Review

Enhancement of economical value of nopal and its fruits through biotechnology

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Abstract

In Mexico, the nopal (*Opuntia* spp.) for human consumption as vegetable and for production of prickly pear fruits is cultivated in several thousands of hectares. This crop has been associated to our culture since the pre-Hispanic age and its demand has increased in the last years due to its remarkable nutraceutical potential and to the content of some key nutrients such as calcium, iron and vitamin C, among others. Nopal is a crop with a high capacity of adaptation to different environmental conditions, mainly arid and semiarid. It represents a very good alternative for the agriculture in these areas, where the soils are poor or are becoming poor and result in very low yield of traditional cultivars. This crop is increasing its economical value in exportations and is becoming a very important alternative for the people who live in the production areas. However, the plant still has undesirable characteristics such as mucilage and spines, pathogen sensitivity, and high number of seeds in its fruits with a poor postharvest life that limits the production and consumer acceptance. We herewith review the efforts that have been done in order to improve the nopal characteristics and its fruit through biotechnology.

Key words: Prickly pear, *Opuntia*, fruit ripening, genes, nutraceuticals

Abbreviations

ABA	Abscisic acid
ACC	Aminocyclopropane
AFLP	Amplified fragment length polymorphism
ANA	Naftalenacetic acid
BA	Benzyladenine
BAP	Benzylaminopurine
β–gal	Beta–galactosidase
cDNA	Complementary DNA
cpSSR	Chloroplastic simple sequence repeat
DAP	6–(γ,γ–Dimethyallylamino) purine
EST´s	Expressed sequences tags
GA_3	Giberelic acid
GUS	Glucuronidase

Ha	Hectares
HPLC	High performance liquid chromatography
IBA	Indole–3–butyric acid
ISSR	Internal simple sequence repeat
ITS	Internal transcripts spacers
kDA	Kilodaltons
KIN	Kinetin
masl	Meters above sea level
mRNA	Messenger RNA
MS	Murashige and Skoog
ORF	Open reading frame
OsPG	*Opuntia* sp polygalacturonase cDNA
PCR	Polymerase chain reaction
PME	Pectin metil esterase
PG	Polygalacturonase
Pro	Proline
RAPD	Random amplified polymorphic DNA
rbcL	Ribulose biphosphate carboxilase large subunit gene
RFLP	Restriction fragment length polymorphism
RH	Relative humidity
RT–PCR	Reverse transcription–PCR
2,4–D	2,4–Dichlorophenoxyacetic acid

Botanical aspects

Nopal (*Opuntia* spp.) is endemic to Mexico but it is becoming an interesting alternative as a fruit and forage crop for semiarid areas of the world. Few varieties, originated from the Mexican germplasm, supply the world market. Nopal is propagated asexually for commercial purposes, but seed propagation is essential for breeding. A main constraint to breeding is apomixis, which has been reported in numerous Opuntias including those of horticultural interest like *O. ficus–indica.* Apomixis makes difficult the screening of individuals obtained from crosses and complicates the genetic studies (Mondragon–Jacobo, 2001). Nopal is a plant from the *Cactaceae* family, endemic to some regions of the American continent, where it mainly grows in the arid and semiarid areas. In Mexico nopal plants belong to the genera *Opuntia* and *Nopalea*, which include a huge number of species of different ripening behavior (Table 1) (Flores–Valdés *et al*., 1995).

Table 1. Cactus pear development and ripening.

Classification	Early ripening	Mid ripening	Late ripening
Days after flowering	80–115	115–130	135–140
Morphospecies	Naranjona, Reyna	Blanca cristalina, Burrona	Cardona, Charola

History

The use of nopal in Mexico initiates in the ancient Mesoamerican civilizations, when the people used to collect cladodes and fruits from wild materials, for feed and medicinal purposes (Velázquez, 1998). The Spanish conquerors spread nopal in America and Europe; they grew the plant in Portugal and in the south east Spain where it was distributed to the world. Now it is cultivated in Italy, Argelia, Morocco, Tunisia, Greece, Israel, India, Philippines, China, Australia, South Africa, Brazil, Argentina, Colombia and USA (Barbera *et al*., 1995; Pimienta–Barrios, 1990). In several

countries the cladodes are not consumed but the fruit is very popular (Muñoz de Chávez *et al*., 1995).

Uses

Nopal has played an important role at the economic and social aspects in our country through its history. It was used for the pre–Spanish people as a source of food, and for medicinal and construction purposes (Granados and Castañeda, 1991; Mizrahi *et al*., 1997; Pimienta–Barrios, 1994; Sáenz, 2000; Velázquez, 1998). Up to now, the uses have increased with the processing of the plant and its derivatives.

Nopal as crop

In Mexico, there are approximately 3 million ha of nopal plants, wild and cultivated (Flores–Valdés, *et al*., 1995); the plant is found in desert and arid zones, but it is also found in warm and template zones, from the sea level to 3000 masl. The main population of wild materials is located at the center of Mexico (Flores–Valdés, 2002).

The wild materials from these regions are used mainly as source of forage for cattle. The materials for human consumption were developed during thousand years at the agostaderos and familiar orchards, in a constant man selection (Flores–Valdés, *et al*., 1995). At the middle of the last century the nopal began its importance as crop and in the last decades has increased its consumption (Pimienta–Barrios, 1994; Flores–Valdés *et al*., 1995; Rodríguez–Salazar and Nava–Cedillo; 1999). At present there is an area of about 10,500 ha of nopal plantations in Mexico.

Nopal breeding

In the ancient culture the nopal growers used to pick up the cladodes from the most vigorous plants and took them to the family orchards, where the plants cross breeding took place in a natural way; the new materials were selected by the growers (Pimienta–Barrios, 1990; Flores–Valdés and Olvera, 1996). The cladodes and fruits have some characteristics that diminish their acceptance by the consumer: the presence of spines, the excessive mucilage, and the high number of seeds in the fruits; in addition to these aspects, there are some agronomic problems as plagues and pathogens (González *et al*., 2003; Flores–Valdés, 2002). Some of the traits to be considered for nopal breeding are: to disminish the number of spines and glochids, to disminish the mucilage content and acidity, to reduce the cuticle; and to improve the flavor, and to increase the nutrimental quality and postharvest life of the fruit.

There are few reports related to nopal breeding and all of them have been done by traditional breeding. The national market is harboring at least 15 nopal genotypes with different specific characteristics distributed all around the country, but there are also wild materials that present other attractive properties for the consumer. It is necessary to generate new genetic materials trough traditional breeding; however the process requires several events of mating, which represent long selection periods, genetic segregation, along a juvenile period with a very slow growth which usually represents a strong limitation. To improve breeding efficiency molecular markers have been used (Table 2).

Gene isolation and proteomics

Fruit ripening

Fruits development and ripening are unique to plants and they represent an important component of human and animal diets. Fruit ripening is a complex process that involves changes in color, aroma and texture as result of the participation of several events (Table 3). Some other changes are not visible but they are very important for the whole process; these changes involve respiration, ethylene synthesis and the gene and protein expression (Carrillo–López *et al*., 2002).

Table 2. Reports related to molecular markers analysis in *Opuntia.*

Reference	Species	Number of analyzed materials	Type of análisis	Comments
Wang *et al*., 1998	*O. ellisiana*, *O. lindheimerii*, *O. cochinellifera*, *O. hyptiacantha, O. Picus–indica*	5	RAPD (random amplification of polymorphic DNA)	First molecular assay. Molecular assays are feasible in *Opuntia*
Mondragon–Jacobo, 2001		17	RAPD	Apomictic characterization with molecular markers
Labra *et al*., 2003	*O. ficus–indica, O. megacantha*	29	cpSSR (chloroplastic simple sequence repeat) AFLP (amplified fragment length polymorphism)	Characterization with two different techniques *O. ficus–indica* should be considered as a domesticated form of *O. megacantha*
Luna–Paez *et al*., 2007	*O. ficus-indica, O. joconostle, O. robusta, O. lasiacantha, O. megacantha, O. albicarpa, O. streptacantha, O. undulata, O. cochinera, O. hyptiacantha*	22	RAPD ISSR (internal)	The molecular classification was related with the current taxonomic groups described

Fruit ripening is a controlled process where it is important the cellular communication, the influence of plant growth regulators and the environmental signals. At ripening fruits undergo many changes which, although variable among species, generally include modification of cell wall ultrastructure and texture, conversion of starch to sugars, alterations in pigment biosynthesis and accumulation, and heightened levels of flavor and aromatic volatiles (Brady, 1987).

Table 3. Changes occurring in fruit ripening.

Biochemical changes	Events
Color	Loss of chlorophyll
	Synthesis and accumulation of pigments (carotenoids and anthocianins)
Texture	Solubilization of pectin/cellulose
	Starch degradation
	Cell wall enzymes activity
Flavor and aroma	Accumulation of sugars and organic acids
	Production of volatiles
	Alcohol esters synthesis
Control of pathways	Increase in respiration
	Ethylene synthesis
	Altered regulation of existing metabolic pathways
Gene expression	Specific mRNA synthesis
	Small and interference RNA appearance
Protein expression	Synthesis *de novo* of ripening–specific proteins

Based in respiration and ethylene production, prickly pear is classified as non–climacteric fruit. The main information generated on fruit ripening has been done in climacteric fruits, principally tomato, whereas the bibliography in non–climacteric fruit is scarce, although very important information is coming with the genetic sequence of grape (Jaillon *et al*., 2007).

The first reports on prickly pear (*O. ficus–indica*) were related to its basic characterization, the proximate composition of pulp, skin and seeds (El Kossori *et al*., 1998). Prickly pear is a neglected nutritional source which should be more widely used because of its potential nutrient contribution (Cruz–Hernández and Paredes–López, 2010; Rosas–Cárdenas, 2008).

The isolation and characterization of a major albumin from the seeds of *O. ficus–indica* were done by Uchoa *et al*. (1998). This protein has a molecular mass of 6.5 kDa and was isolated by a combination of gel filtration chromatography and reverse–phase HPLC. The amino acid composition of this protein was determined and it was shown to have similarities with the amino acid composition of several proteins from the 2S albumin storage protein family.

Carrillo–López *et al*. (2002) at our laboratory evaluated the activity of four cell wall hydrolases, pectinmethylesterase (PME), polygalacturonase (PG), cellulase, and β–galactosidase (β–Gal), which was measured in fruit skins of two prickly pear varieties, Naranjona and Charola, during storage at 18°C and 85–95% relative humidity (RH). The results suggested that PG and cellulase in Naranjona and PG and β–Gal in Charola are the main enzymes responsible for cell wall hydrolytic and ultrastructural changes in skins of stored prickly pears (Carrillo–López *et al*., 2002).

After this previous work, it was important to isolate and analyze the expression of genes that encode enzymes associated to fruit softening, ethylene synthesis and fruit nutrition as first attempt (Table 4). The first report for gene expression was the isolation of the genes associated with the ethylene synthesis (ACC synthase and ACC oxidase) (Collazo–Siqués *et al*., 2003) The isolated genetic sequences showed a high identity when compared to tomato and pineapple, the mRNA expression was induced during prickly pear ripening, as described in other non–climacteric fruits (Cazzonelli *et al*., 1998).

In another study with ripening–related genes, the polygalacturonase (PG; EC 3.2.1.6.9) expression was analyzed; PG has been the most widely studied cell wall hydrolase in fruit ripening. Degenerate oligonucleotides corresponding to conserved regions from reported sequences were used as primers for RT–PCR to amplify mRNA extracted from middle ripe fruit. Cloning and characterization of a cDNA *OsPG* showed a 282 bp product with a predicted sequence of 94 amino acids. The peptide exhibited a high identity with other fruit PGs previously reported. Northern blot analysis of the messenger showed a transcript induced during prickly pear ripening. It was found by Southern blot analysis that there is one copy of this gene. The *OsPG* mRNA expression results sensitive to ethylene, cold storage and wounding (Rosas–Cárdenas *et al*., 2007). The mRNA expression analysis in different prickly pears, with different behavior (early, mid and late ripening), results in different patterns depending on the morphospecies (Figure 1). It suggests that fruit ripening in prickly pear is very complex.

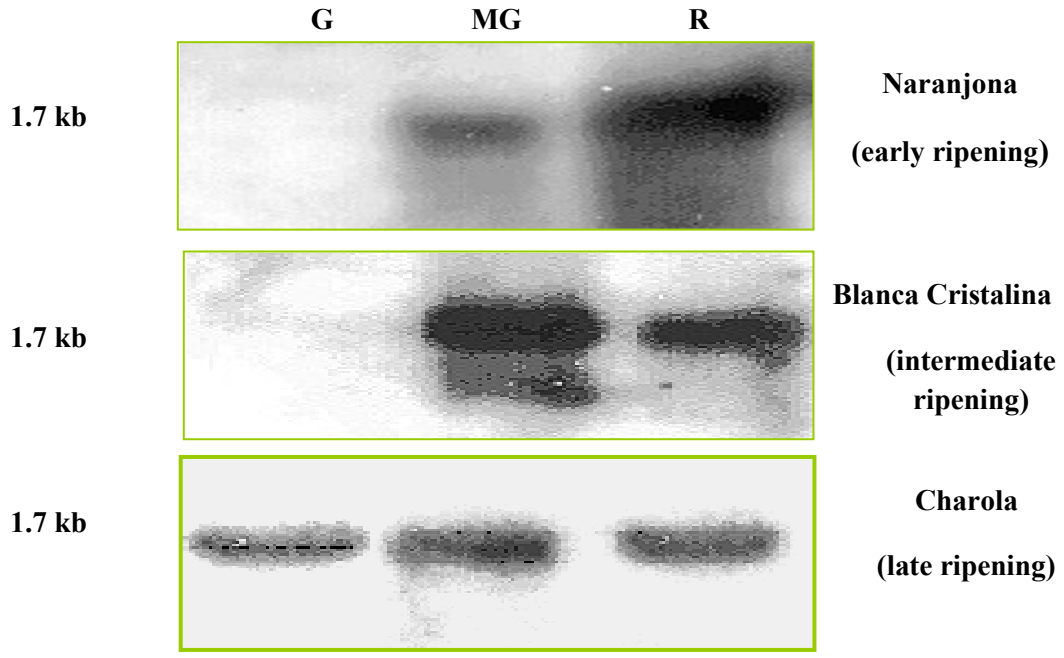

Figure 1. Polygalacturonase mRNA analysis in prickly pear with different ripening behavior. Ten µg of total RNA were analyzed in denaturing conditions, transferred to nylon membranes and hybridized with an homologous probe. The size of the mRNA is indicated (1.7 kilobases) G, green stage; MG, mature green stage; R, ripe stage.

In our group, some other genes have been analyzed (Table 4), and their mRNA expression pattern seems to be not induced during fruit ripening (ie., cellulose, PME); all the data have a strong coincidence with the enzymatic activity already reported by Carrillo–López *et al*. (2002).

Drought tolerance

An important characteristic of *Opuntia* is its adaptation to different environments mainly in areas with poor soils, and with a high drought level. In order to analyze the *Opuntia* response to drought (Silva–Ortega *et al*., 2008), it was measured the proline content, and the activity and the expression level of delta 1–pyrroline–5–carboxylate synthetase (P5CS: g–glutamyl kinase, EC 2.7.2.11 and glutamate–5–semialdehyde dehydrogenase, EC 1.2.1.41), a key regulatory enzyme involved in the biosynthesis of proline, in cactus pear (*Opuntia streptacantha*). Treatment with NaCl of *O. streptacantha* young plants resulted in a decrease in the cladode thickness and root length, and in a

significant and gradual accumulation of Pro in young cladodes, in a time– and concentration–dependent manner. P5CS activity was reduced in all times as a consequence of salt treatment, except at the sixth day at 75 and 150 mM of NaCl, where a slight increase was observed. An open reading frame (ORF) fragment of p5cs gene was isolated. The deduced amino acid sequence of the P5CS protein exhibited 90.4% of identity with another P5CS protein. RT–PCR analysis revealed that the Osp5cs mRNA was induced by salt stress at 9 and 11 days of treatment. Furthermore, ABA–induced Osp5cs gene expression was observed in cladodes of cactus pear young plants. Also, a correlation between the transcript up–regulation and the Pro accumulation under salt stress was observed. The authors suggested that Pro accumulation might function as an osmolyte for the intracellular osmotic adjustment and might be playing a critical role in protecting photosynthetic activity in *O. streptacantha* plants under salt stress (Silva–Ortega *et al*., 2008).

Table 4. Genes identified in nopal.

Gene	Origin/tissue	Process	State
ACC synthase	Fruit	Fruit ripening	Inducible
ACC Oxidase	Fruit	Fruit ripening	Inducible
Polygalacturonase	Fruit	Fruit ripening	Inducible/Constitutive
B–galactosidase	Fruit	Fruit ripening	Inducible
Cellulase	Fruit	Fruit ripening	Constitutive
PME	Fruit	Fruit ripening	Constitutive
1–pyrroline–5–carboxylate synthetase	Cladode	Drougth tolerante	Inducible

Interesting findings have shed light on the molecular basis of developmental ripening control (Giovannonni *et al*., 2001). They have suggested common regulators of climacteric and non–climacteric ripening physiology, and defined a new role for MADS box genes in the late stage of floral development. Analyses of fruit–ripening mutants and ripening–related gene expression suggested higher levels of a developmental regulatory cascade that remains to be defined.

Examination of the molecular basis of ethylene signaling in tomato has demonstrated conservation of the basic model defined in *Arabidopsis*, yet with modifications in gene family composition and expression that may represent adaptations to promote successful fruit development and seed dispersal. The continuing development of genomics tools, including ESTs, cDNA microarrays, and proteomics in prickly pear will accelerate the generation of new discoveries in fruit development and ripening research (Giovannoni, 2003; Giovannonni, 2007).

Proteomic analysis

The technological advances have started to be applied at the post–genomic era, where the research focus gradually will move from genes and genomes to proteins and proteomes, for function analysis (Cruz–Hernández and Paredes–López, 2010; Vihinen, 2001). The data integration of the biological systems will help to establish a fundamental model to understand the evolution, development and adaptability of the organisms (Rose *et al*., 2004).

Up to now, a huge quantitative biological knowledge is available for the study of relevant molecules. This knowledge is based on the development of techniques for a large scale study of those molecules; one of these techniques is the proteomics which allows the quantification and identification of hundred or thousands of proteins in an organism (Bertone and Snyder, 2005). For this reason, the genomic and proteomic analyses are the essential steps to understand a process, an

organelle or an organism. These tools could be useful for analysis of the metabolism, protein–protein interactions, signal transduction pathways, etc. (Vihinen, 2001). Similar to the genomic profiles, the proteomics could analyze and classify the temporal patterns of protein accumulation that occurs in developmental processes (Cánovas *et al*., 2004). The proteomic strategies may help to characterize protein modifications, which cannot be predicted by a genomic sequence (Mann and Jensen, 2003). With this in mind, in our laboratory we have been working in the establishment of the conditions for proteomic analysis in prickly pear. Three different morpho–species with different ripening behavior (early, mid and late ripening) were chosen (Rosas–Cárdenas, 2008).

The conditions for the differential expression analysis trough 2–D electrophoresis were established (Figure 2). We isolated the proteins from three different materials; the protein concentration increased through ripening, which indicates an active protein synthesis. The main proteins were found in a 5–7 pH range and with a 20–80 kDa of molecular weight. Around 1,000 proteins were detected at the ripe stages in all materials.

We also isolated and purified some of the differential expressed proteins; their comparison in data bases showed identity with proteins associated to fruit ripening, such as fatty acid synthesis, anthocyanins synthesis, and photosynthesis (Table 5). With a continuous peptide analysis will be possible to find other transcendental proteins, which may allow us to have a better idea of fruit ripening in prickly pear, and it will eventually give us a chance to propose some strategies for its control.

The final goal is to identify the specific function for the peptides, which will help to identify the differences between these materials and with other fruits, and to understand fruit ripening through the proteomic maps of each fruit. The analysis and expression of the proteins will help to understand the *Cactaceae* biology, through the identification of the proteins and their comparison with other plants sequences.

Plant propagation and plant regeneration

The establishment of an *in vitro* protocol for plant regeneration that allows the massive and controlled propagation of nopal is the first step in a breeding program through genetic engineering. Nopal is propagated clonally by cladode cultivation; seed propagation is not common because the genetic segregation of the offspring and the long juvenile period of the plants; it makes the system non suitable for plant breeding, although these problems could be solved through tissue culture methods (de la Rosa–Hernández and Santana–Amaro, 1998; Llamoca–Zárate *et al*., 1999a). The reports on tissue culture in *Opuntia* are scarce (Table 6); the successful ones are related to the micropropagation through axillary buds and are described in this section.

Escobar *et al*. (1986) in *Opuntia amyclaea* Tenore reported the response of shoot induction after cut plantlets (in transversal and longitudinal way) and exposed them to different BA concentrations (1, 10, 25 and 50 μM). The longitudinal cut treatment with 50 μM BA, supplemented with 10 mM IBA, showed the highest number of lateral buds. They multiplied 25,000 plantlets with roots in 100 days.

Mohamed–Yaseen *et al*. (1995) propagated *O. ficus–indica* through axillary buds activation. They evaluated the response of buds cut in a distal, intermediate and proximal sections, at three BA concentrations (4.4 μM, 8.8 μM and 17.6 μM), in NAA 0.5 μM. They reported that the intermediate cut with a BA 8.8 μM concentration changed the latency of the lateral buds, resulting in the best bud percentage.

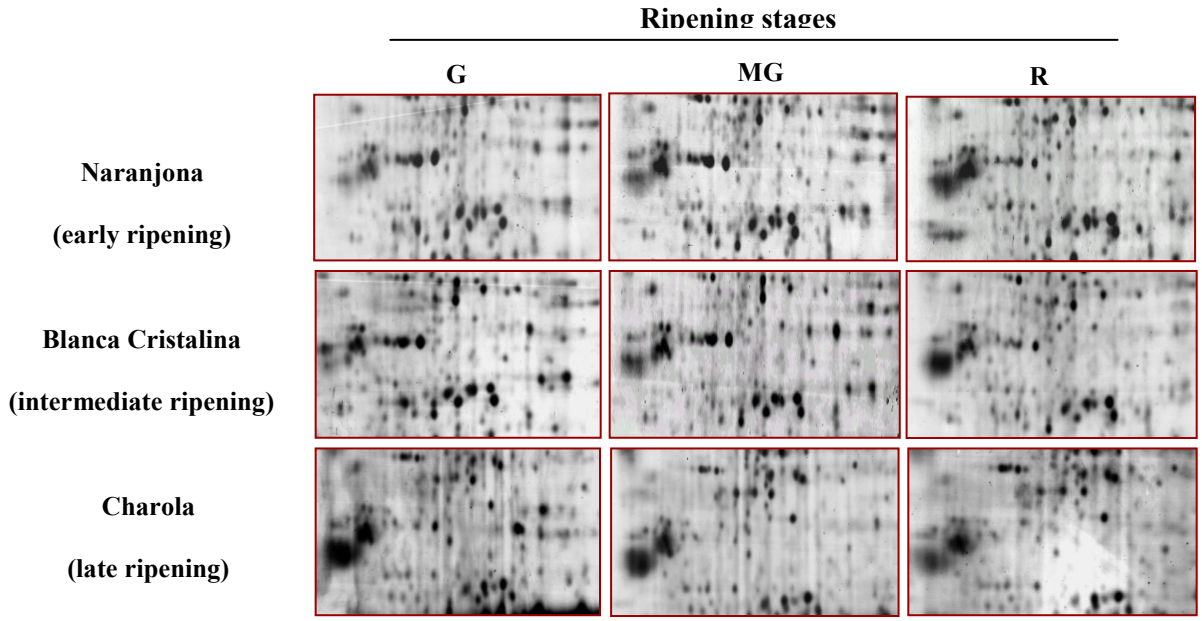

Figure 2. 2–DE analysis of proteins expressed during prickly pear ripening in a range of 25–35 kDa MW and 5.0–6.5 pH. Seven hundred µg of proteins were analyzed by isoelectrofocusing and molecular weight in acrylamide gels, the proteins were identified with Coomassie colloidal reagent and the MW and pI values calculated with commercial standards.
G, green stage; MG, mature green stage; R, ripe stage.

Table 5. Identified proteins at the prickly pear proteome.

Protein	Sequence coverage (%)	pI	MW
Omega–6 fatty acid desaturase, chloroplastic *Glycine max*	18	5.4	21
Ribulose–1,5–bisphosphate carboxylase/oxygenase *Lycopodium annotinum* (Stiff)	24	6.2	25
Chalcone—flavonone isomerase OS Canna generalis	24	6.0	21
Small heat shock protein Hsp23.5 precursor *Triticum aestivum* (wheat)	22	6.2	25
Zinc finger, CCHC–type; Zinc finger, SWIM–type Medicago truncatula (Barrel medic)	18	5.8	18
NBS–type putative resistance protein *Glycine max* (soybean)	55	6.2	25
Putative mitochondrial inner membrana protein *Oryza sativa* (japonica cv group)	40	6.0	21

Table 6. Plant tissue culture reports in *Opuntia.*

Author	Specie	Technique	Description
Escobar *et al*., 1986	*Opuntia amyclaea*	Micropropagation	Plant multiplication
Mohamed–Yaseen *et al*., 1995	*Opuntia ficus–indica*	Micropropagation	Plant multiplication
Santacruz–Ruvalcaba *et al*., 1998	*Opuntia ficus–indica*	Embryogenesis	Embryogenic callus and globular embryos
Llamoca Zárate *et al*., 1999a	*Opuntia ficus–indica*	Callus production	Callus production and cell suspension
Llamoca Zárate *et al*., 1999b	*Opuntia ficus–indica*	Micropropagation	Bud formation and multiplication
Llamoca–Zárate *et al*., 1999	*Opuntia ficus–indica*	Embryogenesis	Heart and torpedo embryos
Juárez *et al*., 2002	*Opuntia ellisiana*	Micropropagation	Plant multiplication
García–Saucedo *et al*., 2005	*Opuntia ficus–indica*	Organogenesis	Plant regeneration
Estrada–Luna *et al*., 2008	*Opuntia lanigera*	Micropropagation and fertigation	Plant multiplication

Llamoca–Zárate *et al*. (1999a) evaluated in *O. ficus–indica* cv Gigante the effect of three growth regulators at different concentrations; they found that the best bud forming percentage was scored at BA 2.2 μM, with or without GA_3. At BA 8.8 μM the lowest bud forming frequency was found. They concluded that GA does not help to the bud forming frequency. The results in all these works suggest that the response to the growth regulators is linked to the genotype and the specific work conditions.

In the case of somatic embryogenesis, Santacruz–Ruvalcaba *et al*. (1998) induced embryogenic calli and globular embryos, but they did not regenerate plants from *O. ficus–indica.* Llamoca–Zárate *et al*. (1999b) induced callus and recovered cell suspension cultures in this same species using cotiledonary and hypocotil tissues, but they did not recover regenerated plants.

Different sterilization protocols were tested in *O. ellisiana* (Juárez and Passera, 2002); areoles were isolated in laminar airflow cabinet, and cultured on Murashige–Skoog medium, supplemented with sucrose and different BAP and IBA combinations. In the most efficient growth treatment, plantlets reached 100% shooting after 35 days of culture, and a mean length of 10.2 mm after 49 days of culture. A 100% rooted plantlets was obtained in a medium containing 5 mg l^{-1} IBA, after 12 days of culture. Acclimatization was achieved under greenhouse conditions, showing 100% plantlet survival.

Cladode explants from three *Opuntia* genotypes (García–Saucedo *et al*., 2005) were cultivated in MS medium containing BA and GA_3. Shoots produced were used as secondary explants and BA added at different concentrations to induce shoot development; 0.5 mM BA was the best for bud formation. Satisfactory rooting occurred when IBA was added to the medium, and plants were successfully established in soil and adapted to greenhouse conditions (Figure 3).

The conditions to micropropagate the ornamental prickly pear cactus *O. lanigera* Salm–Dyck through axillary shoot development from isolated areoles were established. For the shoot proliferation stage different explant orientation (vertical and horizontal), type of cytokinin (BA, DAP and KIN), and concentrations (0, 1.25, 2, 5, 5.0 and 7.5 mg l^{-1}) were evaluated. Carbohydrate concentrations (0.25, 0.5, 0.75 and 1.0%) were studied to optimize individual shoot growth. The

micropropagation protocol described and the conditions to grow the plants produced 12,500 plantlets in average after 12 months of culture (Estrada–Luna *et al*., 2008).

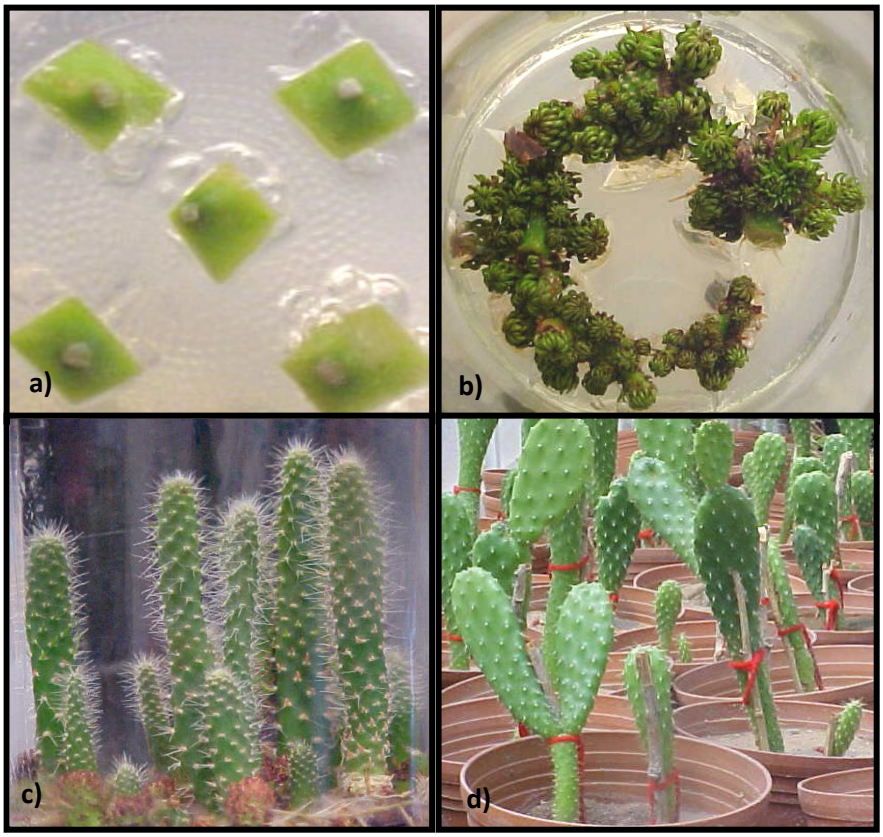

Figure 3. Nopal plant regeneration through organogenesis. Detached cladodes from 'Blanco Sin Espinas' genotype were used as starting material. a) meristems, b) bud propagation, c) bud elongation, d) Plants at greenhouse.

Gene transfer

The principal requirements for plant transformation include a target tissue, an efficient plant regeneration system, selectable markers for tissue and plant selection, a method for gene transfer and a high percentage of regenerated fertile transgenic plants. Two different methods for gene transfer have been developed: biological methods that include viral vectors and transformation via *Agrobacterium tumefaciens*; and the physical methods include microinjection, introduction of DNA to protoplasts and particle bombardment (Birch, 1997; Christou *et al*., 1996). Several crops have been modified through genetic engineering using any of these methods, but in nopal very few approaches have been assayed.

***Agrobacterium tumefaciens* transformation**

A system for genetic transformation was developed for an elite material of nopal (*O. ficus–indica* L., cultivar 'Villanueva') by *Agrobacterium tumefaciens*. A direct infection with a hypodermic syringe to the meristematic tissue (areoles) was done; transgenic plants were recovered using 100 mg l^{-1} kanamycin for selection. GUS activity (Jefferson et al., 1987), PCR and Southern blot were used for histochemical and molecular analysis of regenerated transformed plants, and the

transformation frequency obtained by the system reported here was of 3.2% (Silos–Espino *et al*., 2006).

Particle bombardment in *Opuntia*

Based on somatic embryogenesis, friable callus cultures were initiated from cotyledons and hypocotils of *O. ficus–indica*. Propagation and growth of calli were observed on medium supplemented with 2, 4–D, picloram and casein. Cell transformation was achieved by particle bombardment with a high pressure PDS–1000 He instrument (Bio–Rad). Kanamycin resistance, GUS expression, and PCR analyses indicated successful integration of foreign DNA into the cells of somatic embryos recovered; no plants were regenerated (Llamoca–Zárate *et al.*, 1999b).

Future prospects

The nopal and its products need a better attention in order to give a valued-added to this crop; it is a multipurpose plant which is very important in the life of the people who live in the areas where it is grown. Three main aspects are now in the focus for increasing its value as a crop:

1. Natural products and health foods have recently received a lot of attention, both by health professionals and the common population, for improving overall well–being, as well as in the prevention of diseases including cancer. In this line, all types of fruits and vegetables have been re–evaluated and recognized as valuable sources of nutraceuticals. The great number of potentially active nutrients and their multifunctional properties makes cactus pear fruits and cladodes perfect candidates for the production of health–promoting food and food supplements. Although traditionally appreciated for its pharmacological properties by the Native Americans, cactus pear is still hardly recognized because of insufficient scientific information. However, recent studies on *Opuntia* spp. have demonstrated that cactus pear fruit and vegetative cladodes are excellent candidates for the development of healthy foods (Feugang *et al*., 2006). An interesting study would be the improvement of nutraceuticals content through gene transfer; several pathways of secondary metabolism have been discovered and some assays with transgenic plants have demonstrated the successful expression of these molecules.

2. Plants which traditionally served as food and fiber are now being engineered as novel biomanufacturing systems, with particular attention focused on the creation of plants for producing compounds of pharmaceutical or fuel value. Transgenic plants, containing immunogenic proteins, have been created by introducing coding sequences from human pathogenic viruses or bacteria linked to plant regulatory sequences into transformation vectors. Stepwise progress has been achieved in these studies (Artzen, 1997; Artzen, 2008). Nopal can be used as a bioreactor for the expression of proteins or molecules with economical importance.

3. The first wave of plant genome sequencing has passed, and now there is another era in plant genomics research. The new strategy is based on a mixture of economic and scientific needs (Jackson *et al*., 2006). Up to now, two fruit crops have been sequenced and some others are underway, which will greatly help for elucidation of the ripening process and the phylogenetic relationship with other plants. Nopal is waiting to be sequenced; the information will help to understand several mechanisms for plant adaptation to different environments, and it will give us a clue in relation to fruit ripening in order to control the process and eventually improve the postharvest life of fruits.

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