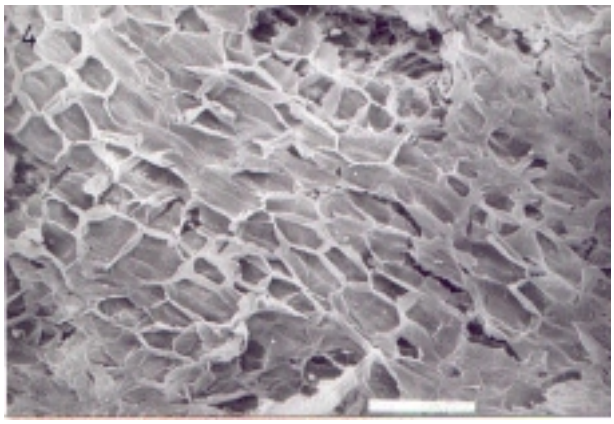

Figure 4. SEM Micrograph of Seed of Red-fruited O. ficus-indica
with the Cuticle Forming a Reticule with Sharp Edges
Bar = 100 Fm

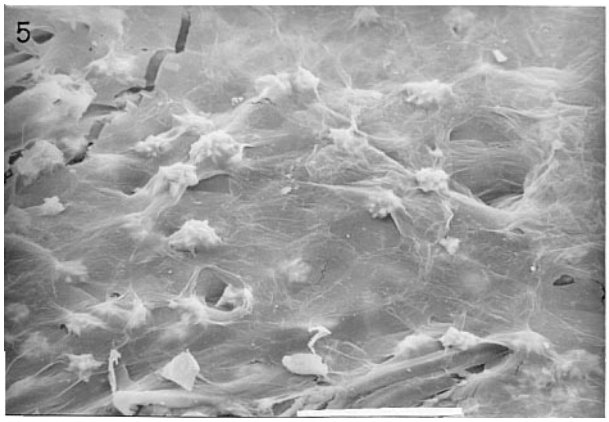

Figure 5. SEM Micrograph of Seed of Red-fruited O. ficus-indica
with Wax Granules Present in the Micropylar Region
Bar = 100 Fm

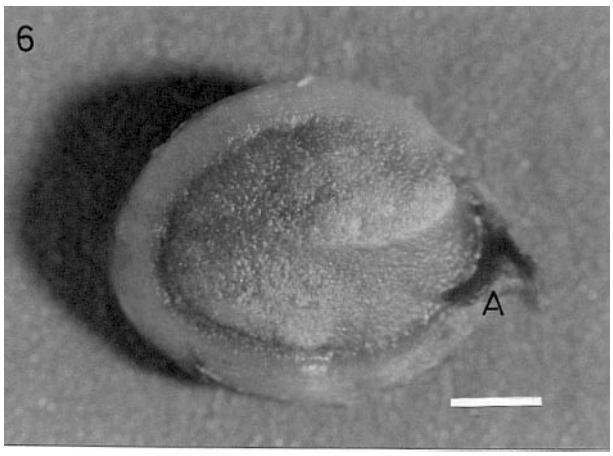

Figure 6. Seed of Orange-fruited *O. ficus-indica* with a Ribbon-like Aril
(A) in the Apex of the Micropyle
Bar = 1 mm

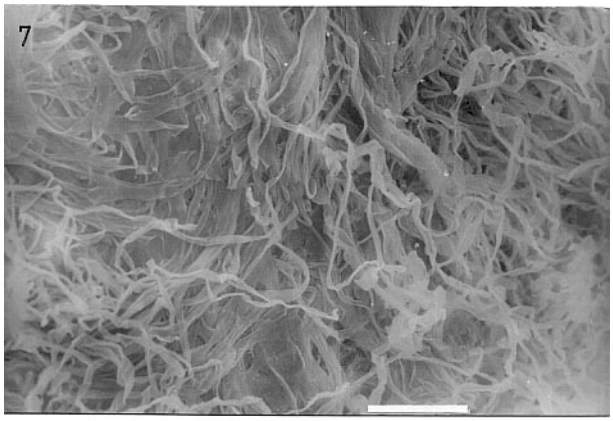

Figure 7. SEM Micrograph of Seed of Orange-fruited O. ficus-indica
with the Epicuticular Wax Forming an Aggregate Coat
of Randomly Oriented Threads
Bar = 100 Fm

Figure 8. SEM Micrograph of Seed of Orange-fruited O. ficus-indica
with Wax Warty Granules Appearing in the Micropylar Region
Bar = 100 Fm

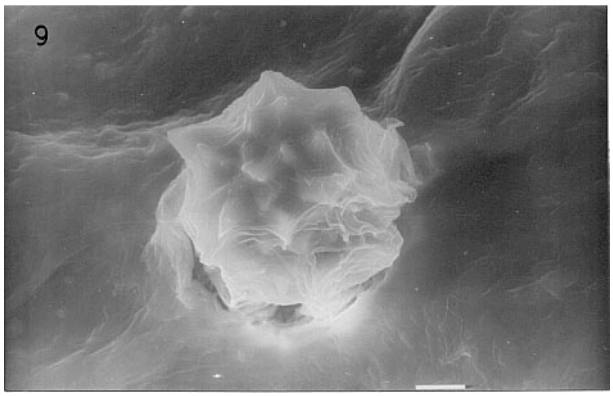

Figure 9. SEM Micrograph of Orange-fruited *O. ficus-indica*
with Closeup of the Warty Granule
Bar = 10 Fm

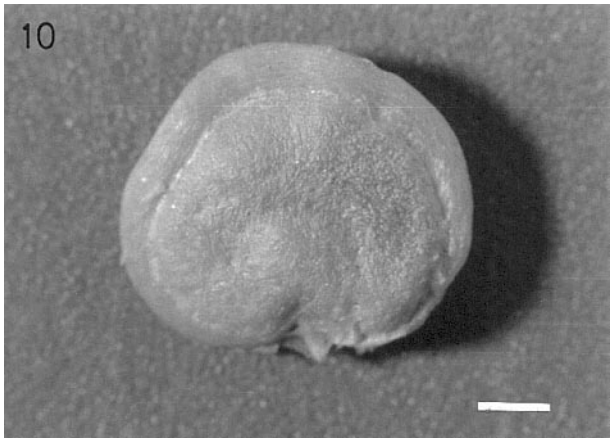

Figure 10. Rounded Seed of Yellow-fruited *O. ficus-indica*
with the Shiny Surface Evident
Bar = 1 mm

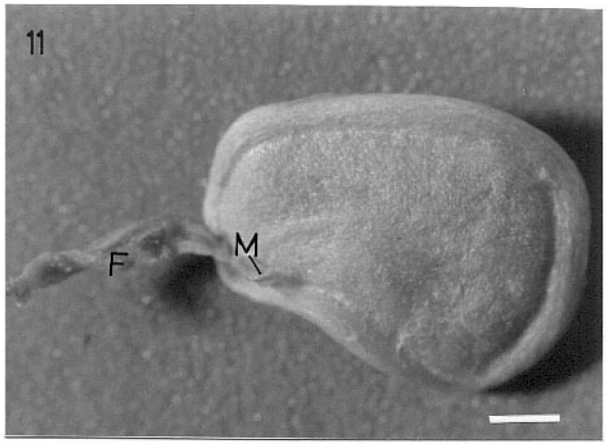

Figure 11. Seed of the Yellow-fruited O. ficus-indica
Indicating a Pointed Funicular End
Micropyle (M) and Funicule (F)
Bar = 1 mm

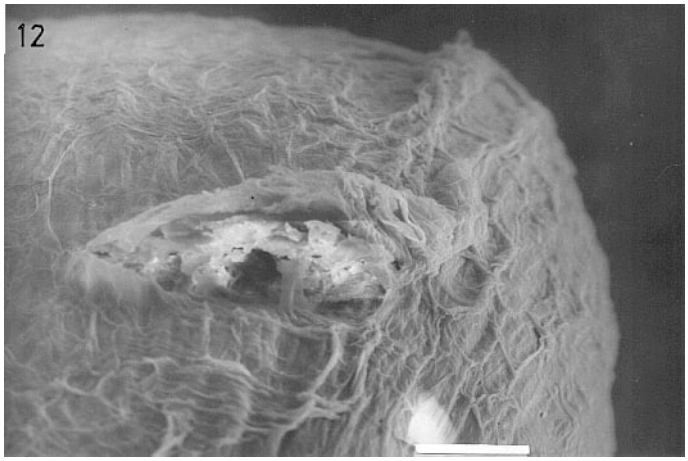

Figure 12. SEM Micrograph of Seed of the Spineless Yellow-fruited
O. ficus-indica Indicating Rough Epicuticular Wax
near the Micropylar Region
Bar = 100 Fm

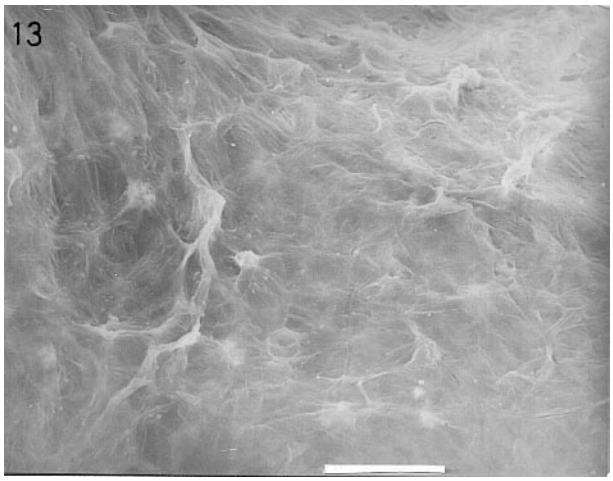

Figure 13. SEM Micrograph of Seed of the Spineless Yellow-fruited O. ficus-indica Exhibiting Few Wax Granules in the Micropylar Region Bar = 100 Fm

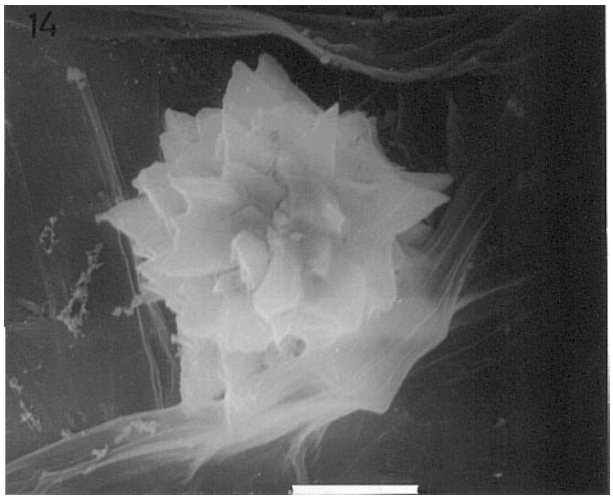

Figure 14. SEM Micrograph of Seed of the Spineless Yellow-fruited O. ficus-indica Exhibiting a Wax Granule Bar = 10 Fm.