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ORIGINAL ARTICLE

Escontria chiotilla (Cactaceae): fruit development, maturation and harvest index

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Abstract – Introduction. The edible fruit of jiotilla (*Escontria chiotilla* (Weber) Rose), a cactus endemic to Central Mexico, mature asynchronously and are characterized by the presence of bracts in the fruit peel (pericarpel). In order to establish a harvest index, a series of physical, chemical, and morphological classical parameters coupled with histological observations were determined at four different stages of fruit development from anthesis to mature fruit (S1–S4). **Materials and methods.** Flowers and fruit samples at each stage were collected for analysis from randomly selected plants from the desert shrub. **Results and discussion.** The single sigmoid growth curve exhibited major and minor changes between S2 and S3, S3 and S4, respectively. Growth cessation and maturation began at S3, the maximum concentration of total sugars occurred in S3, and the maximum value of firmness decreased in S3 and S4. A thinning of the fruit peel due to aerenchyma compression occurred in the transition period S3-S4 and fruit firmness was the mechanical parameter more closely associated with it. Poor relationships with ripening stages were shown by the other physicochemical parameters measured. **Conclusions.** We propose two morphological-histological indicators of harvest: 1) Fruit peel thickness, and 2) Color and texture changes of the bracts. The infundibuliform flower adapted to confine, protect and improve the aqueous reserves in the ovary of the cactus species becoming a special berry with a successful strategy for saving water during fruit development and ripening. This strategy involves structural and physiological adaptive traits to survive in arid lands.

Keywords: Mexico / jiotilla / *Escontria chiotilla* / edible cactus / growth models / plant histology / shelf life

Résumé – *Escontria chiotilla* (Cactacée) : développement du fruit, maturation et indication du stade de récolte. Introduction. Les fruits comestibles de la chiotilla (*Escontria chiotilla* (Weber) Rose), un cactus endémique du centre du Mexique, mûrissent de manière asynchrone et sont caractérisés par la présence de bractées sur le péricarpe (la peau) du fruit. Afin d'établir un indice du stade de récolte, une série de descripteurs physiques et chimiques, ainsi que des paramètres morphologiques classiques couplés à des observations histologiques ont été analysés à quatre différents stades du développement des fruits, depuis la floraison jusqu'à complète maturité (S1–S4). **Matériel et méthodes.** Des échantillons de fleurs et de fruits à chaque stade du développement ont été collectés pour analyse sur des arbustes choisis au hasard dans le désert. **Résultats et discussion.** La courbe de croissance des fruits en forme de simple sigmoïde a révélé les changements majeurs et mineurs qui se sont opérés entre les stades S2 et S3, et S3 et S4, respectivement. L'arrêt de croissance et de maturation a débuté au stade S3, alors que la concentration maximale en sucres totaux et la valeur maximale de fermeté ont diminué en S3 et S4. Un amincissement de la peau du fruit dû à la compression des aérenchymes, s'est produit dans la période de transition S3-S4, phénomène fortement associé au paramètre mécanique de la fermeté des fruits. Les autres paramètres physico-chimiques mesurés ont présentés de faibles corrélations avec le stade de maturation du fruit. **Conclusions.** Nous proposons deux indicateurs morpho-histologiques du stade de récolte : 1) l'épaisseur de la peau du fruit, et 2) le changement de couleur et de texture des bractées. La fleur infundibuliforme,

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adaptée à confiner, protéger et élaborer les réserves aqueuses dans l'ovaire des espèces de cactus, devient une baie particulière ayant développé une stratégie efficace pour économiser l'eau au cours du développement du fruit et de sa maturation. Cette stratégie implique des traits adaptatifs structuraux et physiologiques lui permettant de survivre dans des terres arides.

Mots clés : Mexique / chiotilla / *Escontria chiotilla* / cactus comestible / modèle de croissance / histologie végétale / aptitude au stockage

1 Introduction

Escontria is a monotypic endemic genus from Central Mexico belonging to the family Cactaceae [1–3]. *Escontria chiotilla* (Weber) Rose. It is a common element of the tropical deciduous and desert-shrub forests located at the arid and semiarid areas of the states of Michoacán, Guerrero, Puebla and Oaxaca [4, 5]. In these communities, jiotilla (*E. chiotilla*) is a dominant species because of its ability to adapt to arid conditions. As such, its presence contributes to the persistence of the shrub in places where extreme temperatures and low rainfall might otherwise lead to a loss of plant cover and bare soils [6, 7]. This species is also one of the most culturally and economically important cactus in these regions of Mexico [8].

Jiotilla is a columnar arborescent plant with 3 cm – long infundibuliform flowers. The fruit is non – climacteric [9] and edible, consisting of red – purple berries that are sweet in taste and have a high content of betalains – a particular class of pigments with antioxidant properties [10–13]. As in the case of Myrtillocactus fruit [14] and *Opuntia* spp. [15] despite their nutritional value, striking color and commercial potential, jiotilla remains an underutilized species for several specific reasons. First, fruit mature asynchronously and no harvest index has yet been specified which allows an easy recognition of commercial fruit maturity in the field. This limits both fruit conservation and the establishment of suitable conditions for long shelf life and commercialization [9]. Second, jiotilla fruit has traditionally been collected from the wild and no orchard exists, probably due to its unsuccessful vegetative propagation [16, 17]. This has resulted in the establishment of an incipient forestry management that has so far only limited itself to promoting conservational measures [8]. Third, the fruit are mainly consumed in local villages of the South West and the Mixteca Baja regions of Mexico, where they are frequently sold in regional markets. Therefore, although highly valued locally, they are practically unknown in the rest of the country [17]. Lastly, jiotilla has scarcely been studied and there are no scientific publications that specify a particular harvest index that might serve as the basis for recognizing fruit maturity in the field.

The most commonly employed parameters for fruit harvest indices are: pulp percentage, dry matter content, shape of the fruit or fullness, external color, titratable acidity, total content of sugars and/or total soluble solids. Physical, mechanical, chemical and physiological properties of mature fruit had been widely reported in non-climacteric Cactaceae fruits of the genus *Selenicerus, Hylocereus* and *Opuntia* [18–28]. Recently, non-destructive parameters measured in dragon fruit, *Hylocereus polyrhizus,* were analyzed by principal component analysis and the PC1 component used to establish a single maturation index [22]. Limited experimental work has been done in fruit development of some cactus species [19, 29, 30].

Studies describing a fruit harvest index based on histological features are scarce [31–34] and have not yet been reported in jiotilla. We hypothesized that the maturation index could be improved by coupling physicochemical analyses to structural histological changes. This paper presents the general characteristics of *E. chiotilla* fruit, describes its maturation process and identifies its physical, chemical, morphological characteristics. This work includes histological observations of fruit development from anthesis to ripening coupled with the physicochemical analysis conducted during the same period, in order to establish a harvest index. We also report some preliminary observations about the postharvest life of jiotilla fruit.

2 Materials and methods

2.1 Sampling for morphological and histological observations

The collection of *Escontria chiotilla* flowers and fruits in May and June 2004 was conducted using ten randomly selected plants from the desert shrub near Chazumba, Oaxaca, located in the Tehuacán – Cuicatlán Valley of Mexico (18° 11′ N, and 97° 41′ W). The climate in this area is predominantly arid in the lowlands, with an annual mean temperature of 25 °C and rainfall of 1,340.7 mm [35]. Rainfall in May and June 2004 was 87.4 mm and 262.7 mm, respectively. Sixteen flowers were collected at anthesis which we termed stage zero (S0) along with 35 fruits at S1, S2, S3, S4 stages, an S3-S4 transition stage, and an over mature fruit stage (S5).

Additional samples were collected as needed in the same period to confirm observations. The developmental stages were differentiated based on their morphological and histological characteristics and the number of weeks after anthesis. These were: S1, 4 weeks; S2, 5 to 9 weeks; S3, 10 to 12 weeks; and S4, over 12 weeks after anthesis; with an over mature state S5 (*figure 1* and *table I*). Long timing weeks were selected because jiotilla is not a cultivated species and plants are characterized by floral asynchrony, long fructification periods that extend from April to October [17] with remarkable variations between months of rainfall during fruit production.

Descriptions of fruit tissues were made from at least 3 flowers and 3 fruits of each stage of development, beginning with the pericarpel surface and ending with the fruit interior (locule), with three regions that could be broadly described as: a, the external surface; b, an intermediate region; and c, the region adjacent to the locule (*figure 2*).

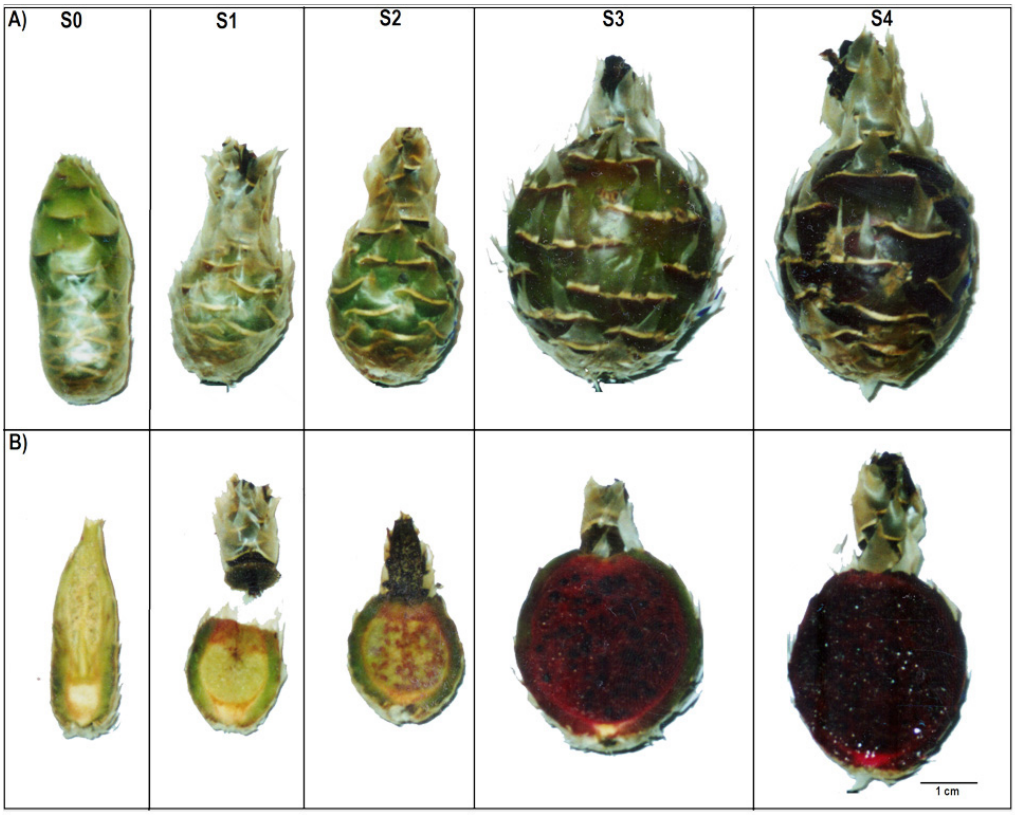

Figure 1. Flower and fruit of *Escontria chiotilla* (Weber) Rose during the stages of development considered for this study: A) External view, where bracts are visible; B) Longitudinal sections at the corresponding stage of development. S0: Flower at anthesis; S1: 4 weeks after anthesis; S2: 5–9 weeks after anthesis; S3: 10–12 weeks after anthesis; and S4: over 12 weeks development after anthesis.

Seeds from a sample composed of 15 fruits were visually counted one by one. The weight of seeds per fruit was determined using an analytical balance and the size measured using Imaging Tool v. 3.0 and confirmed with the scanning electron microscope (SEM) (Zeiss, Model DSM 940).

2.2 Morphological and histological parameters

Flower and fruit samples were fixed using a formalin - acetic acid - ethyl alcohol (FAA) solution and dehydrated by stepwise immersion in an ethyl alcohol and distilled water dilution series (50:50, 70:30, 85:15, 90:10 and 100). Subsequently, they were embedded in paraffin [37, 38] and LR White resin, and sliced using either a rotary microtome or an ultramicrotome in order to obtain 10 μm and < 2 μm slices, respectively. Specific staining was done using toluidine and safranin-O/fast green fixative [38]. Bright-field and phase contrast microscopies were then used for sample visualization, with the morphological observation of bracts specifically made using a SEM after the preparation of such structures by conventional techniques [38, 39].

2.3 Physical and chemical parameters

The polar and equatorial diameters of the fruit were measured using a vernier scale. Fruit firmness was determined using a MacCormick penetrometer (Fruit Tech, Yakima Washington) equipped with an 8 mm probe and the force in pounds converted into Newtons. Flesh and pericarp color were measured with a Hunter – Lab colorimeter (ColorFlex®, Hunter Associates Laboratory, USA).

The dry matter, protein, and moisture contents of fruit pulp were determined following procedures described in A.O.A.C. [36], along with the percentage pulp (w/w). Additionally, in more mature fruits (stage S3 onwards), the following were also measured: total sugars, from extracted juice using the 3,5-dinitro-salicylic acid (DNS) spectrophotometric method; total soluble solids (TSS), with an Atago field refractometer; and titratable acidity (TA), (expressed as % citric acid) by potentiometry using 0.1 N sodium hydroxide.

2.4 Statistical analysis

All data from physical and chemical analyses, as well as those from morphological and histological assessments, were subjected to ANOVAs using the statistical program NCSS version 2010 and SPSS version 18, respectively, with multiple comparisons of means performed by the Duncan method ($\alpha = 0.05$).

Table I. Physical characteristics of *Escontria chiotilla* flowers at anthesis and fruit at different stages of development. Different letters in each column indicate significant differences ($P < 0.05$, $n = 30$) in the comparison of means by the method of Duncan (means ± standard deviation).

Stage of development (in weeks after anthesis)	Fruit fresh weight (g)	Pulp/peel (in % FW)	Surface color (%)	Polar diameter (cm)	Equatorial diameter (cm)	Firmness (N)	Peel thickness (mm)	Pulp color
S0 – Flower at anthesis	3.02 ± 0.36 a	ND[y]	green 100%	ND	ND	ND	ND	white yellow
S1: 4	4.50 ± 0.90 b	20.60a/79.4b	green 9–99%	2.10 ± 0.28 a	1.72 ± 0.12 a	62.27 ± 0.94b	3.70 ± 0.53 c	white yellow
S2: 5–9	7.05 ± 1.13c	27.51b/72.5b	dark green 70% green 30%	2.51 ± 0.25 b	2.13 ± 0.17 b	68.19 ± 0.78 b	2.50 ± 0.45 b	white yellow
S3: 10–12	17.90 ± 2.23 d	61.13/38.9a	green 15% purple 85 %	3.21 ± 0.35 c	3.03 ± 0.43 c	57.82 ± 0.57 b	1.70 ± 0.20 ab	red-purple
S4: > 12	20.87 ± 3.53d	68.09d/31.9a	purple 100%	3.50 ± 0.89 c	3.20 ± 0.23 c	44.48 ± 0.29 a	1.20 ± 0.20 a	deep red-purple

[y] ND: not determined.

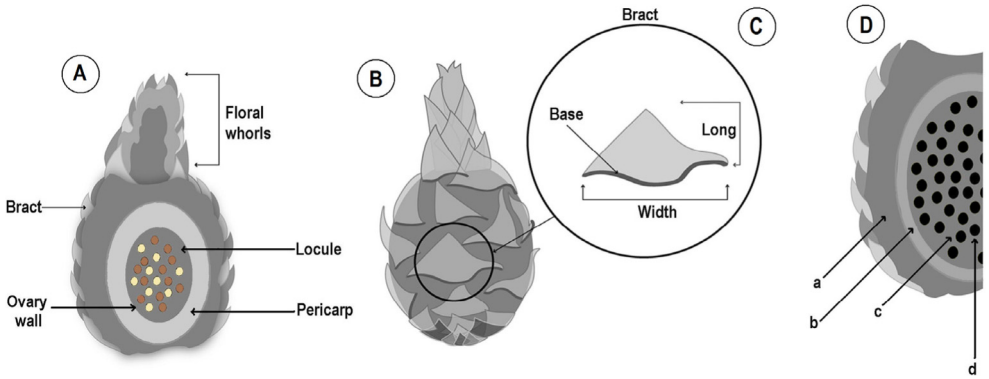

Figure 2. Schematic representation of the fruit of *Escontria chiotilla*. A) Longitudinal section at the S1 stage; B) External morphology at the S1 stage; C) Bract; D) Longitudinal section during the S3-S4 transition period recommended for harvest. a: pericarpel external surface; b: compressed (collapsed) aerenchyma; c: pulp; d: seeds.

3 Results and discussion

3.1 Physical and chemical parameters

The growth pattern of jiotilla was characterized by a single sigmoid curve (*figure 3A–3B*). In contrast, changes in fruit size and fresh weight measured in *Opuntia ficus-indica* showed a double sigmoid curve with three distinct phases along a 80–90 day growth period [18]: Phase I – rapid growth starting shortly after anthesis; Phase II – suspended fruit growth and gain in fresh and dry weight of seeds; and Phase III – final growth after the onset of color change. Several reasons for this difference in growth patterns could be:

1) Jiotilla is a not a cultivated species and we are studying naturally pollinated samples where the fertilization of ovules is not as uniform as in flowers pollinated by hand. Therefore, we observed that seeds developed asynchronically as indicated by the presence of seeds of heterogeneous color in the same fruit at the stage 2: immature – light brown and mature – shiny black (*figure 1*, S2).
2) Seeds of jiotilla are smaller (1.0–1.2 mm) and softer, and accumulates less dry mater than those of *O. ficus-indica*. Indeed, seeds are only 4.5% of the total weight in *E. chiotilla* compared with 5–10 % reported for *O. ficus-indica* fruit [27]. This fact would explain why Phase II, responsible for the gain of seeds weight in *O. ficus-indica*, is not evident in *E. chiotilla*.
3) Long timing periods of sampling could have masked Phase II corresponding to seed growth.

The entire period of jiotilla fruit growth was 140–175 days that is approximately as long as that reported for yellow pitaya (120–162 days, depending on local temperature) [19].

The contents of dry matter and protein in fruit were observed to peak at S1 and S2, respectively, only to decrease later on during fruit development because of the diluting effect of pulp growth (*figure 3A*). During fruit development the total content of water in the fruit remains almost stable (83–87%) (*figure 3A, 3B*). The water transported by the vascular bundles can also move from the pericarpel tissues to the ovary wall and to the growing pulp. These dynamic changes are observed in the variations of the percentage of peel and pulp of

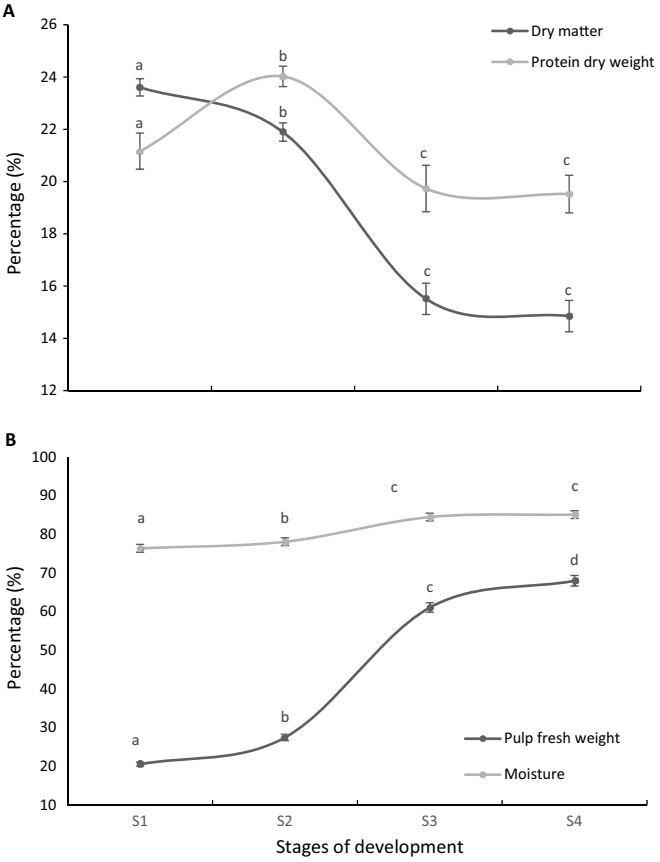

Figure 3. Growth curves of *Escontria chiotilla* fruit. Each point represents the mean of three measurements ± SD. Different letters indicate significant differences in the comparison of means by the Duncan's method ($\alpha = 0.05$).

E. chiotilla (*table I*), and reported in other cactus berry species such as *O. ficus indica, S. megalanthus, H. polyrhizus, H. undatus* [19, 20, 30, 39].

The greatest changes in the growth pattern in jiotilla occurred between the stages S2 and S3, with little or no change occurring between S3 and S4, when growth was nearly

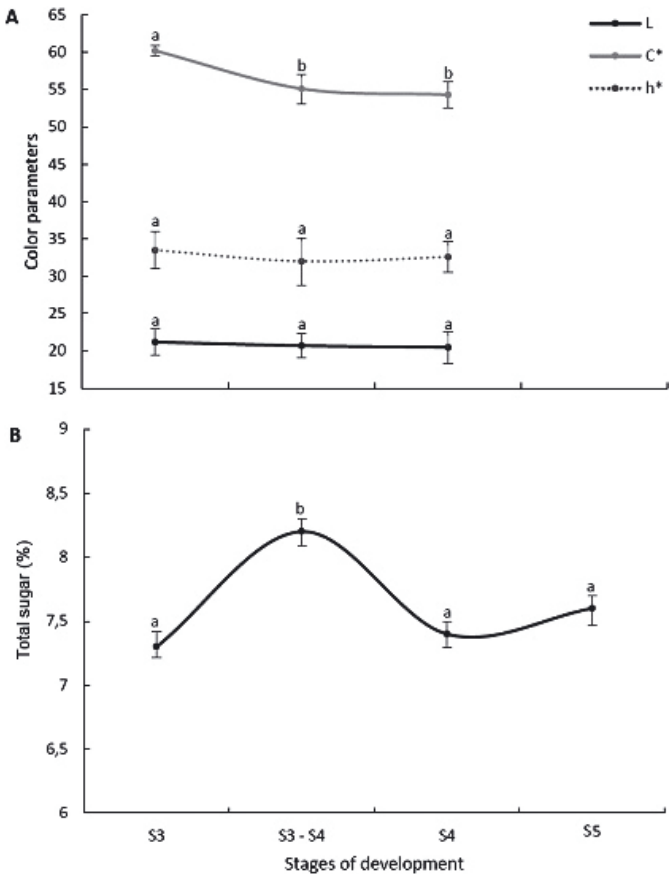

Figure 4. Changes in color (A) and total sugars (B) during fruit development in *Escontria chiotilla*. Each point represents the mean of 30 and 3 measurements for A and B, respectively ± SD. Different letters along curves indicate significant differences in the comparison of means by the Duncan's method ($\alpha = 0.05$).

complete as indicated by the weight, size and shape of fruit (*table I*). These data suggest, therefore, that fruit maturity began at S3.

Based on these results, we collected new fruits at the S3, S3-S4 (transition period), S4 stages, and over mature stage (S5) to better analyze the parameters associated with the process of ripening. The color measurements in these fruits indicated no difference in the values of L* (lightness) and h* (hue) between S3, S3-S4, or S4; nevertheless the fruits did exhibit a minor change in terms of C* (chroma or saturation), showing a more saturated color at S3 (*figure 4A*). These results allow us to exclude the surface color of the fruit as a reference parameter to define ripening stages of jiotilla fruit. In contrast, surface color has been useful in yellow pitaya (*Selenicereus megalanthus*) [19] and red pitaya (*Hylocereus polyrhizus*) [40] for the same purpose. Jiotilla immature fruit could show an external red purple color just as a consequence of environmental factors such as light and temperature.

The maximum concentration of total sugars occurred at the S3-S4 transition stage (8.2%), and non significant differences were observed among S3, S4 and over mature fruit (S5), which exhibited 7.3, 7.4 and 7.6%, respectively (*figure 4B*). Sugar content can vary with harvest time. In a previous year, we found average values of total sugars as low as 4.5, 5.1, 5.0 and 4.9% for S3, S3-S4, S4 and over mature, respectively. In spite of the significant difference in the level of total sugars between years, the behavior was the same in both years: the level increased from S3 to S3-S4 and then decreased. A similar behavior was reported for yellow pitaya by Nerd and Mizrahi (1998), at commercial maturity different cacti berries have the following content of total sugars: *Selenicereus megalanthus* 7% [19], *Hylocereus undatus* 10%, *H. polyrhizus* 9% [20], and *O. ficus-indica* 12–15% [27, 28, 39, 41].

The average values of TSS (9.0, 10.5, 8.0, and 10.5%) and TA (1.0, 0.8, 1.0 and 1.2%) in S3, S3-S4, S4, and S5 fruit, respectively, did not contribute to clearly differentiate any of these stages of development.

3.2 Harvest index based on physical and chemical parameters

The above results indicate that the most appropriate developmental stage for fruit harvesting is the S3-S4 transition since fruit at this stage has the highest content of sugars. Also, since it is less developed than S4, it additionally possesses a greater potential for postharvest life. We observed that jiotilla fruit harvested at stage 4, has a shelf life of 6 days when stored at 25 ± 3 °C, and of 9 days when stored at 7 °C. However, for the purpose of elaborating a practical and reliable harvest index, the color, TSS, and TA values of fruit – parameters easily measured in the field by producers – all failed to accurately identify this S3-S4 transition.

3.3 Morphological and histological parameters of flowers at anthesis

E. chiotilla flowers weighed an average of 3 g. The ovary itself was observed to be unilocular and contained at least 1,034 ovules calculated on the bases of the number of seeds counted per fruit. The amphytropous ovules, similar as three species of *Hylocereus* [42] have conspicuous funiculi. Also, it was noted that the combined pericarpel and wall ovary structure attained its maximum thickness at S0 (*table I*) as it was reported previously for other species [43–45]. The number of ovules is 270 in *O. ficus-indica* and 7,200 in *H. undatus* [18].

A cross section of a flower's pericarpel at anthesis, revealed the following three structures: a monolayered epidermis (*figure 5*, S0a), a multilayered parenchyma with some tannin – containing cells (*figure 5*, S0b), and a parenchyma with abundant calcium oxalate druse, that lied adjacent to the ovary locule. Inside this locule, developing ovules could also be observed (*figure 5*, S0c).

From flower until the last stage of fruit development, the pericarpel epidermal cells remained structured as a single layer, with an approximate thickness of 30 μm and a cuticle of 8–10 μm. Below this layer, larger cells of about 60 μm in diameter were observed belonging to the chlorophyllic parenchyma – the tissue responsible for giving the pericarpel its green color – which was composed of 3 to 4 layers. Many of these cells also contained a large quantity of

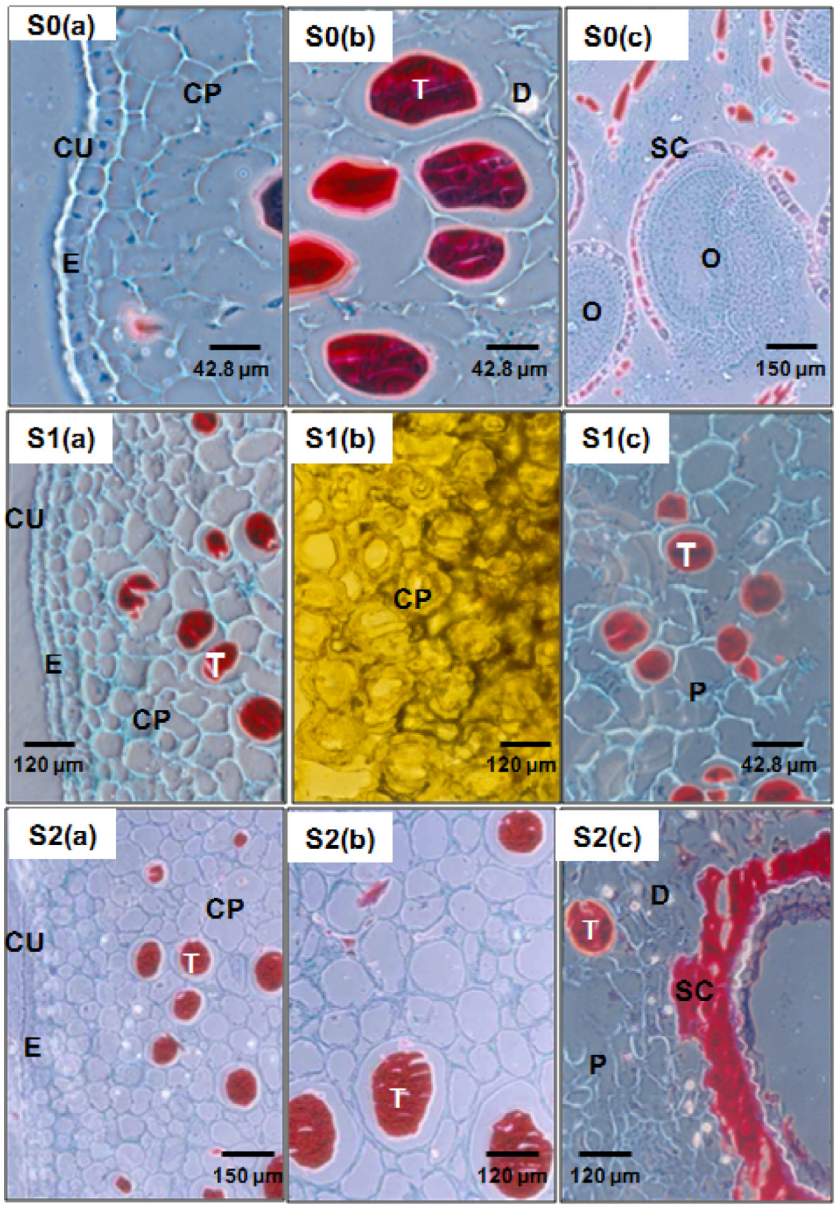

Figure 5. Cross sections of *Escontria chiotilla* fruit at different developmental stages. S0: Flower at anthesis; S1: At 4 weeks post-fertilization; S2: At 5 to 9 weeks post-fertilization. S0: a: Epidermis and chlorophyllic parenchyma; b: Parenchymal cells with tannins; c: Locule with ovules. S1: a: Epidermis and parenchyma with tannin inclusions; b: Parenchyma with thickened walls; c: Parenchyma near the locule with cell walls broken. S2: a: Epidermis and parenchyma with druse and tannins; b: Parenchyma with tannins; c: Parenchyma near the locule with druse and developing seeds. CU: Cuticle, E: epidermis, CP: chlorophyllic parenchyma, O: ovule, T: tannins, P: parenchyma, D: druse, SC: seminal cover.

plastids. An aqueous parenchyma was likewise present, which had large, vacuolated, as well as some tannin - containing, cells. This parenchyma harbored cells with eccentric nuclei, which were attached to the cell walls (*figure 5*, S0a), and the parenchyma that laid adjacent to the locule and was typified by the presence of calcium oxalate druse. Finally, a great quantity of amphytropous ovules could be found in the locule attached to the placenta by a series of conspicuous funiculi.

3.4 Fruit at 4 weeks after anthesis (S1)

This stage of development was typified by a slow increase in the weight and size of fruit (*table I*, *figure 3*). Cell multiplication was present in the pericarpel at all strata, but the epidermis remained structured as a monolayer (*figure 5*, S1a), and the cell walls of the chlorophyllic parenchyma were seen to thicken irregularly (*figure 5*, S1b). Additionally, parenchymal cells near the locule were small (25 µm), had cell walls that were generally thin, displayed persistent druse, and harbored condensed tannins which occupied most of the cell lumen (*figure 5*, S1c).

There was also an intense proliferation of cells in the funicular tissues of seeds, particularly those of the funicular epidermis, which subsequently gave rise to the fruit pulp, just as it occurs in other cacti [11,18,29]. The percentage of fruit pulp

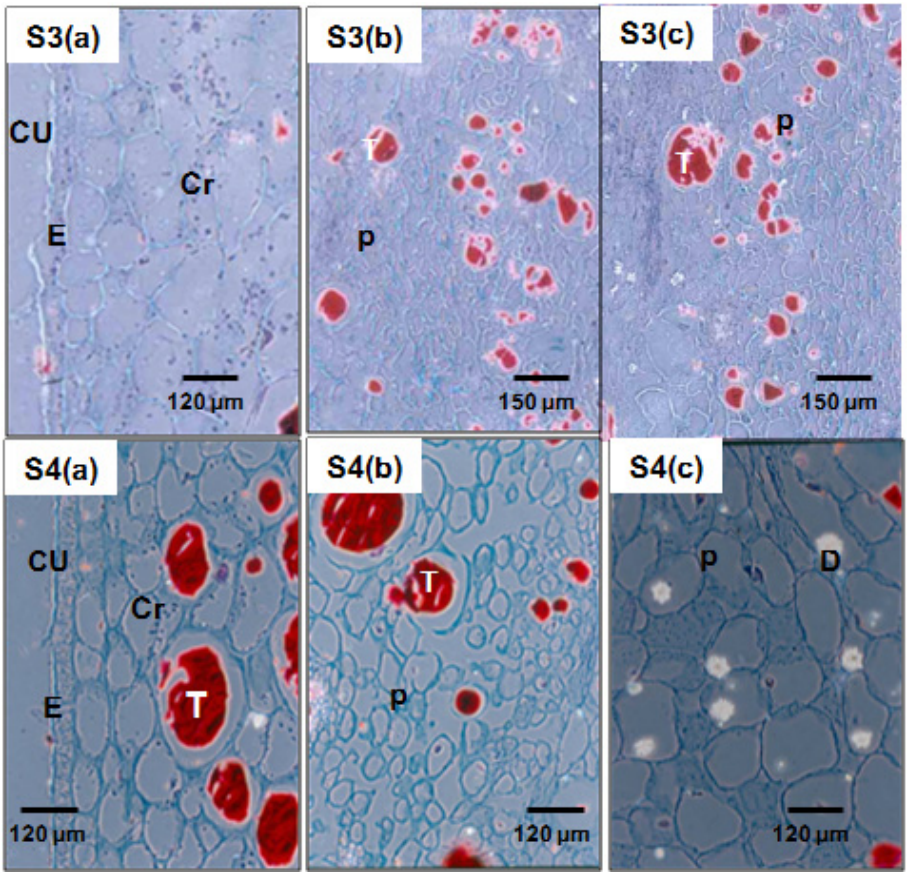

Figure 6. Cross sections of *Escontria chiotilla* fruit at the S3 (fruit of 10–12 weeks) and S4 (fruit after 12 weeks) stages of development as observed through phase contrast microscopy. S3: a: Epidermis with cuticle and chlorophyllic parenchyma, b: Parenchyma with tannin inclusions, c: Parenchyma adjacent to pulp. S4: a: Epidermis and parenchyma with chromoplasts and tannins, b: Aerenchyma before the compression (or collapse) which reduces the thickness of the fruit rind, c: Druse inclusions in the parenchyma adjacent to the pulp. CU: cuticle, E: epidermis, P: parenchyma, D: druse, T: tannins, Cr: chromoplasts.

at this stage was 20.6% (*table I*, *figure 3*) and 79.4% is peel. Firmness was 62.27 N.

3.5 Fruit at 5–9 weeks after anthesis (S2)

Fruit at this stage almost doubled in weight, but the percentage pulp remained low (*figure 3*), and the dark green coloration of the pericarp began to fade (*table I*). Cells of the aqueous parenchyma increased in volume, reaching an average size of 100 µm and they were able to retain the tannins present in their cytoplasm (*figure 5*, S2b). Thus, firmness reaches its maximum value 68.2 N. Finally, the parenchymal cells adjacent to the locule began to lyse, leaving behind many empty intercellular spaces (*figure 5*, S2c).

3.6 Fruit at 10–12 weeks after anthesis (S3)

There was an increase of about 150% in the weight (*table I*) and of 110% in the pulp (*figure 3*) of fruit at S3 compared with fruit at S2, and the polar and equatorial diameters of the former increased significantly (*table I*). There was also an accumulation of pigments, reported as betalains [40, 41, 47], in the parenchyma lying adjacent to the epidermis, giving fruits their characteristic purple color (*figure 1* and *figure 6*, S3a). Additionally, the intercellular spaces of the aerenchyma –a parenchymatous tissue type having large intercellular spaces formed by lysogeny– were observed to increase as a result of the expansion of some cells and the lysis of others (*figure 6*, S3b), and the pericarpel thickness was noticeably reduced (table I; *figure 2*). Thus, fruit firmness decreased slightly to 57.8 N. The intercellular space between parenchymal cells lying close to the fruit pulp became increasingly larger, ranging between 60 and 100 µm, and the druse that were observed at earlier stages of development remained present (*figure 6*, S3c). The locule, on the other hand, ceased to be perceptible as that space gradually became occupied by the pulp and the developing seeds.

An increase in the volume of funiculi made more apparent their participation in the formation of the fruit pulp, which by this stage reached 61% (*table I*, *figure 3*). Also, the appearance of a purple coloration in these cells after 10 weeks of development was observed to be the result of an accumulation of betalains [28], which by the end of S3 were responsible for making this the predominant color of the fruit pulp. In *Hylocereus*, a high correlation between color surface

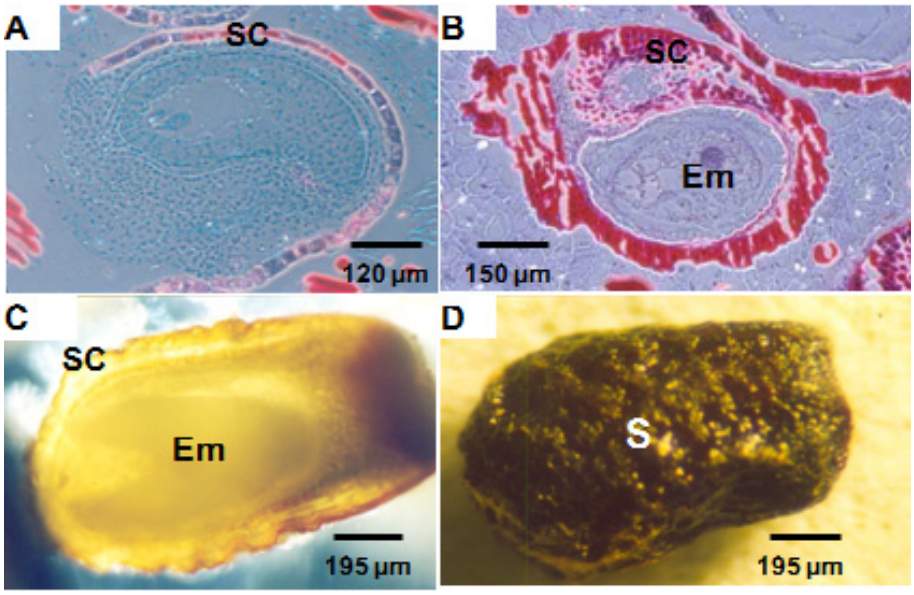

Figure 7. Seed development in the fruit of *Escontria chiotilla*. A) Ovule; B) Seed with globular embryo; C) Heart-shaped seed embryo; D) Mature seed. T: tannins, SC: seminal cover, Em: embryo, S: seed.

a concentration of betalains in the peel and pulp were reported (R^2 = 0.99) [20, 40]. However, it was not unusual for the pulp to still retain, in a few places, some shades of white (*table I*, *figure 1*). Lastly, seed development was asynchronous, and an increasing number of them exhibited a dark seed coat (*figure 7*).

3.7 Fruit over 12 weeks after anthesis (S4)

Fruit became fully mature after 12 weeks (*figure 6*, S4), reaching a maximum weight of almost 20 g on average; in addition, they also attained their maximum polar and equatorial diameters (*table I*). In pericarpel tissues, a series of major changes were characteristic of the transition to S4:

- In epidermal cells, the cuticle reached a maximum thickness of approximately 15 µm (*figure 6*, S4a).
- The aqueous parenchyma now transformed into an aerenchyma collapsed (*figure 6*, S4b). This event could be perceived at first glance as a noticeable thinning of the fruit rind.
- Then the aerenchyma, normally white and spongy, was compressed to < 2 mm and as a result, the thickness of the pericarpel was reduced to a minimum (*table I*).
- In the parenchyma that lies adjacent to the outer pericarpel epidermis there was an increase in the pigments that give the fruit their deep-purple color (*figure 6*, S4a; *table I*).
- The pulp represented, on average, 68% of the fruit (*table I*) and the accumulation of pigments reached its maximum.
- The number of druse present in tissues adjacent to the pulp noticeably increased (*figure 6*, S4c). The firmness decreased to 44.5 N at a similar stage of development in *H. undatus*, and the *H. polyrhizus* firmness reported was 23.5 N and 26.4 N, respectively [20].

3.8 Seeds

Mature fruit contained 1, 034 ± 285 seeds of a shiny-black color that varied in length from 1.0 to 1.2 mm. During seed development, the seed coat hardened as a result of the accumulation of lignin in the cell walls, eventually constituting the sclerenchyma. There was also an accumulation of tannins, which were responsible for the color change from reddish brown to shiny black (*figure 7*) and the outer epidermis produced a striated cuticle (*figure 7D*). Most seeds reached full development at S3; however, the histological changes associated with this stage cannot be taken as indicative of harvest maturity given the asynchronous development that characterizes the seeds of jiotilla.

3.9 Bracts

The number of bracts on the pericarpel ranged 35–40 per fruit, and remained constant throughout the development from flower to mature fruit. Bracts were vascularized and measured approximately 0.9 cm in width and 1.2 cm in length. Additionally, they consisted of two types of epidermis: abaxial and adaxial, with parenchymal tissue in between. Cell disruption and death occurred with each subsequent developmental stage, so that by S4 the only living cells present were located in the bract base (*figure 2*, *8*).

SEM micrographs revealed a gradual accumulation of tannins in bract tissue, which conferred them strength but diminished flexibility as fruit development proceeded. Because of the cell expansion that occurred during pericarpel growth longitudinal fractures appeared in bract tissue beginning at 10−12 weeks after anthesis.

The presence of papery bracts is a specific attribute of jiotilla not present in the fruit of any other cacti [46, 47]. These

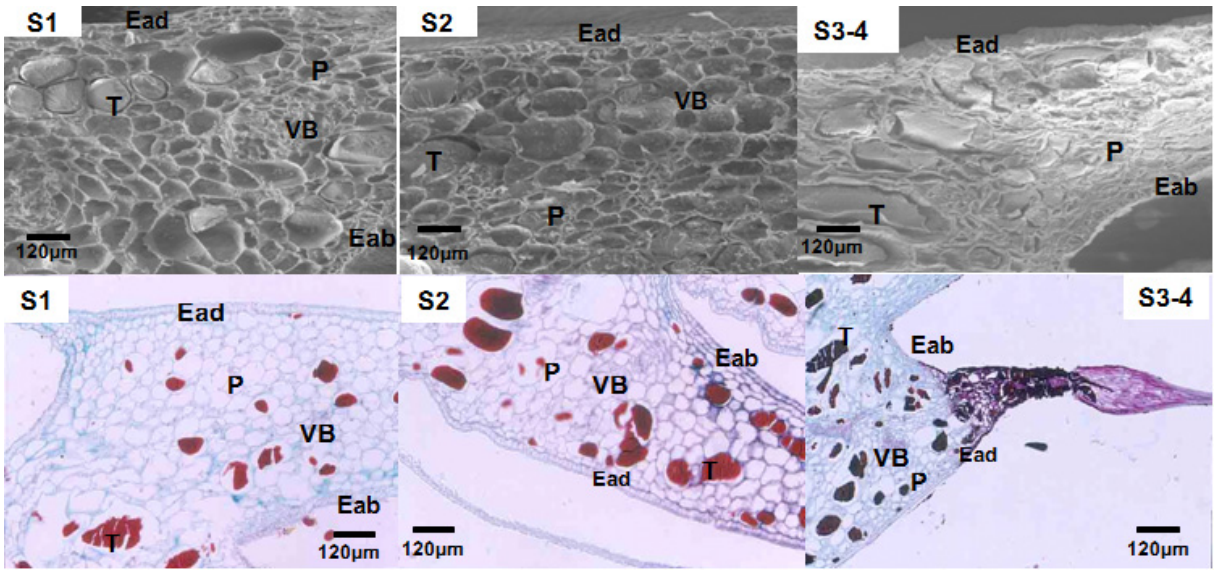

Figure 8. Cross-sectional anatomy and morphology of bracts of *Escontria chiotilla* at the S1, S2, and S3-S4 stages of development as observed through scanning electron microscopy. Shown are the changes in the morphology of cells as a result of the expansion of the fruit surface. Eab: abaxial epidermis, Ead: adaxial epidermis, T: tannin, P: parenchyma, VB: vascular bundle.

structures have the potential to act as discontinuous films reducing dehydration and the incidence of mechanical damage during postharvest handling. This protection would be persistent because bracts never detach from the peel of fruit unless mechanical techniques are applied.

3.10 Harvest index based on morphological and histological parameters

When non-climacteric fruit are harvested prematurely, they fail to mature, have poor aroma, and possess an acidic as well as an overall undesirable taste. In *E. chiotilla*, fruit mature asynchronously so that it is possible to find, even on the same plant, a full range of developmental stages, from floral bud to mature fruit. Both harvesters and consumers can easily detect the morphological and histological indicators of harvest proposed in this paper. These are: 1) a thinning of the fruit peel, which occurs in the transition period S3-S4 (*figure 2D*) and is produced by a compression (collapse) of the aerenchyma, and 2) a change in the coloration of the bracts, which acquire a brownish tone and become brittle, thus indicating the proper timing for fruit harvest. Firmness is the mechanical parameter more closely associated with the process of maturation, and also it could be useful as a harvest index.

Other morphological parameters, such as the change in fruit shape (fullness of the fruit) that results from an increase in the equatorial diameter (*figure 1*) and the dark color of seeds can be used as auxiliary indicators of harvest.

4 Conclusions

The stem structures, which are adapted to confine, protect and improve the aqueous reserves (aqueous parenchyma) in the ovary, are one of the successful traits of the cactus species. Its berry becomes a special fruit with quite a successful strategy for saving water: the liquid can move from the pericarpel, originally an adapted structure of the stem, to the ovary wall, and from both to the growing pulp. By only measuring the water content of the fruit it would be difficult to explain why the total content of water does not change much during fruit development as we observed in jiotilla. The total content of water of the fruit cannot be used as a harvest index because it remains uniform and balanced throughout long periods of development – an exquisite adaptive trait to survive in arid lands. However, when fruits initiate the ripening process there is no reason to maintain stored water in the peel and by that time the aqueous parenchyma is transformed into aerenchyma, and the excess of water translocated to the growing pulp. As the pulp grows, the aerenchyma is compressed with the subsequent change in fruit texture. Thus a good harvest index can be based on the decrease of fruit firmness and peel thickness.

Some additional observations complete the astonishing adaptive traits of cactus fruit to drought. It has been reported that rainfall is associated with an increase in peel weight [27] and that ripened fruits can remain on the plant for several months waiting for agents to disperse their seed as observed in jiotilla. What else could be a better reward than water and sugar in the desert land for the animals? This is an important characteristic of cactus fruit that ensures seed dispersion. *Escontria chiotilla* fruit have neither spines nor glochids, unlike *Opuntia ficus-indica* berries, but they have an additional mechanism to reduce water loss: persistent scales or papery bracts, a unique trait in the Cactaceae family, which can be also used as a harvest index. These advantages establish *E. chiotilla* as an endemic and dominant species in the desert shrub of the Tehuacan Valley in Mexico.

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