Investigation of the genetic diversity of prickly pear (*Opuntia ficus*-indica) cultivars in Taif by using RAPD-PCR

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Abstract: Prickly pear (*Opuntia* spp.), a member of the family Cactaceae, thrives in low annual rainfall countries. Besides being consumed as food or beverages, the plant is appreciated for its anti-diabetic, anti-inflammatory, analgesic, antiviral and anti-oxidant properties. This study was carried out to molecularly characterize two prickly pear cultivars (Red Toti and Yellow Shafawi), growing in Shafa region of Taif, KSA. Twelve 10-mer primers were used to amplify DNA by randomly amplified polymorphic DNA (RAPD) PCR. Out of the RAPD primers used, 7 primers were found useful for distinguishing the two cultivars. The different RAPD primers produced a total of 71 amplicons (110 bp to 2500 bp in size), of which 23 were polymorphic among the two studied cultivars. The genetic similarity between the two cultivars was 43% based on Jaccard's coefficient. Biclustering of RAPD profiles and RAPD amplicons improved the display of the RAPD markers. Certain RAPD markers identified in this study might be associated with the biochemical and morphological traits that distinguish Red Toti and Yellow Shafawi cultivars.
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1. Introduction

Cactus pear or prickly pear, a member of the Cactaceae family (Reyes Aguero *et al*., 2005), originated from arid and semi-arid regions of Mexico, and was introduced into North Africa in the 16th century (Griffiths, 2004). More than 1500 known species of cactus are in the genus *Opuntia* (Hegwood, 1990).

Prickly pear (*Opuntia spp*., Cactaceae) is an important crop in the semi-arid lands. The prickly pear is a fleshy berry, consisting of a thick pericarp with a number of clefts of small prickles, reddish purple, yellow or white in color, with a luscious sweet pulp intermixed with a number of small seeds (El-kossori *et al*, 1998 and S´aenz, 2000). Cactus pear fruit is characterized by a high sugar content (12 to 17%) and low acidity (0.03 to 0.12%) (Elhadi and Mondragon, 2011). The fairly high sugar content and low acidity of the prickly pear fruit (Joubert, 1993; Munoz de Chavez *et al*., 1995) make it very sweet and delicious. On the basis of fruit development, there are prickly pears of early, intermediate, and late ripening, depending on the time required for full fruit development (Pimienta-Barrios, 1994).

Taif is known for the cultivation of roses and fruits, particularly honey-sweet figs, grapes, pomegranates, and prickly pear. Studies on the molecular biology of prickly pear are limited (Maria *et al*., 2002). Most research has demonstrated wide genetic diversity of this genus. Fernandez *et al*., 1994 suggested the need for plant breeding and genetic approaches to improve tissue composition of this plant for human health issues.

Most of the published information available on biodiversity of the cultivated cacti in the world comes from allozyme studies (Boyle and Anderson, 2001). Microsatellite markers developed to study the population genetic structure of some of cacti species revealed possible polyploidy (Helsen *et al*., 2007). RAPD-PCR markers proved to be useful for germplasm discrimination as well as for discovery of patterns of variation in Tunisian *Opuntia ficus-indica* cultivars (Ne´jia *et al*., 2007). The method has proven useful for species identification, elucidation of genetic relationships of numerous plant species (Williams *et al*., 1991 and Levi and Rowland, 1997). Amplified Fragment Length Polymorphism (AFLP) markers were used to provide a quantitative assessment of genetic similarity and diversity among several *Opuntia* species (Snoussi *et al*., 2009).

In this study, RAPD-PCR was chosen, because of its simplicity and applicability to a wide range of species, to molecularly characterize prickly pear cultivars from Taif. The ultimate objective is to identify molecular markers suitable for further investigations on improving the agronomic traits by using marker assisted breeding programs.

2. Materials and Methods

2.1. Plant material and sample preparation

Two local cultivars of prickly pears (*Opuntia ficus* indica L.) were used in this study; i.e. Yellow Shafawi and Red Toti. The two cultivars were grown under rain-fed conditions in private farms located in Shafa region, Taif Governorate, Kingdom of Saudi Arabia. Full ripe prickly pear fruits were harvested at 75 days from fruit setting. The ripe fruits were stored at 4°C between harvest and sample preparation. Representative fruits were washed for two minutes under tap water with a nailbrush and peeled manually after removing of uncolored top and bottom sides. Peel, pulp, and seeds were freeze-dried in Alpha 1–2 Christ LD plus freeze-dryer (Martin Christ, Osterode am Harz, Germany), ground separately into a fine powder in a coffee grinder, passed through a 100-mesh sieve, and stored at -20ºC until analysis. For preparation of juice, fresh pulp (containing seeds) was cut into small pieces, ground for 2 minutes, filtered using double rings filter paper 102 (15.0 cm), and the juice stored at -20°C until analysis.

2.2. Biochemical analysis

Crude protein analysis (%) was performed according to AOAC (2006). Moisture content (%) was measured by calculating the loss in weight on drying X 100 and the result was divided by the weight of the sample. According to AOAC (1994), TSS was measured as suger percentage and expressed as °Brix at 20°C in the pulp juice, and titratable acidity was evaluated and expressed as percentage of citric acid. The pH was determined in the fresh pulp juice by using a pH meter (Model: pH 211, HANNA Instruments).

2.3. Genomic DNA extraction and RAPD assay

Genomic DNA was prepared from the cladodes of two prickly pear varieties (Red Toti and Yellow Shafawi) by using the silica-based DNeasy Plant Mini Kit (Qiagen, GmBH, Germany). Twelve 10-mer primers (Table 1), were synthesized by Operon (Operon, A Qiagen Company, Qiagen GmbH, Germany), and used for RAPD-PCR typing. All primers used were resuspended in TE buffer and stored at -20ºC in aliquots to use in PCR. A 10 µM (10 pm /µl) working solution of each primer was prepared and used in PCR.

Table 1: RAPD primers used in this study.

Primer	Sequence (5' to 3')	Primer	Sequence (5' to 3')
A-13	CAG CAC CCA C	D-05	TGA GCG GAC A
B-06	TGC TCT GCC C	D-14	CTT CCC CAA G
B-15	GGA GGG TGT T	E-05	TCA GGG AGG T
B-17	AGG GAA CGA G	M-05	GGG AAC GTG T
B-18	CCA CAG CAG T	M-17	TCA GTC CGG G
C-19	GTT GCC AGC C	O-09	TCC CAC GCA A

A RAPD reaction mixture (25 ul) contained 10 mM Tris-HCI pH 7.5, 50 mM KCI, 2 mM $MgCl_2$, 0.1 mM dNTPs, 15 pmoles of the RAPD primer, 30 ng of genomic DNA and 0.8 U of *Taq* DNA polymerase. Amplification was carried out in a Gene AmpPCR System 9700 (Perkin Elmer, England) for 40 cycles, each consisting of a denaturing step of 1 minute at 94°C, followed by annealing step of 1 minute at 36°C and an extension step of 2 minutes at 72°C. The last cycle was followed by 7 minutes of long extension at 72°C. The amplification products were separated by gel electrophoresis in 1.5% agarose in 45 mM Tris-borate, 1 mM EDTA buffer (pH 8.0), and containing ethidium bromide at 0.5 µg / ml at a constant voltage of 5 V/cm. PCR products were visualized under UV light and photographed using a gel documentation system (Bio-Rad® Gel Doc-2000).

2.4. Statistical analysis

RAPD-PCR analysis

RAPD bands were scored as 1 denoting presence or 0 denoting absence. The similarity between the DNA profiles was calculated with the band-matching Jaccard's coefficient that ranges from 0 to 1.0, where 1.0 represents 100% identity (presence and position) for all bands in the PCR fingerprints being compared. A pairwise similarity (or distance) matrix was developed and cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic averages (UPGMA) method (Multivariate Statistical Package, MVSP 3.1, Kovach Computing Services, Wales, UK). Cluster analysis and biclustering were performed by using the Cluster and Tree View program of Stanford University, USA (Eisen *et al.*, 1998).

2.5 Biochemical analysis

Results are expressed as mean ± SE of samples.

3. Results and Discussion

The results of the biochemical analyses (moisture content, crude protein content, TSS, titratable acidity, and pH) of fruit samples of the two prickly pear cultivars are presented in Table (2).

The main fruit traits of the two cultivars are presented in Figure (1). The distinguishing traits between the Red Toti and Yellow Shafawi cultivars are respectively, red and yellow to white skin color; red and yellow pulp color; thin and thick skin; and late and early-intermediate ripening time.

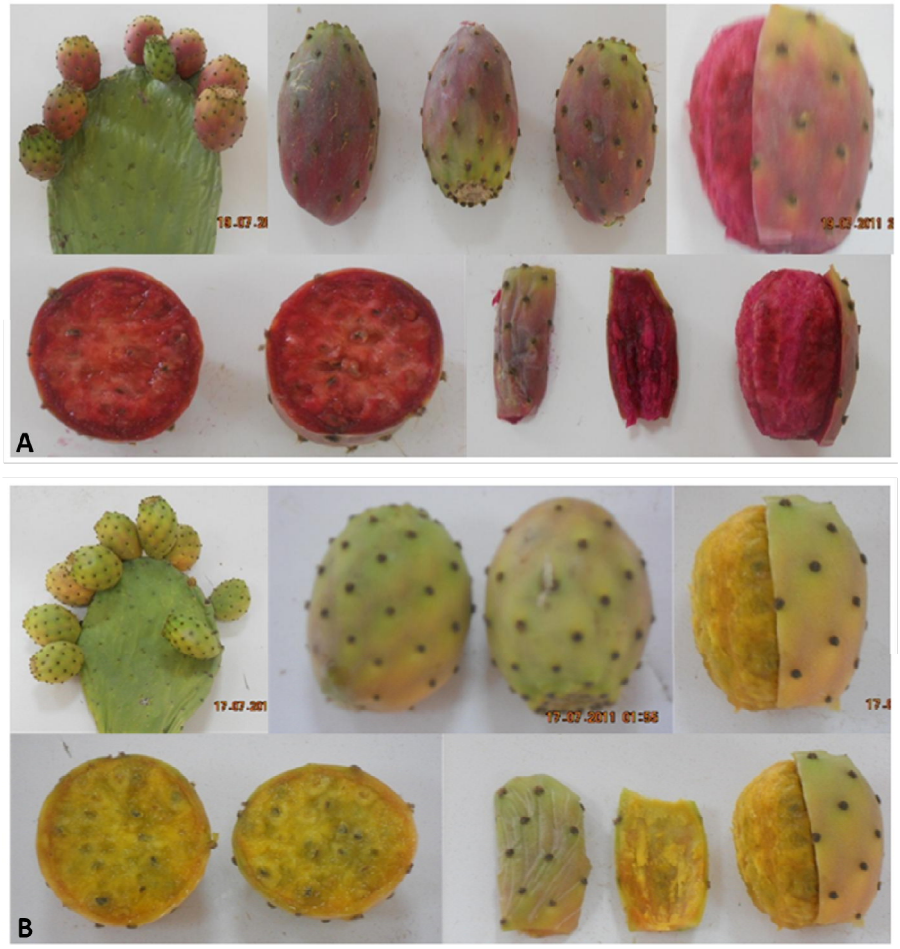

Figure (1): Ripe fruits of Red Toti (A) and Yellow Shafawi (B) cultivars.

Table (2): Biochemical analysis of the two prickly pear cultivars (Mean ± SE):

Fruit samples	Moisture content (%)[a]	Crude protein content (%)	TSS % sugar (°Brix)	Titratable acidity (% citric acid)	pH value
Red Toti peel	82.07±4.7	ND	ND	ND	ND
Red Toti pulp/juice	85.94±4.6	3.95±0.097	17.15±2.08	0.051	5.54
Red Toti seed	ND	20.95±0.55	ND	ND	ND
Yellow Shafawi peel	92.38±4.7	ND	ND	ND	ND
Yellow Shafawi pulp/juice	84.73±4.7	3.85±0.095	11.95±1.71	0.105	5.89
Yellow Shafawi seed	ND	15.70±0.39	ND	ND	ND

[a]Moisture content measured as % w/w of dry matter. ND: Not Determined.

The pulp and peel (skin) contained high amount of water. The moisture content of the peel (skin) of Yellow Shafawi cultivar was higher than that of the Red Toti cultivar (Table 2). This correlates with the difference in thickness of the skin (thin in Red Toti and thick in Yellow Shafawi, Figure 1). Table (2) shows that the crude protein content of Toti Red seeds was high as compared to Yellow Shafawi seeds. Crude protein content of the seeds was higher than that of the pulp. The results were comparable to those obtained by Sawaya *et al*. (1983). At harvest, the values of total soluble solids were 11.95±1.71 and 17.15±2.08 ºBrix for Yellow Shafawi and Red Toti juice, respectively. These results were between 12–17.5 ºBrix interval the interval reported by S´aenz *et al*. (1998) for total soluble solids in prickly pears. Titratable acidity was measured to estimate the organic acid content in the fruit (Stintzing, 1999). Titratable acidity was high in Yellow Shafawi juice (0.105% citric acid) as compared to Red Toti juice (0.051% citric acid). No significant differences were found between the two cultivars in the pH value of the juice. The pH values were in the range already described in other prickly pear varieties, confirming that this fruit is a low acidic food (Sep´ulveda and S´aenz, 1990; Stintzing, 1999).

Out of the twelve primers tested, seven primers [B-06 (P1), O-09 (P2), D-05 (P3), B-17 (P4), E-05 (P5), D-14 (P6), and M-17 (P7)] were suitable for distinguishing the two cultivars. The seven RAPD primers produced a total of 71 amplicons that ranged in size from 110 bp to 2500 bp. Out of these amplicons, 23 amplicons were polymorphic among the two studied cultivars. The similarity between the two cultivars based on Jaccard's coefficient was 43%. The RAPD primer B-06 (P1) amplified the highest number of polymorphic RAPD amplicons (Table 3).

Table 3: Distribution of RAPD amplicons scored in this study.

RAPD Primer	RAPD Amplicons			
	TR	SY	Monomorphic	Total
B-06 (P1)	4	7	7	18
O-09 (P2)	1	2	5	8
D-05 (P3)	2	0	10	12
B-17 (P4)	1	0	9	10
E-05 (P5)	2	2	5	9
D-14 (P6)	1	0	5	6
M-17 (P7)	1	0	7	8
	23	48	71	

TR: Red Toti. SY: Yellow Shafawi.

The biclustering method used showed that out of the 23 polymorphic amplicons, 12 amplicons were only amplified from Red Toti cultivar, while the remaining 11 amplicons were only amplified from Yellow Shafawi cultivar. The primer B-06 (P1) amplified seven amplicons from Yellow Shafawi cultivar and four amplicons from Red Toti cultivar. The primers D-05 (P3) and B-17 (P4) amplified polymorphic amplicons from Red Toti only (Figure 2). The differences between the Red Toti and Yellow Shafawi cultivars in biochemical (moisture content, protein content, total soluble solids, and titratable acidity) and skin thickness traits could be associated with some of the RAPD markers identified in this study. Of course, investigating of this association will be the future studies.

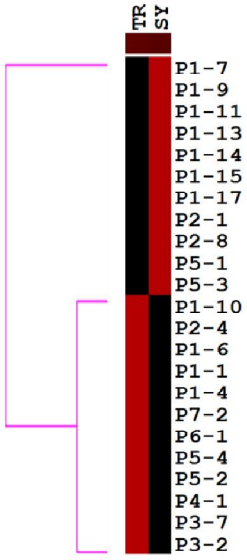

Figure (2): Biclustering of RAPD-PCR data. Rows indicate RAPD amplicons and columns indicate RAPD profiles (TR: Red Toti and SY: Yellow Shafawi). Each RAPD amplicon was assigned a name that begins with the letter P followed by a number (1 to 7) that indicates the primer (B-06, O-09, D-05, B-17, E-05, D-14, and M-17, respectively), and a number that identifies the band position. Red and black colors indicate respectively the presence and absence of the RAPD ampliconin the genome.

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