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RESEARCH ARTICLE

Inter- and intra-specific variation in fruit biomass, number of seeds, and physical characteristics of seeds in *Opuntia* spp., Cactaceae

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Abstract Domestication is an evolutionary process that culminates with the origin of individuals or populations that are morpho-physiologically, chemically and genetically different from their wild, congeneric relatives. The aim of the present study was to quantify the inter- and intra-specific variation in fruit biomass, number of seeds, and physical characteristics of seeds in *O. streptacantha* Lem., *O. hyptiacantha* F.A.C. Weber, *O. megacantha* Salm-Dyck, *O. albicarpa* Scheinvar and *O. ficus-indica* (L.) Mill., the least, intermediate, and most highly domesticated species. The hypothesis was that selecting larger fruits with fewer and softer seeds (normal and aborted) during domestication reduced the interspecific variation in fruit biomass, number of seeds, and physical characteristics of seeds in *Opuntia* spp., Cactaceae. For fruits of 89 variants of the Southern Mexican Plateau, total biomass and biomass of pulp with seeds was quantified; seed length, width, thickness and dehydrated mass were measured, as well as hardness of normal seeds. The total number of seeds was quantified and normal and aborted seeds were counted separately. A randomized design, with 89 treatments (variants) and six replicates and a fruit as experimental unit per treatment was used. The data were analysed by ANOVA, Tukey's multiple comparison test ($p \leq 0.05$), multivariate ordination (principal components analysis) and classification (clustering analysis). Inter-specific analysis indicated that fruits of the most highly domesticated variants (*O. albicarpa* and *O. ficus-indica*) had higher biomass ($p \leq 0.05$), followed by those of *O. hyptiacantha* and *O. megacantha* and wild variants of *O. streptacantha*, and non-significant differences existed in seed thickness or hardness. Fruit and pulp biomass were positively and significantly correlated with seed biomass. Species with greater domestication clustered

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according to their larger fruits ($\geq$117.83 g) and the greater number of aborted seeds ($\geq$65). Domestication of *Opuntia* has also modified seed dimensions and increased the number of aborted seeds per fruit ($\leq$11 to $\geq$65).

Keywords Domestication · Fruit biomass · *Opuntia* seeds · *Opuntia* species · Physical characteristics of seed · Prickly pear

Introduction

Human-induced changes in biota reflect an evolutionary process defined by natural and artificial selection; this process begins with the selection of certain wild individuals or populations for their desired characteristics, and it results in genetic and morphological attributes that differentiate the new individuals from their wild relatives (Pickersgill 2007; Perales and Aguirre 2008). This process is gradual and continuous, and its speed and depth depend on the species (Perales and Aguirre 2008) and on the level of interaction with humans. The combination of morphological, physiological, biochemical and genetic differences between domesticated species and their wild congeneric relatives constitutes the domestication syndrome; in some cases, the ability of the domesticated species to survive in natural environments decreases (Zohary 2004). The attributes that define the domestication syndrome include diverse modifications, such as a decrease in dispersal capacity or in the number of viable seeds, enlargement of organs of interest to humans, modification of sexual reproduction, a decrease in seed latency capacity, and loss of chemical or mechanical protection against herbivores (McKey et al. 2010; Peña-Valdivia et al. 2012; Pickersgill 2007).

Opuntia and *Mammillaria* are the genera with the greatest diversity and widest distribution in the family Cactaceae; *Opuntia* includes 188 species, 78 of which, with both wild and cultivated variants, are native to Mexico (Anderson 2001). Continuous and systematic gathering of *Opuntia* organs of interest to humans has favored the persistence of variants with "nopalitos" (edible cactus pads or cladodes), fruits, or both, with exceptional characteristics, likely because from the start of their humanization variants with these traits were tolerated, favored, or cultivated (Reyes-Agüero et al. 2009).

Measuring degree of domestication within and between species is already very problematic. Reyes-Agüero et al. (2005) were able to identify the level of domestication based on a multivariate analysis of 42 morphological characteristics of the plants, cladodes, nopalitos and fruits of 483 accessions. The cited authors determined that *O. streptacantha* Lem. represented the wild end of the spectrum, followed by *O. hyptiacantha* F.A.C. Weber and *O. megacantha* Salm-Dyck, with an intermediate level of domestication. On the other end of the spectrum, with the highest level of domestication, were *O. albicarpa* Scheinvar and *O. ficus-indica* (L.) Mill. Among the changes that occurred with domestication, these authors identified an increase in pulp sweetness and fruit size, a decrease in the number and hardness of seeds and a change in the proportion of aborted seeds per fruit.

In *Opuntia* genera also an inter- and intra-specific physiological and chemical variability has been observed. García-Nava et al. (2014) evaluated nopalitos of 15 variants of five *Opuntia* species sampled from plants without irrigation for 60 days; much bigger differences were present when they compared turgor potential (fivefold), osmotic potential (up to 55 %), membrane permeability (36 %), firmness (20 %) and tissue water potential (Ψ_W) (12 %) among wild variants of *O. streptacantha*, and this heterogeneity widely contrasted with the homogeneity among cultivars of *O. ficus-indica*. Similarly, López-Palacios et al. (2012) contrasting the content of structural polysaccharides in nopalitos of 14 variants of five species of *Opuntia* documented the greatest differences in mucilages (up to 50 %) within wild variants of *O. streptacantha* and *O. hyptiacantha* than in domesticated species. Meanwhile in a study regarding the polysaccharides and dietary fiber abundance in the most consumed Mexican cultivars of nopalitos, including 10 cultivars of *O. ficus-indica* (L.) Mill., significant and wide variation were observed among the cultivars in structural polysaccharides: mucilages [from 3.8 to 8.6 % dry matter (DM)], pectins (from 6.1 to 14.2 % DM), loosely bound hemicelluloses (from 4.3 to 10.7 % DM), tightly bound hemicelluloses (from 2.2 to 4.7 % DM), cellulose (5.0 to 14.0 % DM), soluble fiber (16.0 to 30.0 % DM), and insoluble fiber (7.8 to 16.2 % DM) (Peña-Valdivia et al. 2012).

In *Opuntia*, the effect of domestication on the morphological characteristics of the seeds has been partially documented (Aguilar-Estrada et al. 2003). The interest in seeds arises due to its relationship with aspects of fruit quality for human consumption (Aguilar-Estrada et al. 2003; Barbera et al. 1994). In an analysis of the Italian cultivars of *O. ficus-indica*, Gailla and Rossa, the size of the fruit depended on the number and weight of seeds per fruit. Pulp weight was positively and significantly correlated with the number of seeds, and the ratio of normal to aborted seeds was constant, independent of their total number in the fruits (Barbera et al. 1994). Aguilar-Estrada et al. (2003) documented that the *Opuntia* fruits have, on average, 229 seeds, of which 186 are normal and 43 are aborted. However, these attributes varied across species, variants, cultivars, and conditions during plant development (Barbera et al. 1994). The pulp of the prickly pear develops from the outside of the epidermal cells of the funicular wrapping and the funiculus of both types of seeds (Pimienta and Engleman 1985), and the pulp content depends on the number of seeds in the fruit. However, in cultivars for fruit production, preferred variants have few, small seeds, with a high abundance of aborted seeds (Aguilar-Estrada et al. 2003; Barbera et al. 1994).

The aim of the present study was to quantify the inter- and intra-specific variation in fruit biomass, number of seeds, and physical characteristics of seeds in *O. streptacantha*, *O. hyptiacantha*, *O. megacantha*, *O. albicarpa*, and *O. ficus-indica*, this identified as the most highly domesticated species. The hypothesis was that selecting larger fruits with fewer and softer seeds (normal and aborted) during domestication reduced the inter-specific variation in fruit biomass, number of seeds, and physical characteristics of seeds in *Opuntia* spp., Cactaceae.

Materials and methods

Botanical material was obtained from commercial plantations, experimental prickly pear plantations, home gardens and wild nopal plants populations in 29 localities of the Southern Mexican Plateau as reported by Reyes-Agüero et al. (2005). Six fruits of 89 variants of five species with extreme and intermediate levels of domestication were collected; the number of variants accessions per species was variable (Table 1). The passport information of these accessions is provided in Supplementary Table S1.

For fruits, total pulp with seeds and seed-only biomass were determined; the percentages of pulp, seeds and skin were also measured. The total number of seeds per fruit and the number of normal versus aborted seeds were quantified; for normal seeds, dehydrated mass, length, width, thickness and hardness were quantified. For these measurements, seeds were separated from the pulp with the help of a domestic blender with blunt blades. The pulp was mixed with 1 L of water, and the blades were activated for 45 s at low speed. The jar contents were poured through a strainer with 48 holes/cm^2. To eliminate the remains of funicular wrappings, the strainer with seeds was put under running water. Seeds were then set in Petri dishes and were left to dry at room temperature in the laboratory (26 ± 2 °C) for 3 days. Dry seeds were kept in paper envelopes at the same temperature.

Fruit and pulp biomass were quantified using a beam balance (Precisa model XB2200C, Switzerland, with ±0.1 g precision). Dry seed biomass was measured with an analytical balance (Scientech model SA120, USA, with ±0.0001 g). Seed dimensions were measured with a caliper (Caliper-Mitutoyo, Japan; digital, with ±0.0001 cm precision); hardness was quantified, with six repetitions per fruit, by the energy necessary to compress each seed to 50 % of its thickness with an universal test machine (Instron Model 1000, USA) and with a load cell of 4.9 kN (500 kg_f).

A completely randomized experimental design was used to assess the 89 treatments or nopal variants, with six fruits as experimental unit. Frequency distribution of fruit size and seed biomass and of size and hardness of seeds were tested for normality based on the graphic residual analysis and the Shapiro-Wilks test with the InfoStat software (Version 2011e) (Balzarini et al. 2008; Di-Rienzo et al. 2011). Variables were transformed when the normality assumption was not met. The data were analyzed with ANOVA, Tukey's multiple comparison test, Pearson's correlation and multivariate Principal Components Analysis (PCA), and clustering (mean distance method). Statistical analyses were performed with the SAS statistical package (version 9).

Results and discussion

Fruit biomass

The range in fruit biomass for the five species included in this study was between 30 and 230 g. The least variable fruits were from *O. ficus-indica* and

Table 1 Nopal species (*Opuntia* spp.), variants and number of accessions (N.A.) included in the study

Species and variants	N.A.	Species and variants	N.A.	Species and variants	N.A.
O. streptacantha Lem.		Chamacuero Monteza	1	Bola de Masa	3
Burra	1	Chirriona	4	Burro	4
Cardón	1	Cuervo Tuna	2	Burrona	5
Cardón Potosino	2	Jagüeño	4	Copa de Oro	11
Charola	2	Jarilla	2	COPENA T12	10
Demshikäjä	5	Jarilla Grande	6	Cristalina	7
Isbiní	1	Jarrilla Käjä	4	Cristalino	5
Jocoquillo	6	Manso Apastillada	4	Dadokäjä	4
Sandía	2	Mieluda	3	Esmeralda	5
Santo Tomás	3	Morada de San Martin	6	Fafayuco	3
Trompa de Cochino	1	Naranjona	2	Gavia	5
		Naranjona Dulce	1	Mango	2
O. hyptiacantha F.A.C. Weber		Naranjona Helia	2	Octubreña	3
Amarilla 24	1	Oshikäjä	1	Papantón	8
Blanca Victoria	1	Pico Chulo	7	Papino	2
Camueso	2	Reventón	11	Reyna	4
Cardón Blanco	5	Roja Saeta	1	Reynita	1
Cardón de la Papas	1	Rojo 10	3		
Jaqueña	5	Rubí Reyna	5	*O. ficus-indica* (L.) Miller	
Jokjä	1	Sangre	1	Copena F1	3
Ladrillo	1	Sangre de Toro	1	Amarilla Milpa Alta	5
Nistokäjä	2	Sangrita	1	Amarillo Huevo	2
Pachón	5	Sgt-INIFAP	1	Atlixco	1
		Tonikäjä	5	Camuesa	2
O. megacantha Salm-Dyck		Torreoja	1	Doctor Mora	4
Amarilla China	1	Tzebekäjä	4	Liso	4
Amarilla Mansa	2			Liso Blanco	1
Amarilla Monteza	2	*O. albicarpa* Scheinvar		Liso Forrajero	13
Amarilla Naranjona	3	Amarilla Olorosa	1	Promotora	1
Amarilla Raleña	1	Amarilla Pera	5	Solferino	7
Apastillada Anaranjada	2	Anaranjado	2	Telokäjä	6
Astikäjä	1	Blanca	4	Telokäja Roja	6
Bola de Masa	5	Blanca Chapeada	3		

O. albicarpa (CV = 25.73 and 26.11 %, respectively). The frequency distribution of fruit sizes was also different among species and was only symmetrical around the mean for *O. megacantha* and *O. albicarpa*. In *O. ficus-indica*, the distribution was biased towards larger fruits with peaks at 110, 130 and 170 g. Biomass variability in *O. hyptiacantha* fruits was high (CV = 42.08 %), without dominance of any one size (Fig. 1A–E).

The intra-specific differences in fruit biomass were significant ($p \leq 0.05$). In all species, the range within variants represented approximately 50 % of the biomass (Fig. 2A). These differences were greater in *O. megacantha* and *O. albicarpa*, as reflected in their frequency distributions.

The inter-specific mean biomass of fruits was also significant ($p \leq 0.05$; the ANOVA analysis information is provided in Supplementary Table S2). The

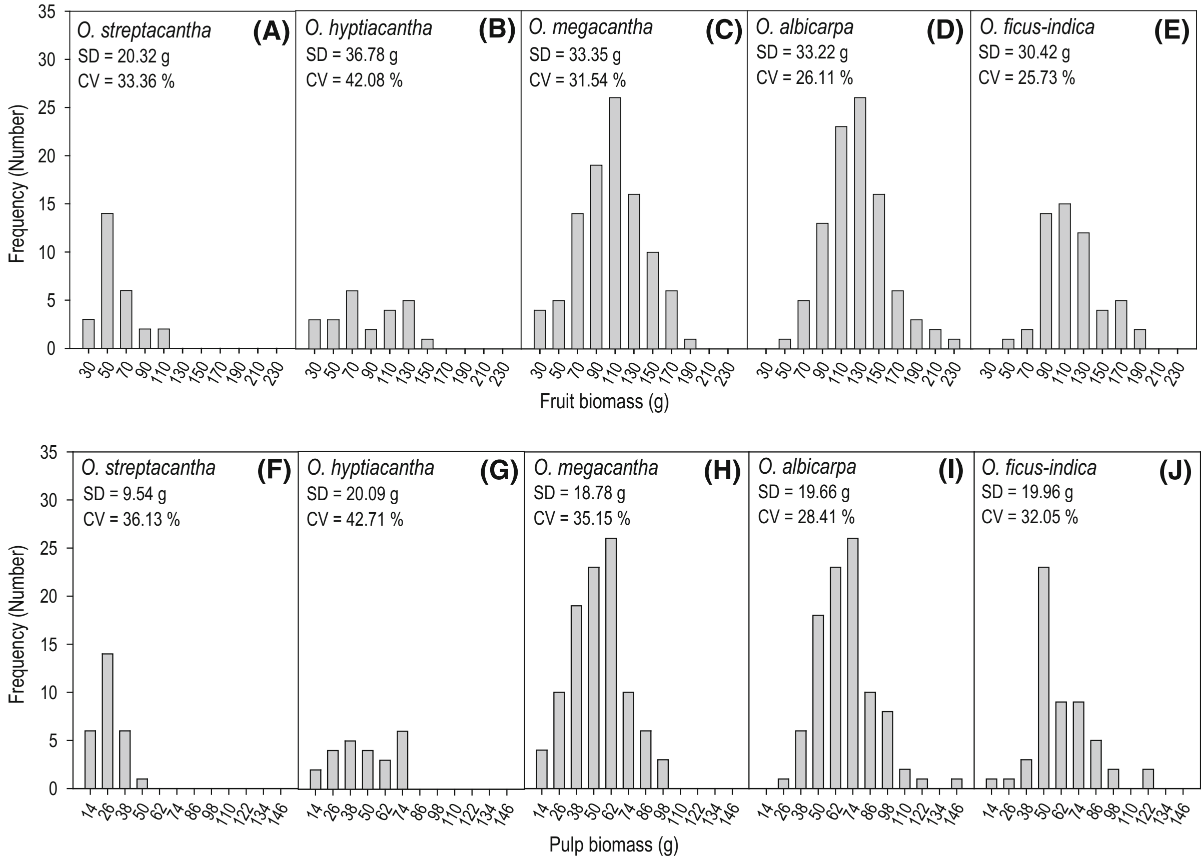

Fig. 1 Frequency distribution of fruit biomass (**A–E**) and fruit pulp (**F–J**) of five species of *Opuntia*. *SD* standard deviation, *CV* coefficient of variation

more domesticate species *O. ficus-indica* and *O. albicarpa* had the fruits with the highest biomass out of the five species, twice that of the fruits of *O. streptacantha*, the smallest fruits (Fig. 3A).

Reyes-Agüero et al. (2009) recorded the range in biomass of *O. streptacantha*, *O. hyptiacantha* and *O. megacantha* fruits (from 23.5 to 116.4, 23.7 to 199.9, and 29.8 to 256.4 g respectively), and the values found in this study fall within these ranges (Figs. 1A–C, 2A, 3A). In contrast, Parish and Felker (1997) found that the fruits of the same species, belonging to variants with commercial value, showed greater biomass (117 and 160 g) (Fig. 3A).

The differences between values in the literature and those found in the present study (Fig. 3A) might have occurred due to the diversity of the variants and the growing conditions before harvest. The weight of *O. ficus-indica* fruits is affected by the time of sprouting of the flower bud and the number of fruits in the cladode. Therefore, the buds that sprout earlier generally form fruits with higher biomass than the cladodes in which only six fruits develop (Inglese et al. 1994; Wessels and Swart 1990).

Opuntia ficus-indica is the most studied and documented species due to its worldwide distribution and high level of domestication (Barbera et al. 1992; Griffith 2004), which is also reflected in its fruit biomass. In fact, Reyes-Agüero et al. (2005), based on a sample of fruits from 72 cultivars in 17 localities from eight states of Mexico, recorded a range in biomass between 86 and 146 g, but there were fruits with only 45 g (a traditional variety without seeds) and up to 223 g. In some cultivars, such as Amarilla Huesona, Amarilla Naranjona and Rojo Pelón, fruits ranged between 114 and 143 g (Pimienta-Barrios 1994); in Gialla and Rosa, they ranged from 135 to

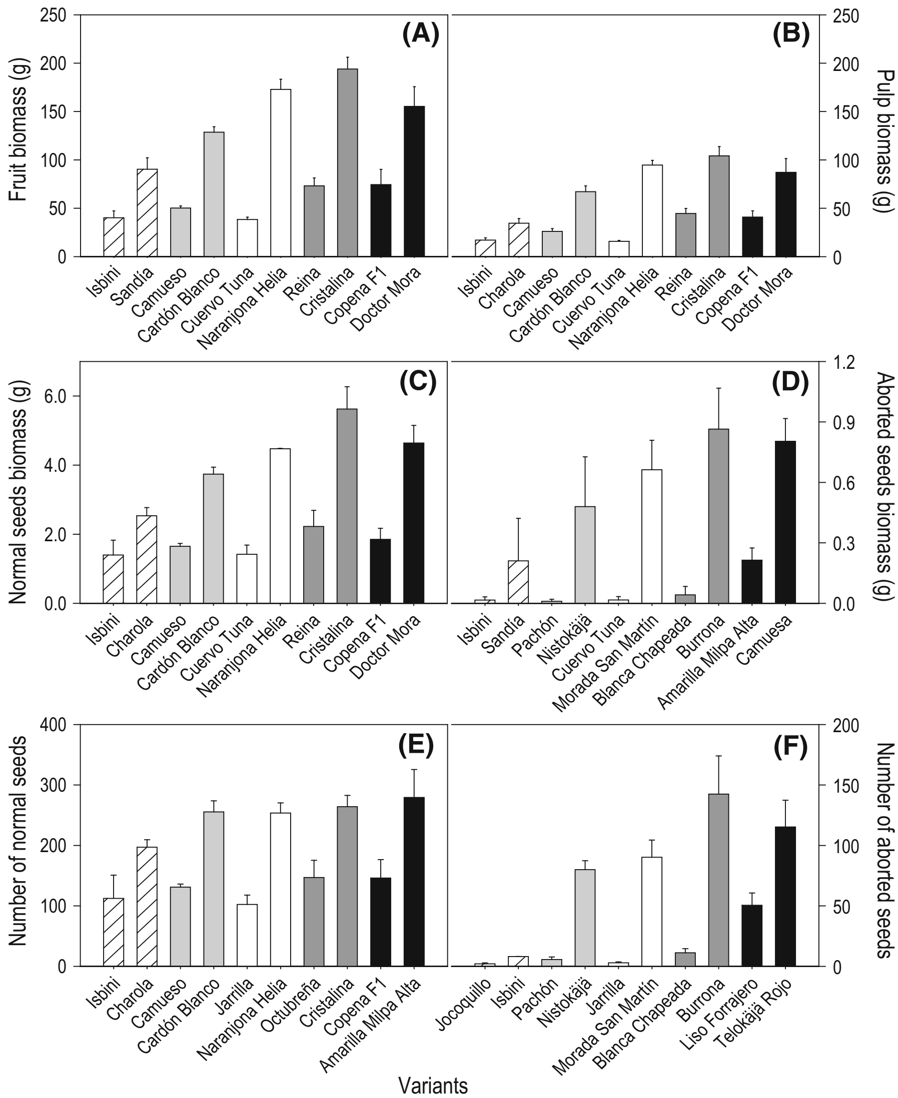

Fig. 2 Two variants with the lowest and highest fruit biomass (**A**), pulp biomass (**B**), normal seed biomass in fruit (**C**), aborted seed biomass (**D**), number of normal seeds (**E**), and number of aborted seeds (**F**) (+SE) of five species of *Opuntia*. *Opuntia streptacantha* Lem. (*hatch pattern*), *O. hyptiacantha* F.A.C. Weber (*light grey*), *O. megacantha* Salm-Dyck (*white bars*), *O. albicarpa* Scheinvar (*dark grey bars*), and *O. ficus-indica* (L.) Mill. (*black bars*)

146 g (Barbera et al. 1994; Schirra et al. 1999) and between 114 and 152 g (Parish and Felker 1997), respectively; in harvests from Argentina, fruits weighed between 112 and 212 g (Felker et al. 2005); in Tenerife Island, Spain, they weighed 98 g (Díaz-Medina et al. 2007), and in Italy, commercial fruits weighed between 80 and 160 g (Inglese et al. 1995). This variation in fruit biomass in *O. ficus-indica* was also recorded in cultivars of the present study (Figs. 2A, 3A).

The results shown (Figs. 1A–E, 2A, 3A; the ANOVA analysis information is provided in Supplementary Table S3) also support the hypothesis of a direct relationship between fruit size and domestication, and confirm records from other authors (Barbera et al. 1992; Díaz-Medina et al. 2007; Felker et al. 2005; Inglese et al. 1995; Parish and Felker 1997; Reyes-Agüero et al. 2005) who reported that the fruits of *O. albicarpa* and *O. ficus-indica* are larger than those of wild or less domesticated species, such as *O. streptacantha*. The increase in fruit size is one of the effects associated with selection by humans (Colunga-García et al. 1986; Reyes-Agüero et al. 2005) and is part of the domestication syndrome in many other species. It has been postulated that the differences in the sizes of fruits between cultivated and wild species are due to differing ploidy (Pimienta-Barrios 1994), but natural extreme fruit size variation, hybridization, and mutations are also

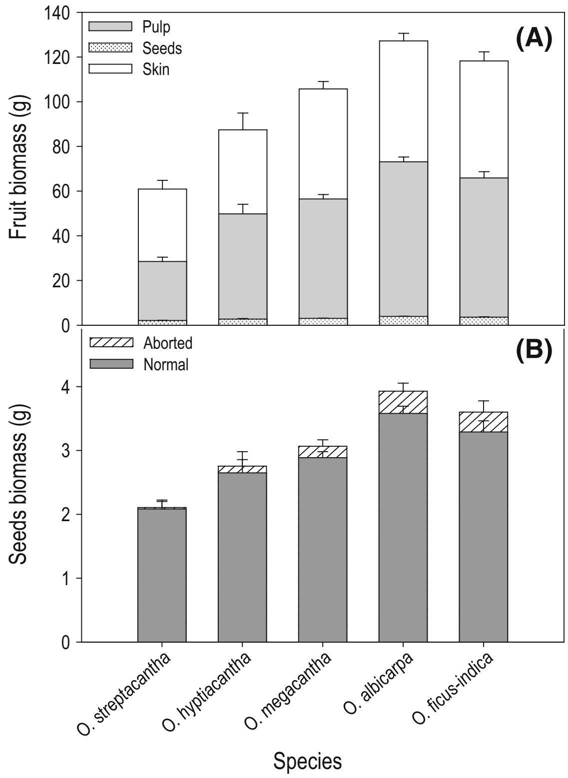

Fig. 3 Fruit biomass (**A**) and seed biomass in fruit (**B**) (+SE) of *Opuntia* species

possible in *Opuntia* genus. During humanization, phenotypes with vigorous cladodes and larger fruits were probably identified and cultivated in home gardens and cultivated parcels in semiarid regions (Colunga-García et al. 1986; Figueroa et al. 1980; Pimienta-Barrios 1994). Therefore, these populations persisted in anthropic environments and contributed to the diversity in fruit size and shape.

Fruit pulp biomass

The range in fruit pulp biomass in the five species was 14–146 g. The fruits of *O. albicarpa* and *O. ficus-indica* were the least variable (CV = 28.41 and 32.05 %), and those of *O. hyptiacantha* showed the greatest variability (CV = 42.71 %). The frequency distributions of pulp biomass, similar to those of fruits, were different between species; it was symmetrical around the mean only in *O. megacantha*, and in *O. hyptiacantha* it had two peaks. The range in *O. streptacantha* was 13–49 g with a mode of 26 g; in *O. megacantha*, *O. albicarpa* and *O. ficus-indica*, the ranges were from 14 to 98, 26 to 146 and 14 to 122 g, and the modes were 62, 74, and 50 g, respectively (Fig. 1F–J).

Intraspecific variation in pulp biomass showed significant differences ($p \leq 0.05$). In *O. streptacantha*, fruits of the variety Isbini showed the lowest amount of pulp, and those of the Charola variant doubled it. Cardón Blanco of *O. hyptiacantha* contained twofold of pulp amount than Camueso. The largest contrast was between variants of *O. megacantha*, Cuervo Tuna and Naranjona Helia, which had almost five times more pulp. In *O. albicarpa*, Reina had significantly less pulp than Cristalina. The extremes in pulp weight cultivars of *O. ficus-indica* were COPENA F1 with the lowest content and Doctor Mora with the highest content. These results indicate that the range in pulp content increased: 23, 52, 100, 76 and 58 % in *O. streptacantha*, *O. hyptiacantha*, *O. megacantha*, *O. albicarpa* and *O. ficus-indica*, respectively (Fig. 2B).

The mean biomass of fruit pulp was different between species, from 26 g in *O. streptacantha* to 62 and 69 g in *O. albicarpa* and *O. ficus-indica* (Fig. 3A). In contrast, it expressed as percentage of fruit biomass was lower ($p \leq 0.05$) in *O. streptacantha* and not significantly different from the remainder of the species ($p > 0.05$; Table 2).

There is limited information on pulp content in fruits of *O. streptacantha* and *O. hyptiacantha*. The whole fruit weight (71 and 76 g) and pulp content (52 and 47 % pulp) estimated by Parish and Felker (1997) was greater than in the present study. In contrast, Reyes-Agüero et al. (2009) recorded fruit pulp percentages between 32.7 and 46.9 % for *O. streptacantha* and between 46.1 and 54.8 % for *O. hyptiacantha*, values similar to that recorded in the present study (Table 2).

Fruit pulp content in *O. megacantha* (Fig. 3A) was also lower than that of certain commercial variants (between 72 and 79 g) and those assessed by Parish and Felker (1997), but it was within the interval (between 12.1 and 132.7 g) described by Reyes-Agüero et al. (2009) for diverse variants of the same species. The relative proportion of pulp in the fruit (from 54 to 57 %), obtained by these authors, was slightly above the found in the present study (50 %; Table 2). The differences in some of the results may be due to the great diversity of variants within the species (Colunga-García et al. 1986; Reyes-Agüero

Table 2 Relative proportion (%) of pulp, seeds and skin in fruits of five species of *Opuntia*

Species	Pulp (%)	Seeds (%)	Skin (%)
O. streptacantha Lem.	44.13b	3.62a	52.25a
O. hyptiacantha F.A.C. Weber	53.69a	3.28a	43.03b
O. megacantha Salm-Dyck	50.36a	2.98b	46.66b
O. albicarpa Scheinvar	54.51a	3.10b	42.38b
O. ficus-indica (L.) Mill.	52.74a	3.07b	44.19b

Values followed by different letters within columns are statistically different ($p > 0.05$)

et al. 2005). The present study confirms the great richness of variants (Table 1) and their variability in fruit biomass (Fig. 2A) and pulp content (Fig. 2B). Moreover, the variants studied by Parish and Felker (1997) partially differed from those included in this study because those authors studied only commercial variants, whereas in this study, variants from very diverse environments were included.

Information regarding the fruits of *O. albicarpa* variants is scarce in the research literature. Pimienta-Barrios (1994) recorded 106 g (56 %) of pulp in the Alfajayucan variant, and Mondragón-Jacobo and Pérez-González (1994) recorded 67 % in the Reyna variant. These values are similar to those reported for the Naranjona Helia variant, with close to 100 g of pulp per fruit (Fig. 2B) but greater than the mean proportion (54 %) of the 22 accessions of *O. albicarpa* included in the present study (Table 2). There are estimations of fruit pulp in *O. ficus-indica* between 46 and 81 g per fruit (Barbera et al. 1994; Díaz-Medina et al. 2007; Parish and Felker 1997; Pimienta-Barrios 1994), but Reyes-Agüero et al. (2009) recorded a wider range, between 14.2 and 170.1 g, which matches the range found in the present study (Figs. 2B, 3A). The relative content of pulp in the fruits depends on the variant and its origin; in the cultivars from Mexico, this parameter varies between 39 and 55 % of the fruit (Parish and Felker 1997; Pimienta-Barrios 1994). This range coincides with results from the present study (Table 2) and suggests that the variability of this trait is relatively stable in *O. albicarpa* and *O. ficus-indica*. In contrast, fruits from Italy are less variable and contain approximately 60 % pulp (Barbera et al. 1994).

In general, the hypothesis of an increase in pulp content in fruits with a greater level of domestication was confirmed (Figs. 2B, 3A), as was a decrease in the proportion of skin (Table 2). The greater proportion of skin in *O. hyptiacantha* and *O. streptacantha* (52.5 and 47.7 %) fruits than in those of *O. megacantha* and *O. ficus-indica* (37.4 and 35.8 %) was recorded by Parish and Felker (1997). Additionally, fruits from cultivars of *O. ficus-indica* with biomass between 140 and 160 g adapted to cold, higher altitude zones showed a greater proportion of skin (59.7 %) than those from warmer environments (50 %) (Felker et al. 2005). According to Bachmann (1978), the size increase of cells or organs correlates with decreases in surface/volume and can alter metabolism and cellular processes such as nutrient absorption. Moreover, smaller cells respond faster to environmental changes, and therefore, in some organisms there is an increase in the proportion of cells in developing organs as an adaptation to the environment. The reduction in the skin proportion that accompanies the domestication of *Opuntia* is not likely to affect the final development of the fruit because photosynthetic activity only supports pulp production before maturity (Pimienta and Engleman 1985) and because a relative reduction in skin occurs mainly due to reduced thickness. In wild species, which frequently develop under stressful conditions, the higher proportion of skin could complement the metabolic activity of vegetative organs and offer greater protection and isolation of the fruit. In this regard, it has been noted that skin tissues are similar to those in cladodes, but the former have better developed mucilaginous ducts and their cells in the crust are organized irregularly (Corrales-García 2003; Pimienta and Engleman 1985).

Seed biomass per fruit

The biomass of normal seeds of the five species varied between 0.3 and 8.7 g per fruit. The frequency distribution of the biomass of this type of seeds in fruits of *O. streptacantha* and *O. megacantha* was symmetrical and uni-modal; *O. ficus-indica* and *O. albicarpa* showed very asymmetrical distributions with very high atypical values (7.5 and 8.7 g per fruit, respectively); *O. hyptiacantha* differed from other species with a flat distribution. In addition to these differences, it was also observed that the mode in the biomass of normal seeds per fruit increased from 2.1 g in *O. streptacantha* to 3.3 g in *O. megacantha* and *O. albicarpa*, and 3.9 g in *O. ficus-indica* (Fig. 4A–E). Ninety percent of the pulp is formed by cells of the parenchyma that originate in the dorsal epidermis of the funicular wrapping; the other 10 % is tissue of the funiculus (Pimienta and Engleman 1985). For this reason, the bigger fruits from species considered more domesticated have higher seed biomass.

Biomass of normal seeds varied ($p \leq 0.05$) within species, and the range increased with domestication. In *O. streptacantha*, the difference between Isbini and Charola with extreme values of normal seeds was 45 %. In *O. hyptiacantha*, the difference between Camueso and Cardón Blanco increased to 56 %, but the greatest differences (between 60 and 68 %) were recorded in *O. megacantha*, *O. albicarpa* and *O. ficus-indica* in variants such as Cuervo Tuna and Naranjón Helia, Reina and Cristalina, and Copena F1 and Doctor Mora, respectively (Fig. 2C).

The biomass of normal seeds per fruit increased from fruits of *O. streptacantha* which had, on average, 2 g of seeds, most of which were normal, to those of *O. albicarpa* and *O. ficus-indica* which had on average 3.5 g of normal seeds (3.8 g of total seeds) (Fig. 3B).

The distribution of biomass frequencies of aborted seeds differed between species, but for all five species, it was symmetrical and not modal; the extremes of the complete interval were 0.06 and 1.62 g per fruit (Fig. 4F–J). Asymmetry to the left indicated that the fruits with low biomass of aborted seeds were frequent in the five species, independent of their level of domestication. The intervals increased with domestication degree, as did frequencies. Fruits of *O. streptacantha* showed, on average, between 0.06 and 0.42 g of aborted seeds per fruit. Those of *O. ficus-indica* and *O. albicarpa* showed greater intervals, with a maximum of 1.0 and 1.6 g, respectively (Fig. 4F–J).

Variants within species showed large and significant differences ($p \leq 0.05$) in the content of aborted seeds; with the exception of *O. ficus-indica*, there were some variants with <0.05 g of aborted seeds per fruit, such as Isbini, a variant of *O. streptacantha*, Pachón of *O. hyptiacantha*, Cuervo Tuna of *O. megacantha* and Blanca Chapeada of *O. albicarpa* (0.04 g on average). In contrast, fruits of *O. ficus-indica* with lower content of aborted seeds, such as Amarilla Milpa Alta, showed 0.21 g on average. A direct relationship was observed between the maximum content of aborted seeds and the level of domestication, only 6 % (0.2 g) in *O. streptacantha* of those present in *O. albicarpa* and *O. ficus-indica* (up to 0.83 g) (Figs. 2D, 3B). However, the relative biomass of seeds per fruit decreased significantly ($p \leq 0.05$), from 3.6 % in *O. streptacantha* to 3.1 % in *O. ficus-indica* (Table 2). This decrease indicates that although there is a significant increase in seed number with domestication, the number of aborted seeds also increases, but with a lower biomass per seed, which contributes little to the total seed and fruit biomass. The changes may also be due to the formation of more pulp for each seed in domesticated *O. ficus-indica* than in wild species.

Total seed biomass in some fruits of *O. streptacantha* and *O. hyptiacantha* was 3.3 g (2.4 %) and 2.9 g (1.8 %), respectively (Parish and Felker 1997). These values are clearly lower than those obtained in the present study (Table 2). In contrast, our data and those of Parish and Felker (1997) referring to *O. megacantha* were similar. Moreover, data from Reyes-Agüero et al. (2009) for *O. streptacantha* (1.2–3.9 g), *O. hyptiacantha* (0.7–6.3 g), *O. megacantha* (0.03–7.9 g) and *O. albicarpa* (0.5–10.8 g) match those of the present study (Fig. 3B).

Seed biomass per fruit in *O. ficus-indica* can vary between 2.2 and 4.3 g (1.8 and 4.9 %) (Barbera et al. 1994; Parish and Felker 1997; Pimienta-Barrios 1994), which coincides with the results of the present study, or between 0.2 and 33.6 g (Reyes-Agüero et al. 2009) and up to 5.9 % (Díaz-Medina et al. 2007), a much wider range than found in the present study (Fig. 4).

Number of seeds per fruit

Normal seeds per fruit for the five species varied between 78 and 438; except for *O. hyptiacantha*, the distribution was symmetrical with the mode between 150 and 222, and the number of normal seeds increased from *O. streptacantha* to *O. ficus-indica*. The greatest variation in the number of normal seeds was for *O. hyptiacantha* (CV = 33.17 %), and the

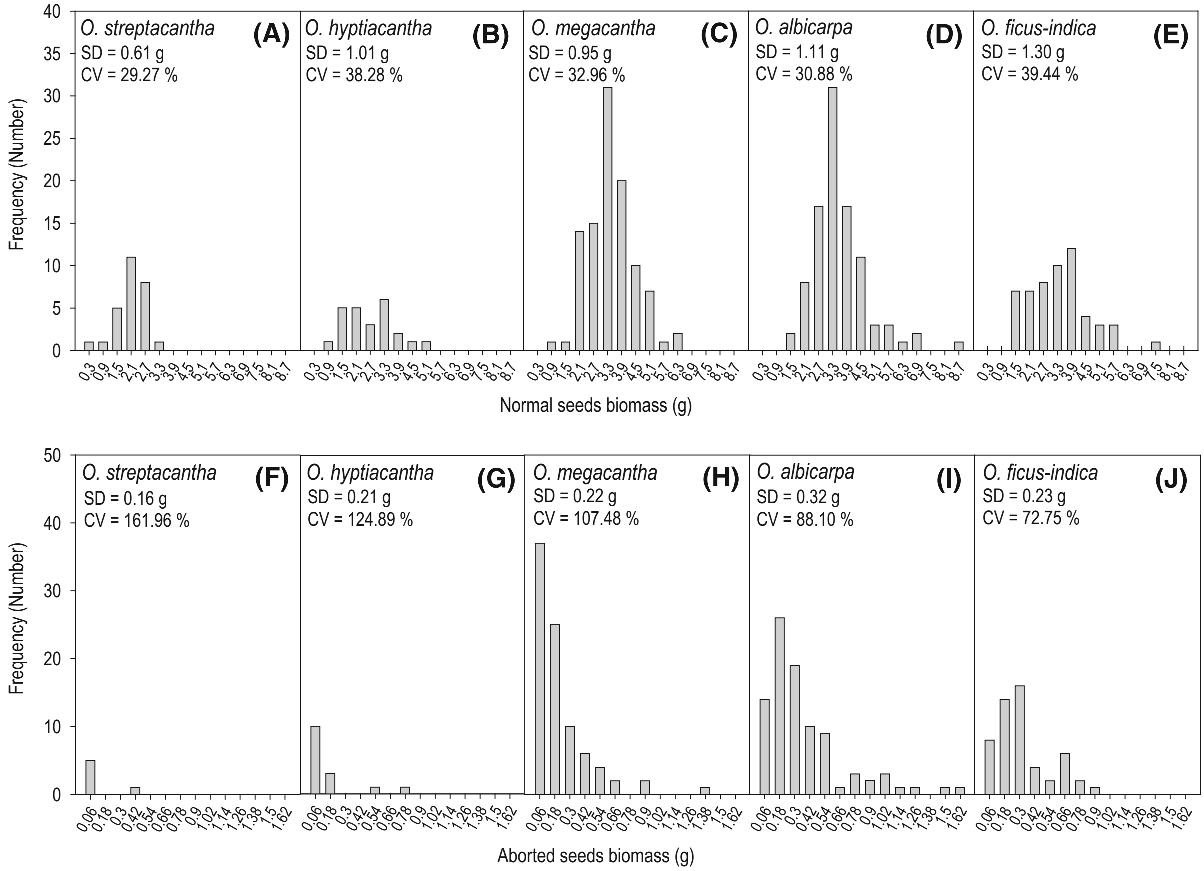

Fig. 4 Frequency distribution of normal seed biomass (**A–E**) and aborted seeds (**F–J**) in fruits of five species of *Opuntia*. *SD* standard deviation, *CV* coefficient of variation

smallest was for *O. ficus-indica* (CV = 24.42 %). The distribution of the number of normal seeds was asymmetrical and skewed towards the left, with two peaks (Fig. 5A–E).

The intra-specific comparison of the number of normal seeds per fruit indicated differences ($p \leq 0.05$) in the five species studied; comparison indicated that variants with fewer and similar ($p > 0.05$) normal seeds were Isbini, Camuezo, Jarrilla, Octubreña and Copena F1, with a mean number of 114 seeds. Among the variants with more normal seeds were Charola, a variant of *O. streptacantha* with 197 seeds per fruit, which was surpassed by other species such as Cardón Blanco, a variant of *O. hyptiacantha*, Naranjona Helia of *O. megacantha*, Cristalina of *O. albicarpa* and Amarillo Milpa Alta of *O. ficus-indica*. These variants did not exhibit significant differences ($p > 0.05$) and had an average of 284 seeds per fruit (Fig. 2E).

Among the species, the number of normal seeds per fruit of *O. albicarpa* and *O. ficus-indica* surpassed by up to 21 % that of *O. streptacantha* (Table 3).

The frequency distribution of the number of aborted seeds per fruit differed slightly among the five species because for all of them, it was asymmetrical and truncated. The general bias to the left indicates that fruits with a low proportion of aborted seeds are the most common in the five species; the range for the five species was between 10 and 230 seeds per fruit (Fig. 5F–J). Moreover, most fruits from *O. streptacantha*, *O. hyptiacantha* and *O. megacantha* showed 10 aborted seeds, and a lower proportion of fruits with 30–150 aborted seeds was more frequent in *O. hyptiacantha* and *O. megacantha*. In contrast, *O. albicarpa* and *O. ficus-indica* fruits with 30–70 and 10–110 aborted seeds, respectively, were the most frequent (Fig. 5F–J).

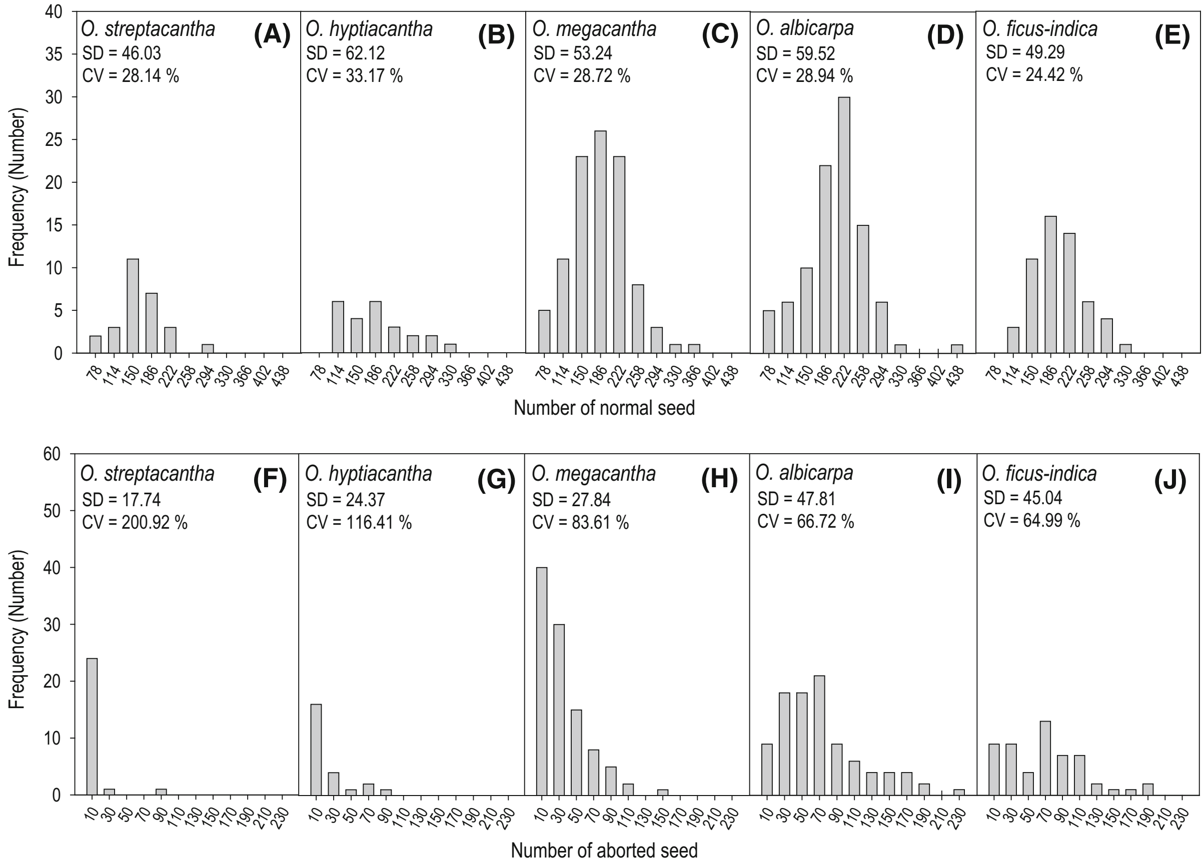

Fig. 5 Frequency distribution of number of normal seeds (**A–E**) and number of aborted seeds (**F–J**) in fruits of five species of *Opuntia*. *SD* standard deviation, *CV* coefficient of variation

The differences in the number of aborted seeds per fruit within species were large and significant ($p \leq 0.05$); in *O. streptacantha*, some variants, such as Jocoquillo, had only (two aborted seeds) one-third of Isbini. These values differed substantially from those of *O. albicarpa* variants, such as Blanca Chapeada and Burrona. They also differed from those of *O. ficus-indica*, as its cultivars with fewer aborted seeds per fruit, such as Liso Forrajero, had 50 aborted seeds, and others, such as Telokäjä Rojo, had up to 115 (Fig. 2F).

Fruits of *O. streptacantha* had the lowest number ($p \leq 0.05$) of all species, and *O. albicarpa* and *O. ficus-indica*, on average, had nine times more. The same trend was observed between species for the total number of seeds (normal plus aborted) per fruit, as fruits of *O. albicarpa* and *O. ficus-indica* had on average 50 % more seeds ($p > 0.05$) than those of *O. streptacantha* (Table 3).

The number of total seeds (Table 3) seems to be similar to other values in *O. streptacantha* and in *O. hyptiacantha* (201 and 191 seeds per fruit) (Parish and Felker 1997). In fruits of *O. megacantha* a higher range (from 248 up to 304 seeds) than that found in the present study (Table 3) was observed (Parish and Felker 1997). The average number of total seeds recorded for *O. ficus-indica* fruits in our study is within the known range (144–342 seeds per fruit) (Barbera et al. 1994; Parish and Felker 1997). In contrast, the ranges from zero to 107, 135 or 306 aborted seeds per fruit of *O. streptacantha, O. hyptiacantha* and *O. ficus-indica*, respectively, and from 2 to 238 or 272 in *O. megacantha* and *O. albicarpa* reported by Reyes-Agüero et al. (2009) were wider than those recorded in the present study; but ranges of 70–319 normal seeds in *O. streptacantha*, from 36 to 559 in *O. hyptiacantha*, from 13 to 404 in *O. megacantha*, from 55 to 402 in *O. albicarpa* and from 10 to 566 in *O. ficus-indica* (Reyes-Agüero et al. 2009) were similar (Table 3).

Seed abundance, size and hardness affect the quality of fresh fruit in *Opuntia* (Inglese et al. 1995; Nobel 2011; Parish and Felker 1997). Thus, in the Canary Islands the fruits of *O. dilleni* (Ker Gawl.) Haw. are not consumed due to the abundance of seeds (Díaz-Medina et al. 2007). However, the seed abundance tends to compensate for small seed size, increasing fruit acceptance (Colunga-García et al. 1986). This trend was observed in *O. albicarpa* and *O. ficus-indica* (Table 3) and coincided with results obtained by Reyes-Agüero et al. (2005), who pointed out that the more domesticated species had fruits with a lower proportion of normal seeds. The fruits of *Opuntia* preferred and selected during domestication likely contained seeds that are easier to ingest, such as aborted seeds (Barbera et al. 1994; Reyes-Agüero et al. 2005). This hypothesis partially agrees with results from the present study because the relative number of aborted seeds was higher in *O. albicarpa* and *O. ficus-indica* (25 %) fruits in comparison with *O. streptacantha* (5 %); however, in these bigger fruits the total number of seeds was also higher (Fig. 5; Table 2). The increase to up to one-third aborted seeds in species with a higher level of domestication compared to wild species was documented by Reyes-Agüero et al. (2005).

The inter-specific relationship between fruit or pulp biomass and the number of seeds, aborted and normal was different in *Opuntia* (Table 4). In *O. streptacantha*, the correlation between pulp and fruit biomass and number of aborted seeds was not significant. Pulp and fruit biomass in *O. hyptiacantha* and *O. megacantha* correlated positively ($p \leq 0.001$) with the number of aborted and normal seeds, as well as with the total seed number; in contrast, in *O. albicarpa* the correlation between pulp or fruit biomass was significant ($p \leq 0.001$) only with aborted seeds. In the species with the highest level of domestication known today, *O. ficus-indica*, none of the correlations mentioned above were significant (Table 4).

The funicular wrap of seeds originates the edible tissue of the fruit (Pimienta and Engleman 1985), and therefore, a better relationship would be expected between seed and pulp content in *O. ficus-indica*. However, the correlation between pulp or fruit biomass with the number of seeds was not significant in *O. ficus-indica* (Table 4). This trend was opposite of that observed by Barbera et al. (1994), who did detect a significant correlation between seeds and fruit pulp, although the correlation was not significant when considering only aborted or normal seeds, as in the present study. Colunga-García et al. (1986) observed a significant correlation between wild fruit biomass and the number of normal seeds (r = 0.747); moreover, they noted that with increased fruit size, this correlation is lost (r = 0.410) due to the increase in the number of aborted seeds, which coincides with results from the present study (Table 3).

Dimensions of normal seeds

The width of normal seeds of the five species analyzed varied between 0.25 and 0.43 cm; its frequency distribution was symmetrical with respect to the mode

Table 3 Seed content per fruit, and normal seed size and hardness of *Opuntia* spp.

Seed characteristic	*O. streptacantha* Lem.	*O. hyptiacantha* F.A.C. Weber	*O. megacantha* Salm-Dyck	*O. albicarpa* Scheinvar	*O. ficus-indica* (L.) Mill.
Normal (number)	167.576c	187.285b	185.353b	205.663a	201.790a
Aborted (number)	8.368c	20.931b	33.296b	71.656c	69.309c
Total (number)	175.944c	208.215b	218.649b	277.319a	271.100a
Width (cm)	0.320d	0.341c	0.352b	0.369a	0.353b
Thickness (cm)	0.160a	0.158a	0.159a	0.160a	0.158a
Length (cm)	0.373d	0.397c	0.421b	0.443a	0.434a
Hardness (kN)	1.525b	1.705c	1.818c	1.754c	1.810c

Values followed by different letters within a line are statistically different ($p \leq 0.05$)

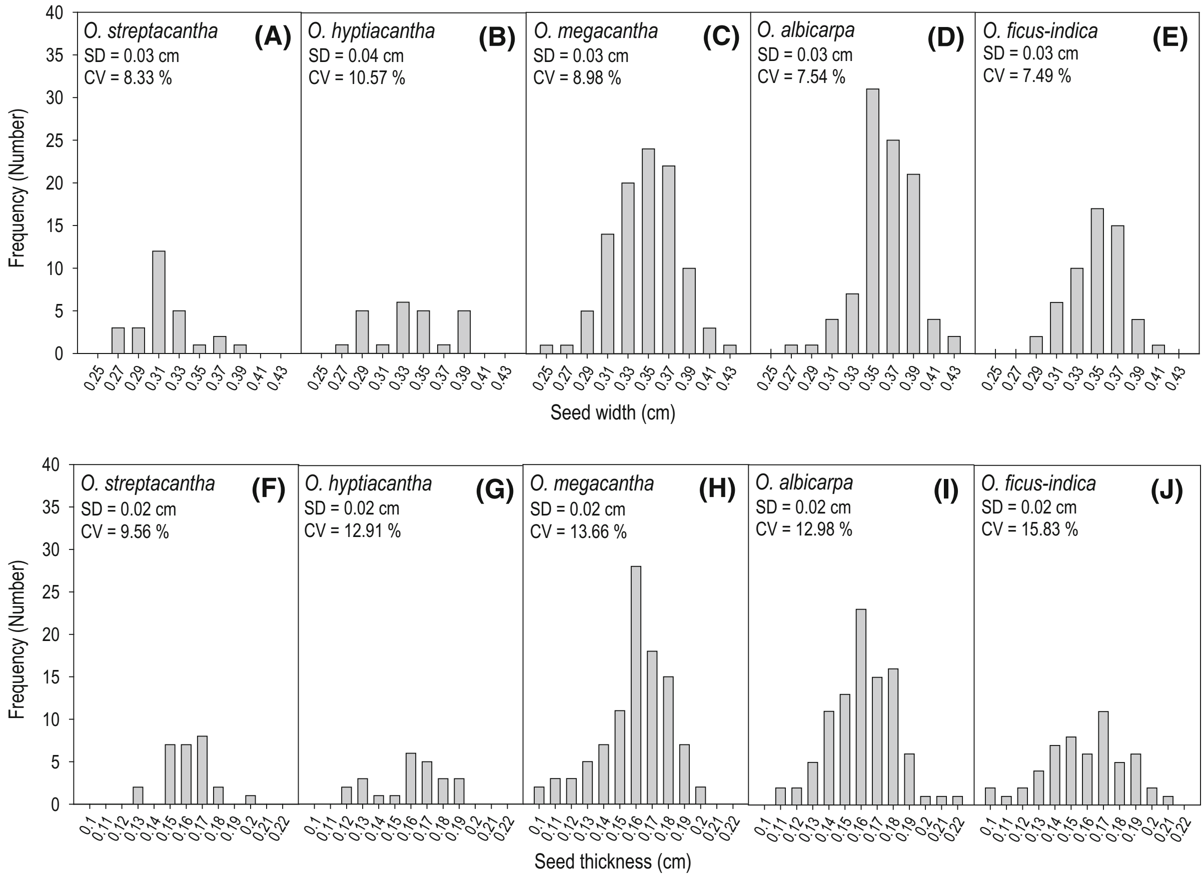

Fig. 6 Frequency distribution of seed width (**A–E**) and normal seed thickness (**F–J**) in fruits of five species of *Opuntia*. *SD* standard deviation, *CV* coefficient of variation

in *O. megacantha*, *O. albicarpa* and *O. ficus-indica*. In *O. streptacantha*, the mode of seed width was 0.31 cm, with another much smaller peak at 0.37 cm; in *O. hyptiacantha*, the distribution of this trait alternated between high and low values (jagged or comb-like distribution) between 0.27 and 0.39 cm (Fig. 6A–E).

In *O. megacantha*, *O. albicarpa*, and *O. ficus-indica* the most frequent seeds with normal characteristics were those with a width of 0.35 cm (Fig. 6A–E). The mean width of normal seeds was a relatively homogeneous trait among variants of each species, because the differences between variants with narrower and wider seeds ($p \leq 0.05$) were very small; the *O. streptacantha* variety Cardón Potosino exhibited narrower seeds and differed from Sandía, one of the variants with the widest seeds. *Opuntia megacantha* variants had the most dispersed values and the widest seeds of this species, such as the variety from Pico Chulo (Fig. 7A). On average, the width of the normal seeds was lower in *O. streptacantha* ($p \leq 0.05$) than in *O. megacantha*, and *O. ficus-indica* but the highest values corresponded to *O. albicarpa* seeds (Table 3).

The mean thickness of normal seeds was not significantly different ($p > 0.05$) between species, ranging from 0.10 to 0.22 cm. The frequency distribution of the thickness of normal seeds differed among species; in *O. streptacantha* and *O. hyptiacantha*, it was truncated; in *O. megacantha* and *O. albicarpa*, it was biased; and in *O. ficus-indica*, it was jagged (Fig. 6F–J).

Among variants of *O. streptacantha* and *O. hyptiacantha* no significant differences were found ($p > 0.05$) in the thickness of normal seeds (Table 3). In contrast, seed thickness of Esmeralda and Cristalina in *O. albicarpa* and Amarillo Huevo and Doctor Mora in *O. ficus-indica* was significantly different ($p \leq 0.05$) (Fig. 7B).

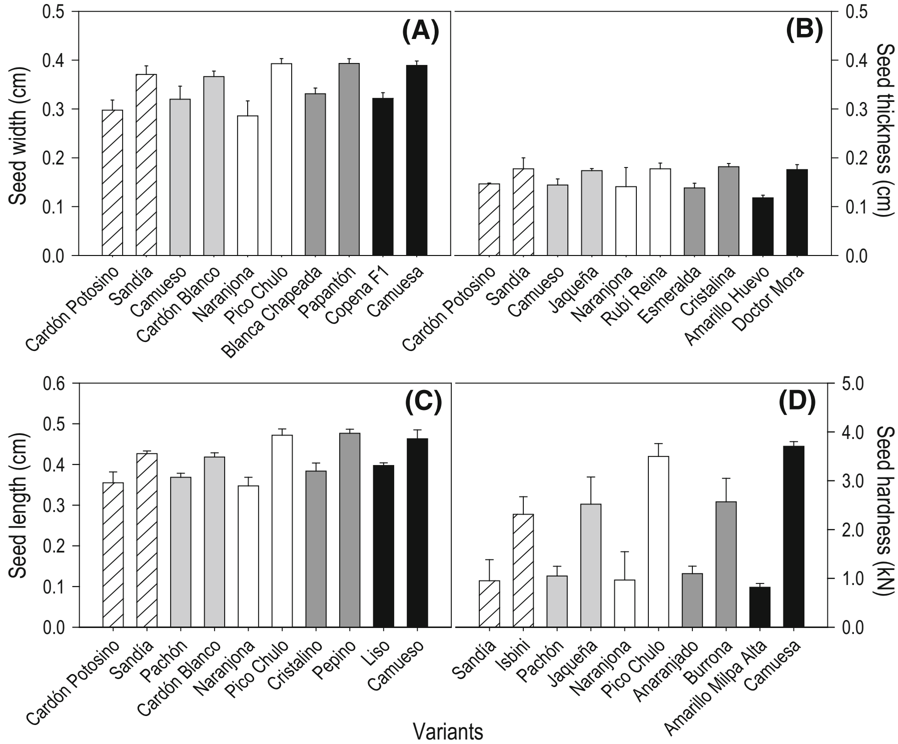

Fig. 7 Two variants with the lowest and highest seed width (**A**), seed thickness (**B**), seed length (**C**), and seed hardness (**D**) (+SE) of five species of *Opuntia*. *Opuntia streptacantha* Lem. (*hatch pattern*), *O. hyptiacantha* F.A.C. Weber (*light grey*), *O. megacantha* Salm-Dyck (*white bars*), *O. albicarpa* Scheinvar (*dark grey bars*), and *O. ficus-indica* (L.) Mill. (*black bars*)

Table 4 Pearson correlation coefficient between pulps biomass and fruit biomass with number of aborted, normal and total seeds of fruits of five *Opuntia* species

	Seed content		
	Aborted	Normal	Total
O. streptacantha Lem.			
Pulp biomass	0.12^{ns}	0.68***	0.72***
Fruit biomass	0.17^{ns}	0.43*	0.49***
O. hyptiacantha F.A.C. Weber			
Pulpa biomass	0.53***	0.78***	0.82***
Fruit biomass	0.55**	0.69***	0.74***
O. megacantha Salm-Dyck			
Pulp biomass	0.38***	0.53***	0.38***
Fruit biomass	0.45***	0.45***	0.45***
O. albicarpa Scheinvar			
Pulp biomass	0.35***	-0.004^{ns}	0.22*
Fruit biomass	0.39***	-0.007^{ns}	0.20^{ns}
O. ficus-indica (L.) Mill			
Pulp biomass	-0.06^{ns}	0.13^{ns}	0.05^{ns}
Fruit biomass	0.07^{ns}	0.22^{ns}	0.20^{ns}

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ^{ns} $p > 0.05$

The length of normal seeds in the five species varied between 0.33 and 0.47 cm, and with the exception of *O. hyptiacantha*, the distribution of this variable was modal. The greatest frequency for length varied from 0.37 cm in *O. streptacantha* to 0.43 cm in *O. albicarpa* (Fig. 8A–E). Seed length varied significantly ($p \leq 0.05$) within species, thus in *O. streptacantha*, seeds of Cardón Potosino were among the shortest and those of Sandía were among the longest. In *O. hyptiacantha*, the difference between extreme values (Pachón and Cardón Blanco), although significant, was the smallest among the five species. In contrast, *O. megacantha* variants exhibited the shortest (Naranjona) and longest (Pico Chulo) seeds. Differences in *O. albicarpa* were intermediate (15–26 %) with respect to differences in other species (Fig. 7C).

Seed length in the five species (Table 3) was within the known interval (between 0.21 and 0.57 cm), as well as seed width (between 0.23 and 0.44 cm) (Aguilar-Estrada et al. 2003; Reyes-Agüero et al. 2009; Rodríguez and Nava-Cedillo 1998). Moreover,

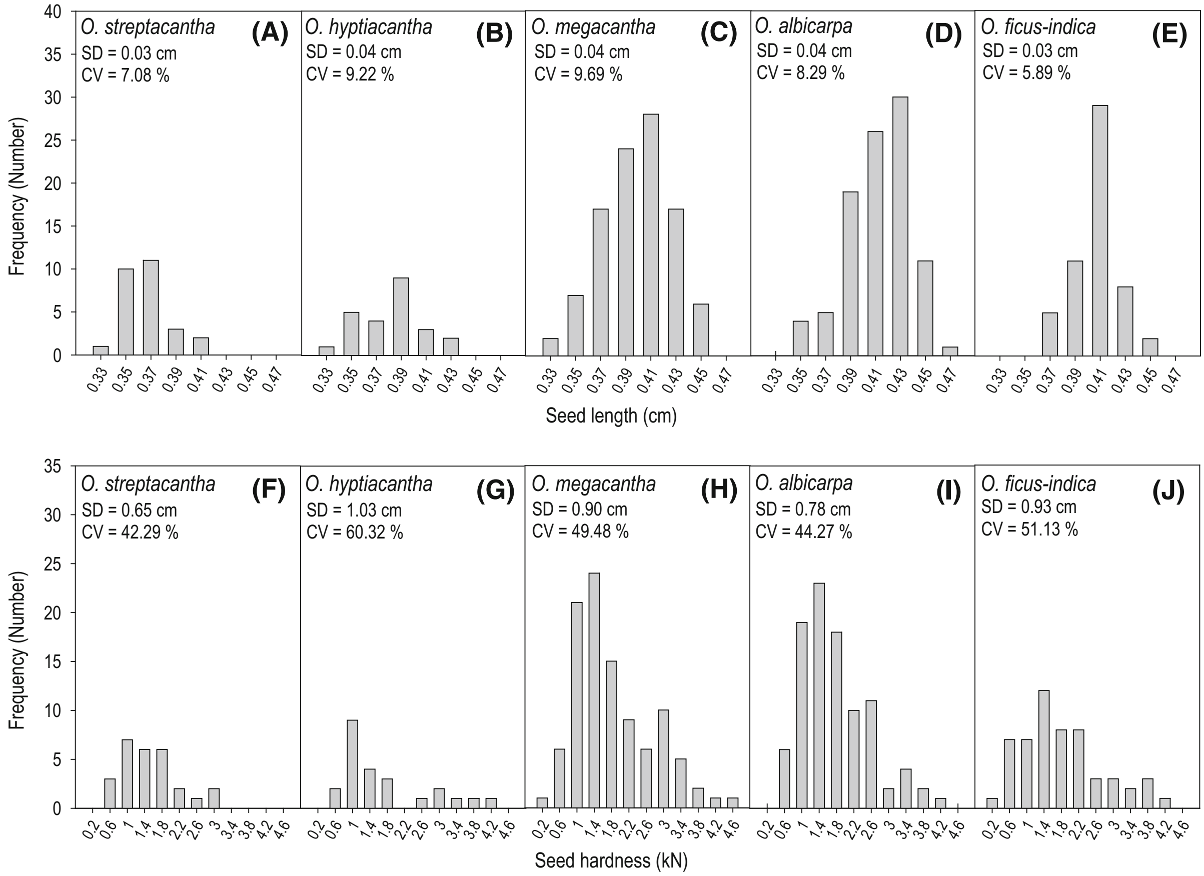

Fig. 8 Frequency distribution of seed length (**A–E**) and normal seed hardness (**F–J**) of five species of *Opuntia*. *SD* standard deviation, *CV* coefficient of variation

Table 5 Pearson correlation coefficient between seed hardness with seed size of five *Opuntia* species

	Seed size		
	Length	Width	Thickness
O. streptacantha Lem.			
Hardness	-0.25^{ns}	-0.07^{ns}	0.03^{ns}
O. hyptiacantha F.A.C. Weber			
Hardness	0.52**	0.52**	0.46*
O. megacantha Salm-Dyck			
Hardness	0.44***	0.46***	0.28**
O. albicarpa Scheinvar			
Hardness	0.20*	0.28**	0.28**
O. ficus-indica (L.) Mill			
Hardness	0.31*	0.53***	0.34*

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$; ^{ns} $p > 0.05$

seed thickness coincided with the average 0.16 cm documented by Aguilar-Estrada et al. (2003) and Reyes-Agüero et al. (2004). That *O. albicarpa* and *O. ficus-indica* had the longer and wider seeds should be associated with their greater domestication, and coincides with the results obtained by Aguilar-Estrada et al. (2003) and Reyes-Agüero et al. (2005). Similarly, Colunga-García et al. (1986) attributed the greater length of domesticated (0.43 cm) than wild (0.39 cm) seeds to fruit gigantism.

Hardness of normal seeds

Hardness of normal seeds of the five species included in this study varied between 0.2 and 4.6 kN. The frequency distribution of hardness in this type of seeds was similar among species. The least variable species

Table 6 Eigenvalues and proportion of the variance explained for the first three principal components, generated from characteristics of fruits and seeds of 89 variants of five *Opuntia* species in a domestication gradient

	Principal component		
	1	2	3
Biomass of aborted seeds	0.0016	0.0043	−0.0033
Biomass of normal seeds	0.0148	−0.0001	0.0072
Biomass of pulp	0.2965	0.1333	0.3065
Hardness of normal seeds	0.0002	0.0039	0.0004
Number of normal seeds	**0.7702**	**−0.5297**	−0.3506
Number of aborted seeds	0.2720	**0.7745**	**−0.5710**
Seed length	0.0002	0.0005	0.0007
Seed width	0.0002	0.0003	0.0005
Total fruit biomass	0.4946	0.3190	**0.6760**
Variance	0.7188	0.9322	0.9953

Variables in bold had higher relative effect in each principal component

in seed hardness were *O. streptacantha* (CV = 42.29 %) and *O. albicarpa* (CV = 44.27 %), and the most variable was *O. hyptiacantha* (CV = 60.32 %) (Fig. 8F–J).

Intraspecific hardness of normal seeds was heterogeneous. Seed hardness variation in *O. streptacantha* was equivalent to the mean difference between variants (43 %). In *O. hyptiacantha*, *O. megacantha* and *O. albicarpa*, some seeds were twice as hard as others, and in *O. ficus-indica*, cultivars such as Camuesa had seeds that were four times harder (3.71 kN) than Amarillo Milpa Alta (0.82 kN) (Fig. 7D). In contrast, no significant variability within species was found ($p > 0.05$) in this trait (Table 3).

Seed hardness of the five species analyzed in this work coincided with the range already known, 1.59–1.68 kN (Aguilar-Estrada et al. 2003; Reyes-Agüero et al. 2005, 2009). Seed hardness can be undesirable in *Opuntia* fruits (Inglese et al. 1995). Aguilar-Estrada et al. (2003) noted an inverse relationship between seed hardness and total seed number in *Opuntia* fruits. Moreover, Reyes-Agüero et al. (2005) mentioned the possibility that the cultural selection that favored fruits with abundant pulp led to an increase in the number of normal seeds with reduced hardness; the results of the present study are not consistent in this respect.

With the exception of *O. streptacantha*, hardness of normal seeds correlated positively and significantly

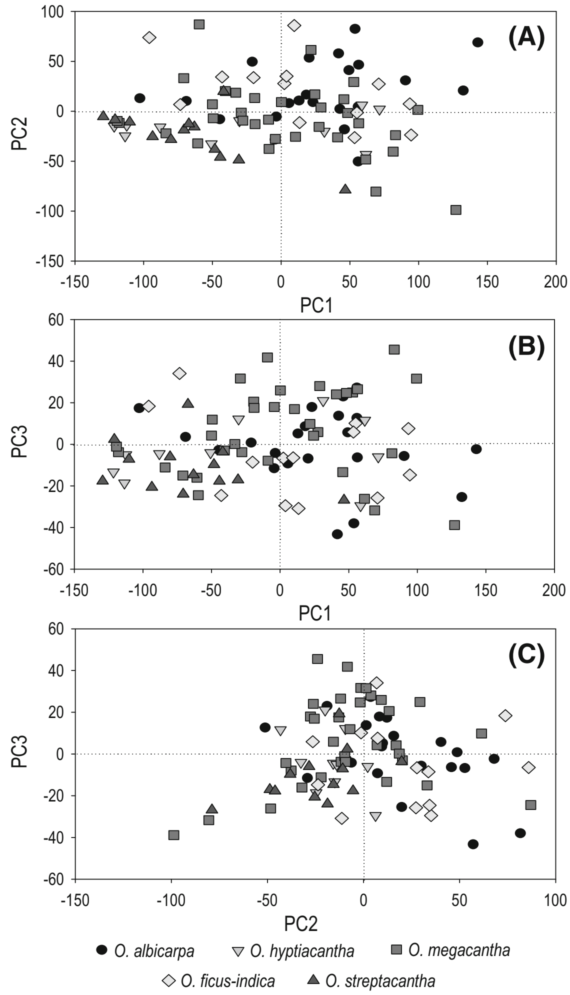

Fig. 9 Ordination of the three principal components (PC) of 89 variants of five Mexican *Opuntia* species, based on fruit and seed characteristics (n = 89)

($p \leq 0.001$) with seed dimensions (Table 5); that is, with the increase in the size of normal seeds during domestication, hardness has also increased. These changes appear to concomitant to the increase of fruits size. These characteristics in more domesticated species seem to compensate for one another, at least partially, with an increase in aborted seeds, which are ingested more easily than normal seeds by humans (Nobel 2011). For this reason, the current search for varieties has focused on lower content of normal seeds, although this goal has not been separated from an increase in aborted seeds (Barbera et al. 1994), which also contribute to pulp production (Pimienta and Engleman 1985). However, in the present study, the correlation between aborted seeds and pulp

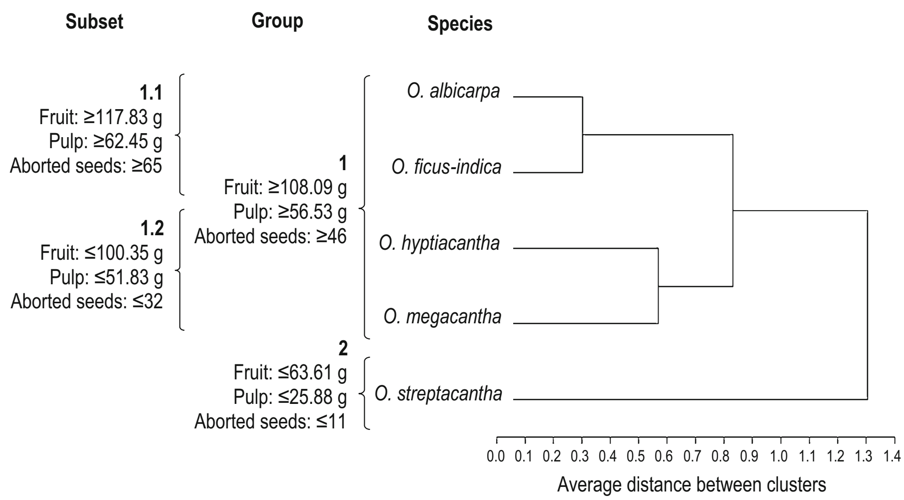

Fig. 10 Classification of 89 variants of five species of *Opuntia* with different degree of domestication, based in fruit and seed characteristics

biomass in the species with the highest level of domestication was not significant (Table 4), which shows that normal seeds have a greater effect on the proportion of pulp. In fact, Pimienta and Engleman (1985) found more prominent funicular wrappings in normal seeds than in aborted ones.

Multivariate analysis

A PCA was performed to identify which fruit and seed attributes could be related to domestication in *Opuntia*. In this analysis, seed thickness was excluded because its correlation with most other variables was not significant. With the other variables, the first three principal components (PCs) explained 99.53 % of the total variance (Table 6).

In PC1, number of seeds was the most important variable and was directly related to fruit biomass; PC2 revealed that the number of aborted seeds was inversely proportional to the number of normal seeds; PC3 showed that fruit biomass was inversely proportional to the number of aborted seeds. Ordination by the first three PCs confirmed that fruit biomass and the number of normal and aborted seeds may be used as markers to differentiate *Opuntia* variants according to the level of domestication. Moreover, the relative importance of these three traits is relatively high, with respect to others, because the PC analysis emphasizes their relative weight in the first three PC (Table 6).

In the graphic representation of PC1 against PC2, *O. albicarpa* and *O. ficus-indica* variants, with higher fruit biomass and number of seeds, were differentiated from *O. streptacantha* and *O. hyptiacantha*. The absence of a trend in the ordination of *O. megacantha* variants was also noted. Graphic representation of PC1 against PC3 also showed a greater affinity in the ordination of *O. streptacantha* and *O. hyptiacantha* variants, as well as of *O. ficus-indica* and *O. albicarpa*. It showed great dispersion of *O. megacantha* variants. Graphic representation of PC2 against PC3 also separated *O. streptacantha* variants, with lower fruit biomass and seed content, from *O. albicarpa* and *O. ficus-indica*; it also showed the dispersion of *O. megacantha* variants (Fig. 9).

The first two main groups generated by cluster analysis clearly separated *O. streptacantha* from the other species. The cluster including *O. albicarpa*, *O. ficus-indica*, *O. hyptiacantha* and *O. megacantha* is characterized by their large fruits (≥56.53 g) with numerous aborted seeds (≥46). In contrast, the other cluster is characterized by its small fruits (≤64.61 g), reduced pulp (≤26 g) and few aborted seeds (≤11) (Fig. 10).

The first cluster was subdivided into two subsets, one of domesticated species (*O. albicarpa* and *O. ficus-indica*) with large fruits, with their biomass equal to or greater than 118 g and a higher number of aborted seeds. The other subset included *O. hyptiacantha* and *O. megacantha* (species with intermediate levels of domestication), with fruits of approximately 100 g and intermediate numbers of aborted seeds (32, on average; Fig. 10).

The results confirm the presence of greater fruit biomass and pulp in species and variants with more intense levels of domestication (Figs. 9, 10). Pimienta-Barrios (1994) attributes this to ploidy, but this factor would explain only a minimum part of the variability in wild populations (Harlan 1992). Luna-Páez et al. (2007) used molecular markers to characterize *Opuntia* species and noted the difficulties of delimiting *Opuntia* species, as observed in the PCA (Fig. 10). This difficulty might be a result of including variants with different levels of domestication within species.

Variation in seed content and proportion of normal/aborted seeds appears to be concomitant to selection for bigger fruits the increase in aborted seeds observed in species such as *O. albicarpa* and *O. ficus-indica* (Figs. 9, 10). The increasing trend observed for the relationship between normal/aborted seeds and domestication agrees with results by Aguilar-Estrada et al. (2003) and Reyes-Agüero et al. (2005) and their suggestion that the increase in aborted seeds is directly related to selective pressures under domestication. In turn, this selective pressure is opposed to the reduction in the number of normal seeds, fruit biomass and seed thickness. Zohary (2004) noted that in perennial domesticated species, such as fruit trees, part of the domestication syndrome is the ease of asexual reproduction in wild species. Therefore, reproduction of an exceptional individual can be achieved by vegetative propagation in what Harlan (1992) calls instantaneous domestication. In this process, the high number of aborted seeds in *O. albicarpa* and *O. ficus-indica* (Figs. 9, 10) favors acceptance of the fruit and is not important for reproduction because these fruits are easily cloned.

Proximity between *O. albicarpa* and *O. ficus-indica* (Fig. 10) has been highlighted based on molecular data (Luna-Páez et al. 2007) and on plant morphological characteristics (Reyes-Agüero et al. 2005). Moreover, the cited authors found certain proximity of *O. hyptiacantha* and *O. megacantha* to *O. ficus-indica*, as the more domesticated species, as was observed in the present study (Fig. 10). This proximity seems to respond to the fact that these species present variants with fruits and seeds that are similar to those of species with higher levels of domestication (Fig. 10).

The high intra-specific morphological (Reyes-Agüero et al. 2005), chemical and physiological variability (García-Nava et al. 2014; López-Palacios et al. 2012; Peña-Valdivia et al. 2012) in present *Opuntia* variants may be originated from the presence of outstanding attributes of human interest and tolerance to environments created by humans in field crops or back yards (Casas et al. 1999; Figueroa et al. 1980) for diverse uses (e.g. production of nopalitos, mature cladodes, fruit and flowers, and host of carmine dye-producing insects *Dactylopius coccus*).

Conclusions

The domestication of *Opuntia* resulted in significant differences in fruit size and in the proportions of fruit components. The fruit and pulp biomasses in highly domesticated species were higher and less variable. Moreover, these species showed a greater number and biomass of normal and aborted seeds, as well as longer and wider normal seeds. Domestication was not significantly related to seed thickness or hardness. The least domesticated species, *O. streptacantha*, has a higher proportion of skin and less pulp than species with a higher level of domestication. Greater fruit biomass results from a higher proportion of pulp and a lower proportion of seeds and skin.

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