Fruit characters among apomicts and sexual progeny of a cross of the Texas native *Opuntia lindheimerii* (1250) with a commercial fruit type *Opuntia ficus–indica* (1281)

Peter Felker[1*], R. Zapata[2], X. Wang[3], Diego Medina[4], Ronald Bunch[1], Andrew Paterson[5]

[1]D'Arrigo Bros, 20911 Harris Road, Salinas, CA 93902

[2]Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina

[3]British Columbia Cancer Agency, 675 West 10th Avenue, Vancouver, BC V5Z 1L3 Canada

[4]Facultad de Agronomía y Agroindustrias, Universidad Nacional de Santiago del Estero, Argentina

[5] Plant Genetic Mapping Laboratory, University of Georgia, Athens, GA

*Author for correspondence: e–mail, peter_felker@hotmail.com

Received 28th May, 2009; accepted 21th December, 2009

Abstract

A wide interspecific cross between two wild, spiny Texas native *Opuntia lindheimerii* Texas A&M University Kingsville (TAMUK) accession 1250 male parents and a spineless commercial *Opuntia ficus–indica* fruit type TAMUK accession 1281 was made to serve as the mapping population for a genetic map. Seedlings (127) resulting from this cross were grown in Santiago del Estero, Argentina and evaluated at four years of age when they were 3 to 5 m tall. At this age, the female parent was spineless, 4 m tall, with greenish pads and large fruits (ca 150 gram) while the male was spiny with bluish pads, and about 1 m tall with small (ca 35 gram fruits). Due to the presence of apomixis (asexual reproduction of seeds without fertilization) in *Opuntia*, we used Random Amplification of Polymorphic DNA (RAPD) markers to test for apomixis. RAPD bands were obtained that distinguished the two males and the female. The lack of "male" RAPD bands in "female looking progeny" confirmed the presence of apomixis in some of the progeny. However, 46% of the progeny had at least one morphological character (spines, small cladodes, bluish cladodes) of the male parent indicating that these were not apomicts. Spine, and disease ratings were measured for all of the 127 "progeny" and fruit characters were measured for 109 progeny. Ten fruits were analyzed for the male and female parents and four fruits were analyzed for the progeny. The fruits ranged from 6 to 15.5% soluble solids, 22 to 197 g fruit weight, 24.4 to 59.2% edible pulp portion, 2 to 22,100 g fruit per plant, 0.63 to 2.86 kg pulp firmness, 3.3 to 10.4 g of seed per 100 g pulp, 4.35 to 6.95 in pH and there were 78 days difference in maturity of the fruits. Several of the progeny had many characteristics of the male parent (short habit and small, bluish cladodes but without spines) and may have potential as cold hardy forage types.

Key words: Cactus, genetics, seeds, soluble solids, DNA.

Introduction

Surprisingly little is known about the genetics of *Opuntias*. Grant *et al*. (1979) found that when flowers of the wild Texas *O. lindheimerii* were artificially self pollinated and bagged flowers they set fruit, but that non self–pollinated bagged flowers did not set fruit. Wang *et al*. (1996) reported emasculation techniques for *Opuntias*, and found that none of the seven *Opuntias* examined set fruit after emasculation without pollination. However, when *Opuntia ficus–indica* commercial fruit types were used as the female parent, from 40 to 70% fruit set was obtained using a different *O. ficus–indica* as the male parent. However such high fruit sets were not obtained when the male parent was *O. lindheimerii* or other non *O. ficus–indica* parents. These studies provided insights into the sterility barriers within *Opuntia*. All of the commercial fruit types, both spiny and spineless, produced fruit with fertile seeds from controlled pollinations with the commercial fruit types (known variously as *O. hyptiacantha, O. megacantha, O. streptacantha, and O. ficus–indica*, among others). *O. ficus–indica* also produced fertile seed using the Texas native species *O. lindheimerii* (1250) as the male parent but not with the spineless cold hardy *O. ellisiana.*

More recent molecular genetic work has cloned genes for one of the fruit softening enzymes (Collazo–Siqués *et al.*, 2003), cloned partial genes for two enzymes involved in fruit pigment development (Felker *et al.*, 2008) and reported techniques for genetic transformation of *Opuntia* (Silos–Espinosa *et al.*, 2006).

In 1995, we initiated a series of crosses to combine the best characteristics among the commercial fruit types and also to develop a genetic map of the most important useful characters in *Opuntia* (Wang *et al.*, 1996; Wang *et al*., 1997). It is easier to map segregants when the parents are more distantly genetically. Thus, we chose to develop a mapping population using a wide hybrid cross between the cold hardy, spiny, Texas native *O. lindheimerii* (TAMUK accession 1250 erroneously noted as 1255 in Wang *et al*., 1997) with bluish pads and small fruits of low sugar contents as the male parent and a spineless not so cold hardy, commercial variety with greenish cladodes, large, high–sugar reddish fruits (TAMUK accession 1281) as the female parent. Apomixis, which is the asexual reproduction of seeds without fertilization, was reported in *Opuntia* by Velez–Gutierrez & Rodriguez–Garay (1996) and Mondragon (2001).

RAPD markers were found that could discriminate between the male and female parents. Unfortunately, these markers found significant apomixis in the progeny and that the two *O. lindheimerii* plants used as the male (denoted 1250–a and 1250–b) were not identical. Since apomictic seedlings are clonally identical to the female parent, presence of any unique male trait in the progeny indicates that that seedling is a true sexual seedling and not an apomictic. Thus the molecular markers and morphological data were used to separate the seedlings into a set of apomictic and non apomictic seedlings. The variation among fruit characters of the apomicts provides useful information on the effect of environmental and edaphic factors on fruit characters of genetically identical *Opuntia*s.

The thornless, non–apomictic seedlings are being evaluated by Juan Carlos Guevara at IADIZA in Mendoza, Argentina for faster growing, more cold hardy, spineless forage varieties (Felker *et al*., 2008).

There was great variation in the cladode, spine and fruit characters of spiny non apomict seedlings of this cross. This variation appears to overlap with taxonomic characters of other North American spiny species, i.e. *O. dillenii, O humifusa, O. vulgaris* and *O. stricta*.

An evaluation of 70 apomictic and 57 non apomictic seedlings for fruit characters, spine characters, resistance to a golden scab disease and date of maturation in a four year old planting of these seedlings in Santiago del Estero, Argentina is reported below.

Materials and methods

The spineless *O. ficus–indica* (1281) was used as the female parent, after emasculation and bagging as described by Wang *et al*. (1996). After removing the perianth covering the stigmas, an entire fruit of the Texas native *O. lindheimerii* (1250) was taken to the emasculated fruit of 1281(female parent) and brushed on the stigma. This type of pollen transfer provided greater fruit set than efforts using isolated pollen. The emasculated flower was then rebagged and this repeated for several days. Two plants of *O. lindheimerii* (1250) collected on the W.A. Maltsberger Ranch near Cotulla, Texas in 1985 were used as the male parent. At the time of pollination, these males were believed to be derived from the same plant.

Dr. Marc Baker at the Southwest Research Institute performed chromosome counts on the two male parents and two non apomictic progeny from pollen.

In July 1998, approximately 10 cm tall, 0.5 cm diameter seedlings of these progeny were transferred from Texas A. & M, University at Kingsville and established in January 1999 under drip irrigation on the Universidad Nacional de Santiago del Estero experimental station near Santiago del Estero, Argentina on a 1.7 by 3.0 m spacing.

The cultural operations included complete weed control in and between the rows. After combinations of glyphosate and hand tools, achieved virtually bare ground, diuron was applied in a pressure regulated backpack sprayer with a boom of four nozzles (8001) spaced 50 cm apart at a concentration of 35 g l^{-1} of 85 % active compound. Diuron was typically applied once in the spring at the onset of the rains, once in mid–summer in the middle of the rainy season and once in late summer/fall to provide residual for the coming year. The plants were fertilized yearly with 110 g per plant of 15–15–15 as suggested by Potgieter (1996, pers. comm.). Control of the burrowing insect cactoblastis (*Cactoblastis cactorum*) was achieved with four yearly sprays of the insecticide carbaryl (Sevin) at a dose of 1 g l^{-1} active ingredient along with a coadjuvant that we believe helps penetrate the cuticle to kill some of the larvae within the cladode. The nematicide carbofuran (Furadan) was applied to the base of the stems to control bacteriophagous nematodes that occurred above the soil line (Doucet *et al*., 2002).

Apomictic seedlings are clonally identical to the female parent (in this case the commercial red fruited TAMUK 1281) with no genetic material from the male parent. As many of the four year old progeny had great resemblance to the female parent, molecular markers known as RAPDs were used test a subset of progeny for apomixes. However, subsequent RAPD data has shown that the two male parents were different genotypes.

The DNA isolation and PCR amplification from degenerate primers was performed by Dr. Mark Massoudi, Ag–Biotech Inc. (Hollister, CA) with genomic DNA being extracted by the method of de la Cruz *et al*. (1997). The first objective was to find RAPD Primers that could distinguish the female (TX1281) and the two male parents (TX1250–a and TX 1250–b). This was accomplished using primers UBC117, UBC 110, GEN 801, GEN 821 and GEN 866 in Table 1. An expanded population of parents and putative progeny were then tested using the five primers above and the following additional primers UBC 122, UBC 126, UBC 308, UBC 322 and UBC 811(Table 1). This new experiment included the female parent, both males, four "female looking putative apomicts" and nine "male looking non apomicts" that included a wide range in morphological and date of fruit maturation characteristics. For these 10 primers, 123 bands were read of which 71 exhibited polymorphism between the parents. Photos of the banding patterns, correlation matrices of various primer bands are available from the author.

Table 1. Primers used to uniquely identify *O. ficus–indica* clone 1981, *O. lindheimerii* 1250–a, *O. lindheimeri* 1250–b, nine "male looking" non apomicts and four "female looking" putative apomicts.

Primers used to separate parents (5' to 3')	
UBC117	TTA GCG GTC T
UBC 110	TAG CCC GCT T
GEN 801	CGA TTC CAC T
GEN 821	TTC TCT GCC T
GEN 866	GGT CGA AAG T
Additional primers used in combination with above to separate parents and progeny (5' to 3')	
UBC 122	GTA GAC GAG C
UBC 126	CTT TCG TGC T
UBC 308	AGC GGC TAG G
UBC 322	GCC GCT ACT A
UBC 811	GAG AGA GAG AGA GAG AC

All the seedlings were visually evaluated for their potential to be apomicts. If the seedlings had any of the male characters, i.e. spines, small pads, small fruit, bluish color they were deemed not to be apomicts. Those seedlings not distinguishable visually from the female could be sexual progeny, but seedlings with any male character could not be apomicts.

As noted below, especially in the cool winter months, the cuticle layer of some of the plants changed from green to a yellowish/brown. If this brown cuticle was peeled off, the green, firm cladode with a normal smell was noted. In the summer with active new growth, the plants outgrew this symptom with the new (but not old growth) appearing normal. Samples with this symptom were sent to the Texas A. & M. College Station plant diagnostic lab as well as to the Universidad Nacional de Tucuman pathology lab. No organism could be consistently isolated from this tissue and the pathologists believed that this was a plant physiological response to stress. In Mexico a similar symptom has been noted as "Mancha de Oro" or gold stain disease. For the sake of simplicity, we here denote this symptom as the "Golden Scab Disease" and an example of the symptoms is shown in Figure 1. This was ranked as the percentage of the total plant covered by this symptom in September 2003 which was late fall in Argentina. It is to be noted that the same symptom occurred in duplicate copies of the progeny in the University of Georgia greenhouse.

Under the summer rainfall conditions the plants grew more rapidly than similar plants in Texas. For example in the winter of 2002 (July), many of the female looking plants were more than 3 m tall and were pruned to keep them under 2.4 m in height.

There were 127 progeny in the trial, of which fruit was obtained from 106 plants. Some of the plants that were severely affected by a Golden Scab disease did not have fruit. However, the spine length was recorded for all of these seedlings. As the date of the first mature fruit occurred over a 3 month period, every Monday, the plants were examined for fruit maturity and harvested when approximately 40% full color occurred. Thus, all the measurements were made on fruits of equal maturity basis, although this occurred over a tree month period. Fruit analyses were made on four fruits of each of the progeny and on 10 fruits of the male and female parents. After the fruits were collected they were sent at night by overnight bus (6 hr) from Santiago del Estero to the Universidad Nacional de Cordoba where they were analyzed.

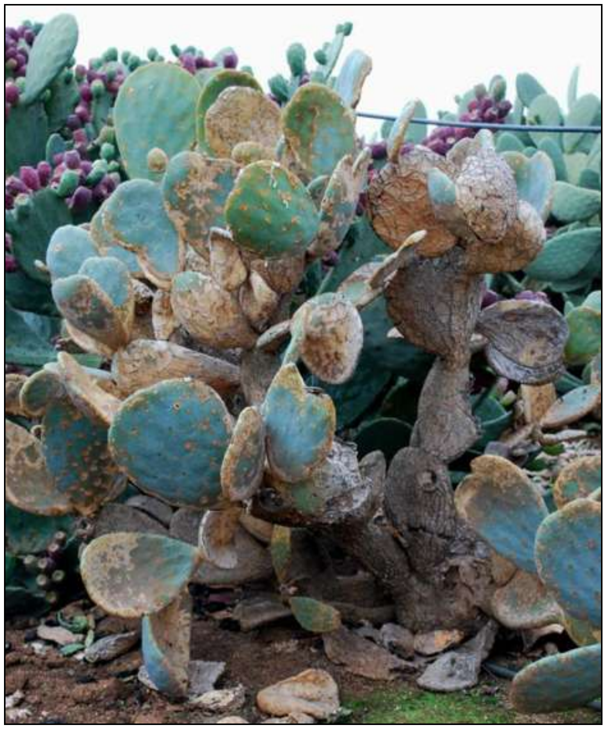

Figure 1. Example of "Golden Scab Disease" on spineless progeny #47.

Fruit sugar soluble solids and pH was made on the homogenized pulp using a temperature corrected refractometer (ATAGO ATC 20E) and pH meter respectively. Fruit firmness was measured on the pulp with a hand penetrometer from www.qasupplies.com/fruitfirtes.html. Seed weight was reported for the cleaned, air dried seeds.

Results

For the 71 polymorphic bands (from the 10 RAPD primers) among the female parent, two male parents, nine male looking progeny and four female looking (apomictic progeny) all but seven of these bands segregated in the progeny. Of the 13 progeny, four had the identical band pattern of the female parent. These four progeny were phenotypically, non–distinguishable from the female parent and were randomly selected from that group. Three progeny had bands from the male 1250–a as well as bands from the female. Six progeny had bands from the male 1250–b as well as the female.

All of the *O. ficu–indica* chromosome counts thus far (Powell and Weedin, 2001) have indicated this species is octaploid. Chromosome counts by Marc Baker found that the both male parents 1250–a and 1250–b were hexaploid and that two of the non apomictic progeny i.e. #42 and #122 were 2n=77.

Photographs of the two males, 1250–a and 1250–b and one of the apomictic progeny #146 are shown in Figure 2. These males had fewer spines than most *O. lindheimerii* as they were selected by Rancher W.A. Maltsberger to plant for forage for his ranch south of San Antonio, Texas. It is to be noted that the males have small bluish pads, while the female has long greenish pads without spines. We have subsequently observed that the fruit from male 1250–b matures considerably later than for 1250–a. The data presented here for the Texas native *O. lindheimerii* accession (1250) male parent #2 (soluble solids mean = 7.8, 95% Confidence Interval (CI) = 0.91), fruit weight (mean = 35.4 g 95% CI= 3.44 g), and seed content (mean =20.4 per 100 g pulp, 95% CI =2.9 g per 100 g pulp), falls within the range of 5 other *O. lindheimerii* plants on the W. A. Maltsberger Ranch (Chavez–Ramirez *et al*., 1997) for fruit soluble solids (mean = 7.8, 95% CI =1.0), size (mean = 46.7, 95% CI = 9.0 g) and seed content (mean = 15.7 g/100 g pulp, 95% CI = 5.7 g/100 g pulp).

Figure 2. Photograph of male parents *Opuntia lindheimerii* 1250–b top left, *O. lindheimerii* 1250–a bottom left, and *O. ficus–indica* apomictic progeny #146 right. Note the small blue color cladodes of the male parents and the greenish color of the female parent and the fact that fruit ripening was more advanced on 1250–a, than 1250–b. All photographs for Figures 3, 4 and 5 were taken the same day.

Photographs that illustrate the variation for the spiny non apomicts in cladode color, shape, spine characters and fruit size/shape are shown in Figure 3. These progeny exhibited considerable differences in spines/areole, spine length, cladode shape, cladode color and as noted below in fruit size. Progeny 108 with a fruit weight of 108 g was more than double that of the male parents. Note fruits on 119, 111 and 49 were mature/overmature while some fruits were immature on 108 and 109.

An example of the spineless, non–apomicts is in Figure 4. The progeny 80, 89 and 130 have the blue color of the male parent while progeny 46 has more of the green color of the female parent. All of these fruits were more than double the size of the male parent with #46 and 80 being the largest at 111 g. As discussed later, progeny 46 had the greatest fruit production of the spineless non apomicts and second highest fruit load after the spiny 143.

Figure 3. Examples of variation in spines, cladode and fruit morphology among the spiny non apomictic seedlings. Top left to right progeny 119, 109, 111, bottom left to right 108, 49. All photos were taken on the same day.

Figure 4. Examples of lack of spines and variation in cladode and fruit morphology among the spineless non apomictic seedlings. Top left to right progeny 42, 89, 46, bottom left to right 130, 80. The extensive number of fruits in the background of 42 were from the same plant. Note the extensive fruit production on #46.

A comparison of spininess, percent of the plant covered by Golden Scab and apomixis classification is presented in Table 2. At least 44.9% of the seedlings were non apomictic with 68 % of the non apomictics being spiny. The fact that the male was thorny, the female thornless and that 68 % of the non apomictics had some thorns would suggest that thorniness is probably a simply inherited trait.

Table 2. Apomixis classification, and segregation of spine length and Golden scab disease on progeny of *Opuntia lindheimerii* (1250) by *O. ficus–indica* (1281).

Progeny/parent	Spine length (cm)	Golden scab rating (0–100%)	Non apomictic seedlings =1	Progeny/ parent	Spine length (cm)	Golden scab rating (0–100%)	Non apomictic seedlings =1
Female (1281)	0	0		39	0	0	
Male (1250)	2	0		40	0	0	
1	4	30	1	41	3.5	100	1
2	0	0		42	0	0	1
3	0	0		43	0	0	
4	0	0		44	4	0	1
6	0	0		45	0	0	
7	0	0		46	0	0	1
8	0	0		47	0	100	1
9	0	0		48	3	100	1
10	0	0		49	4	0	1
11	0	0		50	4	100	1
12	0	0		51	0	0	
13	3.5	0	1	52	0	0	1
14	0	0		53	0	0	
15	0	0		54	0	0	
16	0	0		55	0	0	1
18	0	0		57	0	0	
19	0	0		58	0	0	
20	0	0		59	0	0	
21	0	0		60	4	100	1
22	0	0		61	0	0	
23	3	100	1	62	0	0	
24	0	0		63	0	0	
25	0	0		64	0	0	1
27	0	0		66	0	0	
28	4	0	1	67	0	0	
29	0	0		69	0	0	
30	2	0	1	70	0	0	
33	0	0		71	0	0	
34	0	0		72	0	0	
36	2.5	20	1	73	0	0	
37	0	0		74	0	0	
38	0	0		75	3	100	1

Table 2. Continued

Progeny/ parent	Spine length (cm)	Golden scab rating (0–100%)	Non apomictic seedlings =1
76	0	0	
77	3	95	1
79	0	0	
80	0	0	1
81	0	0	
82	2.5	50	1
83	0	0	1
85	0	0	1
86	0	0	
87	0	0	
88	4	0	1
89	0	0	1
91	0	0	1
92	0	0	
93	0	0	
94	0	0	1
95	0	0	1
96	0	0	
97	0	70	1
98	0	0	
100	0	0	
101	0	0	
102	0	0	
103	3	0	1
104	2.5	0	1
106	3	0	1
107	3	0	1
108	3	0	1
109	3.5	0	1
110	0	0	1
111	2	50	1
112	4	0	1
113	4	0	1
114	4	0	1
115	3	30	1
116	4	0	1
117	4	0	1
118	4	30	1
119	4	0	1
121	4	0	1
122	4	0	1
124	4	0	1
125	0	0	
126	0	0	
127	0	0	
128	2.5	0	1
130	0	40	1
131	0	0	1
132	0	0	
133	0	0	
134	2	0	1
135	0	0	
136	0	0	
139	2	100	1
140	4	0	1
142	0	0	
143	4	0	1
145	0	0	
146	0	0	
148	0	0	
150	0	0	1
Percent with trait	30.7%	13.2%	44.9%

Golden scab was estimated as the total percent of the plant covered. If any trait of the male, i.e spines, cladode shape, fruit shape or cladode color was present, the seedling was classified as being non apomict. Other seedlings could have other salient traits that would not put them in the non apomict classification.

The golden scab disease occurred only in the non apomicts, with a frequency of 29.8 % and occurred both in spiny and non spiny seedlings. Over the four year course of this study, this Golden Scab never affected the large apomictic plants in spite of the fact that in some cases, these plants were on all four sides of severely affected plants. Perhaps as the Texas A&M pathology lab has suggested, these symptoms are not a result of a pathogenic organism but are a result of physiological stress. Alternatively, the female plants may just possess resistance to the pathogens. The progeny with the most severe symptoms of the golden scab disease were more highly correlated with the male parent in having small fruits and cladodes and in being spiny. Some of the very severely affected plants with Golden Scab never produced fruit which was probably due to stress from this disease.

Measurements of various fruit characters are presented in Figures 5–13, grouped according to non apomicts and apomicts.

There was nearly a tree fold range in the fruit size (Figure 5) for the non apomictic fruits, with the male being close to the small end of the range. This would suggest that combination with the larger sized female had a significant effect on fruit size. The largest fruits of the non apomicts, i.e. 115–120 g approached the size of commercial fruits.

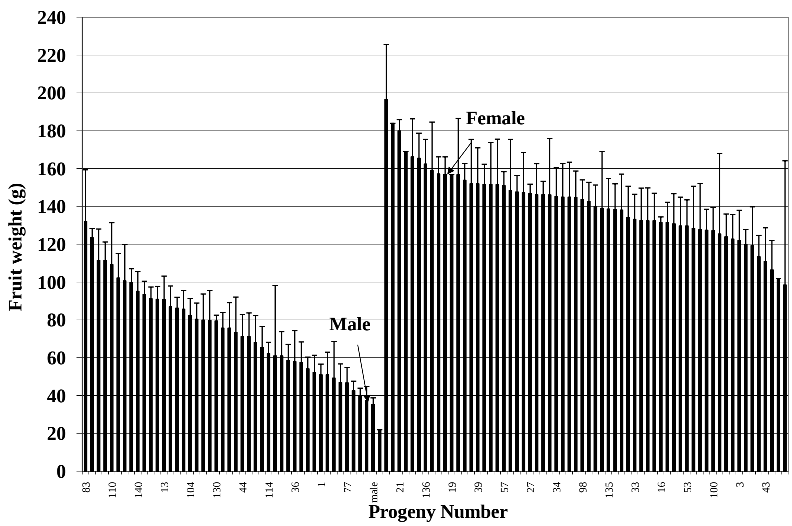

Figure 5. Fruit weight (g) from four year old seedlings of O *lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The first four fruits per plant with greater than 40 % color were harvested for these measurements. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

There was a very large variation in yield per plant (fruit number x fruit weight) as can be seen in Figure 6. One of the spiny, non apomictic progeny had the highest fruit number (progeny 36 had 235 fruit of 58 g). One of the apomictic seedlings #136 had 136 fruit of 162.5 g with the greatest yield of 22.1 kg per plant. In spite of the small fruit size of the non apomicts, the fruit yield per plant was surprisingly similar between the non apomictic and apomictic fruits. Given the 1.7 by 3.0

m spacing, the 22 kg fruit per plant would translate into a yield of 43,300 kg ha^{-1}. Obviously this individual plant productivity without a border buffer will not be obtained with this close spacing in large fields but this does give an indication of high commercial yields.

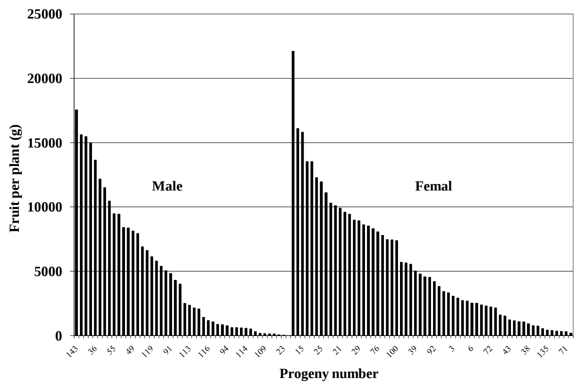

Figure 6. Fruit yield (g) derived from fruit number and fruit weight from four year old seedlings of *O. lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The first four fruits per plant with greater than 40% color were harvested for these measurements. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

Although the range for the pulp percentages was greater for the non apomicts than the apomicts (Figure 7), the male was in the center of the distribution, and it is not clear if the female had much of an effect on pulp %. However, the greatest pulp % in the non apomictic seedlings approached that of the female parent. Inglese *et al*. (1995) has suggested that an edible pulp/whole fruit weight of 55 % is the minimum for a commercial variety. The apomicts were not significantly different from this 55 % and a few the non apomictic seedlings approached this value.

For Brix, the female parent was approximately in the middle of the distribution of the apomictic while the male was at the very low end of the distribution for the non apomictic seedlings (Figure 8). Thus it appeared as if the female had a very significant effect in improving the Brix of the progeny. The greatest Brix of the non apomictic seedlings was similar to that of the distribution of the apomictic seedlings.

Pulp firmness (Figure 9) is an important fruit character with many of the high yielding orange, yellow and red fruits, including the female parent 1281, being too soft at maturity (Felker *et al*., 2005). It has been suggested that the pulp (not peel) firmness of 2 kg as found in the typical Argentine green varieties be considered ideal. In contrast to the beneficial effect of the female in increasing fruit size, Brix and possibly pulp %, the female seemed to have the effect of lowering the firmness in the non apomictic seedlings.

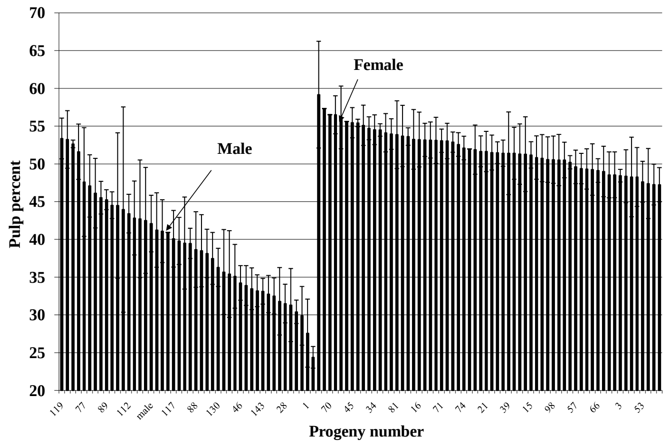

Figure 7. Percent pulp from four year old seedlings of *O. lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The first four fruits per plant with greater than 40% color were harvested for these measurements. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

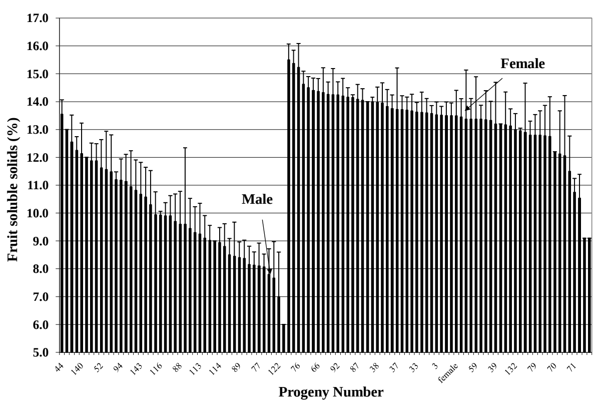

Figure 8. Fruit soluble solids (Brix) from four year old seedlings of *O. lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The first four fruits per plant with greater than 40 % color were harvested for these measurements. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

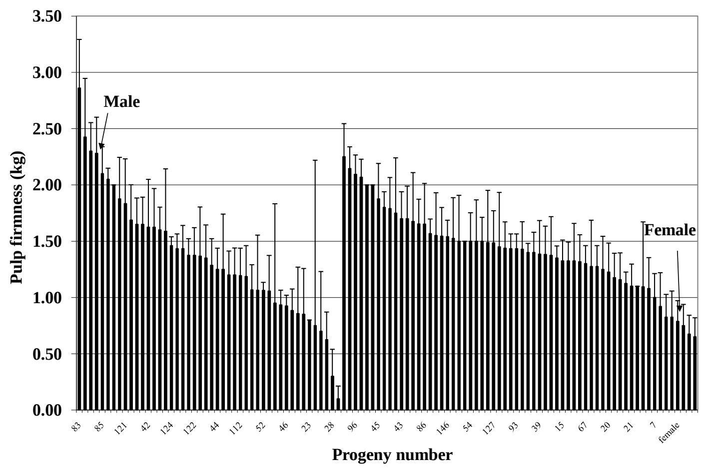

Figure 9. Pulp firmness (kg) from four year old seedlings of *O. lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The first four fruits per plant with greater than 40 % color were harvested for these measurements. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

Fruit pH generally is a very constant fruit character. However, as can be seen in Figure 10, fruit pH varied considerably more in the non apomictic seedlings than in the apomictic ones.

Of all the fruit quality parameters, perhaps none has attracted so much attention as the quest for low seedy varieties which would have either fewer, smaller or softer seeds. Unlike the Israeli parthenocarpic variety BS 1(Weiss *et al*., 1993), or the low seedy varieties 1319 and 1321 (Parish & Felker, 1997), neither of the parents in the cross reported here has promise in introducing low seedy varieties. Nevertheless there was a six fold difference among the varieties in the grams of seeds/100 g fruit (Figure 11). None of the progeny had as great a value for seeds per 100g pulp as the male parent. However some non apomictic seedlings were lower in seeds per 100 g pulp than the set of apomictic seedlings (Figure 11).

Pimienta and Engleman (1985) have stated that since the edible fruit portion develops from the funiculus of the seeds, it will be impossible to develop seedless fruit and that the greater is the seediness the greater is the fruit size. In Figure 12 two highly significant relationships can be seen between pulp weight and seed weight for the apomicts and non apomicts respectively. This suggests that it will be difficult to break this relationship to develop seedless fruits. Ideally what one is looking for are values that are below and to the right of the regression line. The value for seeds/100 gram edible pulp of the male parent was significantly improved (lowered) in the non apomictic progeny by the cross with the *O. ficus–indica*.

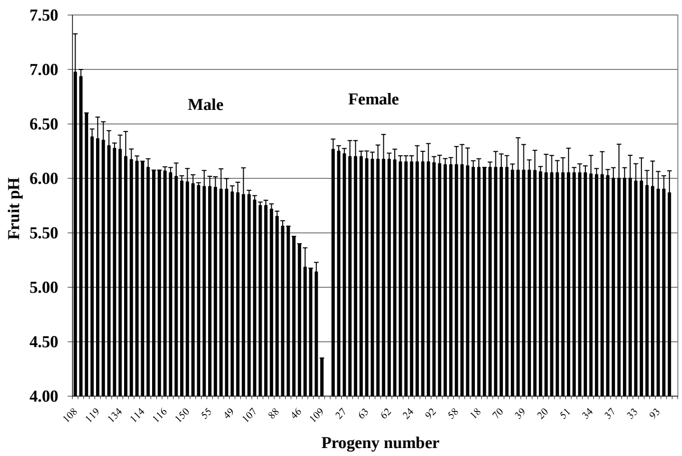

Figure 10. Fruit pH from four year old seedlings of *O.lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The first four fruits per plant with greater than 40% color were harvested for these measurements. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

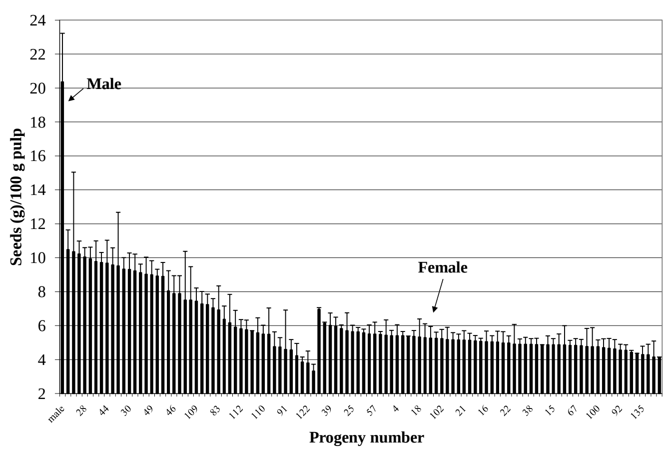

Figure 11. Seed content (g/100 g edible pulp) from four year old seedlings of *O. lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The first four fruits per plant with greater than 40 % color were harvested for these measurements. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

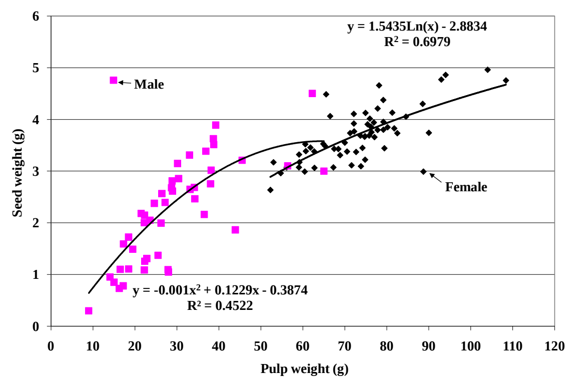

Figure 12. A comparison of edible pulp size (g) versus seed weight (g) of combined apomictic and non apomictic fruits of the cross of *O. lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281.

Barbera *et al*. (1994) examined correlations between fruit and pulp weight of Gialla and Rossa cultivars and also found a highly significant correlation between pulp weight and total dry weight (r=0.826, p=0.001) and between pulp weight and aborted seed dry weight (r=0.469, p=0.001). Given the objective to develop "seedless" fruits, the close correlation between pulp weight and seed content is discouraging. However, the fact that the progeny were intermediate between the high seed/low pulp weight of the male parent and the lower seed weight/high pulp weight of the female parent, suggests that crosses of commercial fruit varieties with low seedy varieties such as Ofer BS1 or Chilean clones 1319/1321 may be able to lower the seed content of commercial varieties.

The fruit of a considerable number of the non apomictic seedlings matured much later than did the female parent as can be seen in Figure 13. In this figure, the date at which the first commercially sized fruit was harvested was denoted as day 0. Thus four fruits were obtained from all the apomictic seedlings within about 20 days of this first harvest. In contrast, some of the non apomictic fruits were not mature for 80 days after the first apomictic fruit harvest. Progeny 23 with spines, 22 g fruit of 9.0 % soluble solids and 100% Golden Scab disease had the longest delay of 78 days after the female parent.

Discussion

Our original objective to develop a population useful for a mapping population was not successful due to the presence of many apomicts. Nevertheless much useful information was learned about possibilities of making a wide cross, the high percentages of apomictic seedlings that result from such crosses, the great range in morphological characters among the apomictic progeny that are normally used for species classification, possibilities for manipulating economically important fruit traits (Brix, fruit weight, pulp %) through controlled crosses and the possible direct use of some progeny for cold hardy spineless forage varieties.

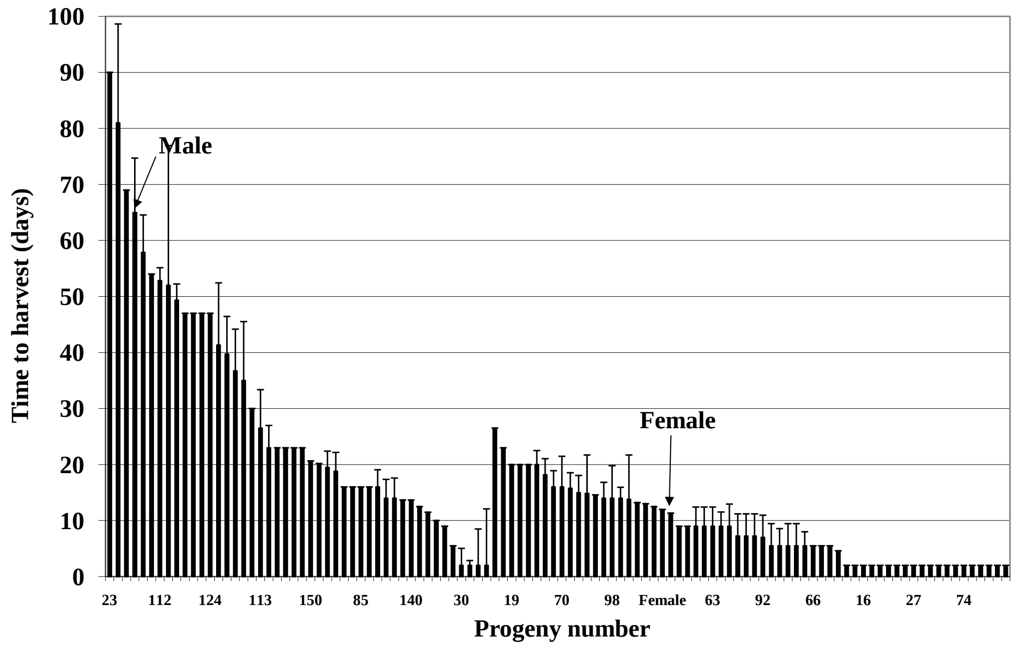

Figure 13. Number of days from the harvest of the first fruits in the trial of four year old seedlings of *O. lindheimerii* 1250–a and *O. lindheimerii* 1250–b x *O. ficus–indica* 1281 grown in Santiago del Estero, Argentina. The results are ranked within groups of non–apomictic plants with some male traits and apomictic seedlings with female traits.

The variation in fruit, cladode and spine morphology among the non apomicts seems to encompass the taxonomic characters for *O. stricta* (http://www.sms.si.edu/IRLspec/*Opuntia*_stricta.htm) and similar spiny red fruited *Opuntias* of low stature in southeastern USA i.e *O. humifusa*, *O. vulgaris* and *O. dillenii*. Due to the inadvertent introduction of the burrowing insect *Cactoblastis cactorum* into the USA and its subsequent spread along the US gulf coast in these low stature, spiny *Opuntia*s it would be helpful to know the genetic affinities of the southeastern USA species(Majure and Ervin, 2007) to their more westward *Opuntia* relatives. Certainly one possibility is that one or more of these could be derived from *O. lindheimerii* that is much more ubiquitous in the arid regions of southwestern USA and Mexico. The classic proof of the validity of such species is that they would not cross with each other and that seedlings germinated from these species would be true to the parental type. However given the presence of apomixis in *Opuntia*, it is possible that a sterile progeny of cross could be propagated clonally by seed and appear to be true to type. While molecular markers have been used for commercial fruit types (Griffiths, 2004; Luna–Paez, 2007; Chessa *et al*., 1996; Uzun, 1996; Oelofse, 2002) we are not aware of any hybridization or molecular classification of these low stature, spiny *Opuntia*s. Given the ease of hybridization of *O. lindheimerii* to *O. ficus–indica*, it is seems highly likely that they could be related or possibly variants of *O. lindheimerii*.

Pimienta (1990) has hypothesized that the current commercial varieties arose over centuries of selection, with the most desirable fruits from wild *Opuntias* being brought to homes, consumed and further selections made from seedlings of defecated seeds that were hybrids with new genetic combinations (Pimienta, 1990). As support for this hypothesis Pimienta noted that the wild types are virtually all diploid (2n=22), while the commercial types are tetraploid, octaploid (Palomino and Heras, 2001) and various deviants thereof (7n=77) (Pimienta, 1990). This work supports this hypothesis since when the hexaploid *O. lindheimerii* parents with small, low Brix fruits were crossed with Octaploid, *O. ficus–indica* large, high soluble solid fruits, the resulting heptaploid progeny had greater Brix and fruit size than the wild *O. lindheimerii* parent.

Pimienta and Engleman (1985) reported that the pulp of the fruit was derived from the funiculus of the seeds, suggesting that it will be difficult to obtain seedless fruits. However, Weiss *et al*. (1993) reported the presence of a parthenocarpic fruit with small nearly aborted seeds. Nevertheless the strong correlations observed here between seeds per 100 g of edible pulp and pulp size suggest that while it may be possible to reduce the seeds per 100 g pulp, it will be very difficult to produce seedless cactus pears.

The presence of spines and the important fruit characters of fruit weight, pulp percent, total soluble solids, fruit seeds and firmness were intermediate between the male and female parents. No transgressive segregants were observed. The female could be considered to improve the fruit quality of the male by increasing fruit weight, pulp percent, total soluble solids, and seeds per 100 g pulp (by lowering it) while the female lowered the fruit quality of the male by decreasing pulp firmness. The opposite view could be taken that the male decreased all the fruit quality characteristics of the female. These results suggest that if a commercial fruit variety were lacking in any one of these traits and if another variety could be found with an improved trait, the progeny would have an improved trait over the existing variety but not equal to the donor parent. However, all the other fruit characteristics of these parents would be intermediate as well. Perhaps with a large enough population the desired combination of characters could be found among the progeny.

Some of these progeny may be useful in their own right. The most cold hardy of the spineless cacti useful for livestock feed is the very slow growing *O. ellisiana* (Felker, 1995). We believe the spiny male parent, *O. lindheimerii*, which occupies the same geographical range as *O. ellisiana* has approximately the same cold hardiness as the *O. ellisiana*. Thus progeny with smaller, bluish cladodes similar to the male parent may also carry more of the cold tolerant genes than the female parent and have the added benefit of being spineless. Juan Carlos Guevara, of the Instituto Argentino de las Investigacion de Zonas Áridas (IADIZA) in Mendoza, Argentina has measured the biomass and mean frost damage of the ten spineless non apomictic segregants (42, 46, 64, 80, 83, 85, 89, 94, 97, and 150) in replicated trials near Mendoza, Argentina (Felker *et al*., 2007) and found that # 46 had more than twice the biomass of *O. ellisiana* with the same high freeze tolerance as *O. ellisiana.*

Given the many imperfections in current *Opuntia* varieties, i.e. large seeds and spines on high Brix/high firmness Mexican Burrona type varieties, low firmness on Italian Gialla varieties, thick peels on high sugar/high firmness Argentine varieties, we believe the priority should be to focus on identification of genes for critically important traits such as: (a) seed size and quantity, (b) cold hardiness, (c) total soluble solids content, (d) fruit firmness (e) yield of fruits, (f) color of fruits (g) resistance to cochineal, bacterial soft rots and black rots and (h) spine characters. It will be of interest to know if all the genomes of these polyploid hybrids contribute equally to the phenotypic characteristics, if the genes segregate independently and if there will be problems with negative correlations between important characters such as yield and fruit sugar content. Additional full sibling crosses will be needed to determine the dominance, heritability and linkage of these characters.

Acknowledgements

The financial assistance of the Fernández Forestry Experiment Station of the Catholic University of Santiago del Estero, and of the USDA cooperative agreement No 58–3148–8–041 are gratefully acknowledged.

References

Barbera, G., P. Inglese, and La Mantia, T. 1994. Seed content and fruit characteristics in cactus pear (*Opuntia ficus–indica* Mill). Scientia Horticulturae 58: 161–165.

Chavez–Ramirez, F., Wang, X., Jones, K., Hewitt, D., and Felker, P. 1997. Ecological characterization of *Opuntia* clones in south Texas: Implications for wildlife herbivory and frugivory. Journal of the Professional Association for Cactus Development 2: 9–19.

Chessa, I. Nieddu, G., Serra, P., Inglese, P. and La Mantia, T. 1996. Isozyme characterization of *Opuntia* species and varieties from Italian germplasm. Acta Horticulturae 438: 45–52.

Collazo–Siqués, P., Valverde, M. E., Paredes–López, O., and Guevara–Lara, F. 2003. Expression of ripening–related genes in prickly pear (*Opuntia* sp.) fruits. Plant Foods Hum. Nutr. 58(4): 317–26.

de la Cruz M., Ramírez F., and Hernández H. 1997. DNA isolation and amplification from cacti. Plant Molecular Biology Reporter 15: 319–325.

Doucet, M.E., Weht, S., and Felker, P. 2002. Nematodes as a possible cause of damage to *Opuntia ficus–indica*. Journal of the Professional Association for Cactus Development 4: 84–89.

Felker, P., Rodriguez, S. del C., Casoliba, R.M., Filippini, R., Medina, D., and Zapata, R., 2005. Comparison of *Opuntia ficus–indica* varieties of Mexican and Argentine origin for fruit yield and quality in Argentina. Journal of Arid Environments 60: 405–422.

Felker, P., Bunch, R.A., Borchert, D.M., and Guevara, J.C. 2007. Potential global adaptivity of spineless, progeny of *Opuntia ficus–indica* 1281 x *O. lindheimerii* 1250 as forage cultivars adapted to USDA cold hardiness zones 7 and 8. Acta Horticulture 11:333–342.

Felker, P., Stintzing F.C., Mussig, E., Leitenberger, M., Carle, R., Vogt, T., and Bunch R. 2008. Colour inheritance in cactus pear (*Opuntia ficus–indica*) fruits, Annals of Applied Biology 152: 307–318.

Grant, V., Grant, K.A., and Hurd, P.D. 1979. Pollination of *Opuntia lindheimerii* and related species. Plant Systematics and Evolution 132: 313–320.

Griffiths, M. P. 2004. The origins of an important cactus crop, *Opuntia ficus–indica* (Cactaceae): new molecular evidence. American Journal of Botany 91(11): 1915–1921.

Inglese, P., Barbera, G., and La Mantia, T. 1995. Research strategies and improvement of cactus pear (*Opuntia ficus–indica*) fruit quality and production. Journal of Arid Environment 29: 455–468.

Luna–Paez, A., Valadez–Moctezuma, E., Barrientos–Priego, A.F., and Gallegos–Vázquez, C. 2007. Characterization of *Opuntia* spp. by means of seed with RAPD and ISSR markers and its possible use for differentiation. Journal of the Professional Association for Cactus Development 9: 43–59.

Majure, L.C., and Ervin, G. N. 2007. The *Opuntias* of Mississippi. Haseltonia 14: 111–126.

Mondragon, C. 2001. Verification of the apomictic origin of Cactus pear (*Opuntia* spp Cactaceae) seedlings of open pollinated and crosses from central Mexico. Journal of the Professional Association for Cactus Development 4: 49–57.

Oelofse, R. M. 2002. Characterization of *Opuntia ficus–indica* cultivars in South Africa. M.S Thesis, Bloemfontein, University of the Free State, South Africa.

Palomino, G., and Heras, H.M. 2001. Karotypic studies in *Opuntia* cochinera, O hyptiacantha and O. streptacantha (Cactaceae). Caryologia 54: 147–154.

Parish, J., and Felker, P. 1997. Fruit quality and production of cactus pear (*Opuntia* spp) clones selected for increased frost hardiness. Journal of Arid Environments 37: 123–143.

Pimienta, E. 1990. El Nopal Tunero. Universidad de Guadalajara. Guadalajara, México. 246 pp.

Pimienta, B.E., and Engleman, M. 1985. Desarollo de la pulpa y proporcion en volumen, de los componentes del loculo maduro en tuna (*Opuntia ficus–indica* (L.) Mill.). Agrociencia 62: 51–56.

Powell, A.M., & Weedin J. F. 2001. Chromosome numbers in Chihuahuan Desert Cactaceae. III Trans Pecos Texas. American. Journal of Botany 88: 481–485.

Silos–Espino, H., Valdez–Ortiz, A., Rascón–Cruz, Q., Rodríguez–Salazar, E., and Paredes–López O. 2006. Genetic transformation of prickly–pear cactus (*Opuntia ficus–indica*) by *Agrobacterium tumefaciens*. Plant Cell, Tissue and Organ Culture 86(3): 397–403. doi10.1007/s11240–006–9123–1.

Uzun, I. 1996. Fruit and cladode isozymes in cactus pear. Acta Horticulturae. 438: 53–56.

Velez–Gutierrez, C., and Rodriguez–Garay, B. 1996. Microscopic analysis of polyembriony in *Opuntia ficus–indica*. Journal of the Professional Association for Cactus Development 1: 39–49.

Wang, X., Felker, P., Paterson, A., Mizrahi, Y., Nerd, A. & Mondragon–Jacobo, C. 1996. Cross–hybridization and seed germination in *Opuntia* species. Journal Professional Association for Cactus Development 1: 49–60.

Wang, X., Felker, P., and Paterson, A. 1997. Environmental influences on cactus pear fruit yield, quality and cold hardiness and development of hybrids with improved cold hardiness. Journal of Professional Association for Cactus Development 2: 48–59.

Wang, X., Felker, P., Burrow, M.D., and Paterson, A. H. 1999. Assessment of genetic diversity among *Opuntia* genotypes, reflected by DNA markers. Journal Professional Association for Cactus Development 3: 3–14.

Weiss, J., Nerd, A., and Mizrahi, Y. 1993. Vegetative parthenocarpy in the cactus pear (*Opuntia ficus–indica* (L.) Mill. Annals of Botany 72: 521–526.