Genomic Relations among 31 Species of *Mammillaria* Haworth (Cactaceae) Using Random Amplified Polymorphic DNA

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Thirty-one species of *Mammillaria* were selected to study the molecular phylogeny using random amplified polymorphic DNA (RAPD) markers. High amount of mucilage (gelling polysaccharides) present in *Mammillaria* was a major obstacle in isolating good quality genomic DNA. The CTAB (cetyl trimethyl ammonium bromide) method was modified to obtain good quality genomic DNA. Twenty-two random decamer primers resulted in 621 bands, all of which were polymorphic. The similarity matrix value varied from 0.109 to 0.622 indicating wide variability among the studied species. The dendrogram obtained from the unweighted pair group method using arithmetic averages (UPGMA) analysis revealed that some of the species did not follow the conventional classification. The present work shows the usefulness of RAPD markers for genetic characterization to establish phylogenetic relations among *Mammillaria* species.

Key words: Mammillaria spp., RAPD, Molecular Phylogeny

Introduction

Mammillaria is one of the largest genera of the family Cactaceae. It is second only to *Opuntia* in size and to none in popularity. Although several hundred species were described in the past, the general consensus of botanical opinion has put them at 200 (Slaba, 1992) and only 62 cultivated/naturalized species were reported from India (Panda and Das, 1995). The genus is widely distributed in Mexico, USA and also with odd species occurring in the West Indies, Colombia and Venezuela (Pilbeam, 1987). They are fairly small cacti, usually globular or elongated, colourfully spined and profusely flowering. The spines differ greatly in shape, size and colour. The flowers are usually small and borne profusely in rings like a garland around the apex of the stem; they are succeeded by smooth, juicy, club-shaped berries, usually of brilliant red colour, which are a colourful sight in autumn. All these make them a permanent source of interest and admiration for both the collectors and the nature lovers.

Since the time of Linnaeus, botanists have tried to come up with a perfect system of classification and naming of cacti but so far without success. Visual descriptions of morphological characters of a plant require long time which could be affected by environmental and soil conditions, cacti in particular being incredibly variable, responding quickly to a change in their environment and looking quite different under different conditions (Riha and Subik, 1992). Local adaptations are often observed in plants of the same genus and species occurring over an extended geographical area and it is easy to see how a single plant in different locations could receive more than one name. Furthermore, the process of evolution as well as natural hybridization resulting from cross-fertilization of related species of cacti is a continuing process even today (Schuster, 1990). In addition, cacti resist the standard herbarium procedure based on dried specimen (Rowley, 1985). All this makes the tasks of the taxonomists very difficult which is evident from the published classifications. Interpretations by different authors of classifications and cactus names are more or less in agreement with regard to the larger groupings, but at the lower levels of plant units, such as genus and species, major differences of opinion exist (Schuster, 1990). This aspect could be clearly observed from the past history of the works done by different taxonomists (Mars-

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den, 1957; Weniger, 1969; Rowley, 1978; Hunt, 1987; Pilbeam, 1987; Lamb, 1991; Slaba, 1992) to further classify the genus *Mammillaria* on the basis of morphological characters like stem, tubercles, spines, flowers and fruit characters, and there existed many discrepancies in their views. Proper identification of the plant and an accurate and authentic system of classification is required for not only academic interest but for the purpose of conservation and to carry out successful breeding programmes. In this context molecular markers, the most powerful tools available today for discerning biosystematic, biogeographic and phylogenetic relationships provide increased accuracy and expanded scope of biosystematic inference and facilitate statistical analysis of phylogenetic trees. These markers are more advanced and have many advantages over the conventional markers. The simple operation without requiring a prior knowledge of species DNA sequences, cost effectiveness of random amplified polymorphism DNA (RAPD), the number of markers and speed of data generated by this have encouraged its application to many types of biodiversity-associated problems and attracted many researchers, particularly those interested in either genetic fingerprinting or the patterns and levels of genetic diversity, since 1990. RAPDs have proven to be useful for species identification, elucidation of genetic relationships of numerous plant species, and parentage testing (Wang *et al*., 1998). It has provided new opportunities for evaluating the genetic variability in many plants ranging from tubers to fruit crops and from rice to forest tree species (Attanayaka *et al*., 2000).

The first step for the application of a molecular approach to any species is to develop a technique to extract high quality DNA. For cacti, the main problem was the binding of water in the extraction buffer by mucilages present in the tissue, producing a gel-like mixture that prevented all further processing (Mondragon-Jacobo *et al*., 2000). Few reports are available on the DNA extraction from cacti and we have some of them (de la Cruz *et al*., 1997; Mondragon-Jacobo *et al*., 2000) and a modified protocol has been reported by us. We have also discussed the usefulness of RAPD technique in studying the genetic variability and phylogenetic relationship among 31 species of *Mammillaria.*

Materials and Methods

Plant material

Thirty-one different species of *Mammillaria* were collected from the cacti germplasm collection of the Regional Plant Resource Centre, Bhubaneswar, Orissa, India. Fresh and young vegetative buds were used for DNA extraction. The spines were removed; the tissues were washed thoroughly with cold distilled water and then blotted to dry. 5 g of tissue were excised from the upper growing apical portion of the plant. The tissues were extracted on the same day of their collection.

Isolation and purification of genomic DNA

Isolation of good quality DNA was initially difficult due to high mucilage content in cacti. 5 g fresh, young plant tissue were ground with 100 mg insoluble polyvinyl polypyrrolidone to a fine powder in a cold mortar with repeated addition of liquid nitrogen. Thawing was avoided to reduce the secretion of mucilage. In the majority of the species DNA was isolated following the protocol of Doyle and Doyle (1990) with little modification. The CTAB (cetyltrimethylammonium bromide) content was increased to 4% in the extraction buffer. But in eight species, namely *M. elongata, M. spinosissima, M. bella, M. herrerae, M. saboae, M. hahniana, M. glassii and M. humboldtii,* this method did not give satisfactory results. For all these species, the protocol of Mondragon-Jacobo *et al.* (2000) was followed with satisfactory result except for two species, namely *M. herrerae* and *M. humboldtii*. Hence another method given by de la Cruz *et al*. (1997) was tried but without any encouraging result. To overcome this problem, a modified method was tried in which the extraction buffer taken was ten times of the sample tissue. The extraction buffer was the same as suggested by Doyle and Doyle (1990) but the CTAB content was increased to 6% and the NaCl concentration was increased to 2.5 M. After incubation, the mixture was centrifuged at 10,000 rpm for 20 min at room temperature. The upper aqueous phase was pipetted out and an equal volume of chloroform/iso-amyl alcohol (24:1 v/v) was added and mixed thoroughly but gently. Again the mixture was centrifuged as described earlier and the supernatant was pipetted out. An equal volume of chilled isopropanol was added, mixed thoroughly and DNA was precipitated. The crude DNA was purified with RNase A (20 μg of RNase A was used per ml

of DNA solution) followed by washing thrice with chloroform/iso-amyl alcohol (24:1 v/v). To test the quality and quantity of the purified DNA, the samples were electrophoresed in a 0.8% agarose gel along with a known amount of uncut λ DNA (Fig. 1) (Bangalore Genei Pvt. Ltd, Bangalore, India) as standard. The sample DNA was diluted to 25 ng μl^{-1} for RAPD-PCR analysis.

RAPD analyses

25 random decamer primers (Operon Tech., Alameda, USA) from A, D and N series (OPA02, 04, 06, 07, 08, 09, 10; OPD02, 03, 05, 06, 08, 11, 16, 20; and OPN01, 02, 04, 05, 06, 08, 09, 10, 13, 16) were used for RAPD analysis. RAPD assays were performed in a final volume of 25 μl containing 10 mM Tris-HCl [tris (hydroxymethyl)aminomethane hydrochloride] (pH 8.3), 1.5 mM $MgCl_2$, 50 mM KCl and 0.01% gelatin, 200 μM of each dNTPs, 0.4 μM primer, 25 ng template DNA and 0.5 unit of Taq DNA polymerase (Bangalore Genei). The RAPD analysis was performed as described by Williams *et al.* (1990) using a programmable thermal controller (PTC-100, M J Research Inc., Watertown, MA, USA). The RAPD analysis was repeated at least twice, in some cases thrice, to be sure about the reproducibility of banding pattern.

The amplified products were separated in a 1.5% agarose gel stained with ethidium bromide (0.5 μg per ml of gel) for 3 h at 55 V. The gels were observed under a UV trans-illuminator, documented in Gel-Doc 2000 (Bio-Rad, California, USA) and photographed.

The banding patterns obtained from RAPD were scored visually as well as by using the 'Quantity One' software (Bio-Rad) as present (1) or absent (0). Jaccard's coefficient of similarity (Jaccard, 1908) was measured and a dendrogram based on similarity coefficients generated by the unweighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal, 1973) and the SAHN clustering was obtained. Similarity matrices obtained with all the primers were compared with the combined similarity matrices developed from the pooled data of 5, 10 and 15 primers to study the efficiency of the primers using the Mantel 'Z' statistics (Mantel, 1967). The grouping of the species was also tested using principal co-ordinate analysis (PCOORDA). All analyses were performed using the computer package NTSYS-pc (Rohlf, 1997).

Results

DNA isolation

The concentration of the total genomic DNA isolated from different species varied from 60 ng μl^{-1} to 1.8 μg μl^{-1} with good quality, which was evident from the agarose gel analysis (Fig. 1).

Fig. 1. Qualitative and quantitative analysis of total genomic DNA by 0.8% agarose gel. M, uncut phage DNA (500 ng); lanes 1 to 19, *M. sheldonii, M. tonalensis, M. elongata, M. spinosissima, M. bella, M. hahniana, M. glassii, M. longimamma, M. baumii, M. matudae, M. carmenae, M. plumosa, M. karwinskiana, M. herrerae, M. compressa, M. albicoma, M. knippeliana, M. pectinifera, M. humboldtii.*

RAPD analysis

25 primers were used for PCR amplification in the present study, out of which 22 primers (listed in Table I) resulted in reproducible and scorable bands; they were considered for the analysis of genetic relationship among the 31 species of *Mammillaria.* Three primers, namely OPA06, OPD06, and OPN01 did not result in satisfactory amplification. 22 primers resulted in the amplification of 621 amplicons of various sizes ranging from 100 bp to 5000 bp (Table I). The RAPD banding patterns using 4 random primers are represented in Fig. 2. The maximum number of bands (39) with the primer OPD08 and the lowest number (20) with the primers OPA02 and OPA08 (Table I) were amplified. Out of these 621 amplified bands, none was monomorphic and only one band of 1000 bp, amplified by the primer OPN04, was shared by all the species except *M. matudae* (Fig. 2). Only 57 (9.18%) bands were confined to a particular species (Table I). The highest number of bands (195) was amplified in *M. matudae* and the lowest (63) in *M. bocasana.*

Genomic relations

The similarity matrix was calculated using Jaccard's similarity coefficient (Jaccard, 1908) for the pooled data of all the primers. *M. winterae* and *M. zuccariniana* showed the highest similarity (0.622) and the lowest (0.109) was observed between *M. karwinskiana* and *M. mammillaris* (Table II). The

Table I. Details of RAPD banding pattern of 31 species of *Mammillaria* using 22 primers.

Name of the primer	Sequence of the primer	Total no. of bands amplified	Range of amplicons [bp]	No. and % of exclusive bands
OPA02	5′ TGCCGAGCTG 3′	20	400–2500	9 (45%)
OPA04	5′ AATCGGGCTG 3′	21	300–2200	2 (9.52%)
OPA07	5′ GAAACGGGTG 3′	27	300–2800	2 (7.41%)
OPA08	5′ GTGACGTAGG 3′	20	250–2500	5 (25%)
OPA09	5′ GGGTAACGCC 3′	37	100–2800	0 (00%)
OPA10	5′ GTGATCGCAG 3′	24	400–3000	0 (00%)
OPD02	5′ GGACCCAACC 3′	28	400–3155	2 (7.14%)
OPD03	5′ GTCGCCGTCA 3′	27	390–3190	4 (14.81%)
OPD05	5′ TGAGCGGACA 3′	37	250–3675	3 (8.11%)
OPD08	5′ GTGTGCCCCA 3′	39	400–4000	5 (12.82%)
OPD11	5′ AGCGCCATTG 3′	33	200–5000	0 (00%)
OPD16	5′ AGGGCGTAAG 3′	24	250–2000	0 (00%)
OPD20	5′ ACCCGGTCAC 3′	31	400–3000	3 (9.68%)
OPN02	5′ ACCAGGGGCA 3′	24	380–3100	1 (4.17%)
OPN04	5′ GACCGACCCA 3′	28	250–3000	2 (7.14%)
OPN05	5′ ACTGAACGCC 3′	36	200–3000	2 (5.56%)
OPN06	5′ GAGACGCACA 3′	24	400–2400	4 (16.67%)
OPN08	5′ ACCTCAGCTC 3′	30	280–3800	4 (13.33%)
OPN09	5′ TGCCGGCTTG 3′	31	350–3125	1 (3.23%)
OPN10	5′ ACAACTGGGG 3′	26	300–3200	5 (19.23%)
OPN13	5′ AGCGTCACTC 3′	30	500–3800	2 (6.67%)
OPN16	5′ AAGCGACCTG 3′	24	300–3000	1 (4.17%)
Total		621	100–5000	57 (9.18%)

UPGMA dendrogram based on SAHN clustering showed two distinct clusters, cluster II consisted of only two species *M. mammillaris* and *M. humboldtii* and the rest 29 species were included in cluster l. Cluster l again was sub-divided into two clusters, cluster lB consisted of only two species *M. bombycina* and *M. bocasana* and the rest 27 species were grouped under cluster lA which was further divided into 2 sub-clusters, cluster lA_1 and cluster lA_2 containing 5 and 22 species, respectively (Fig. 3). The result was compared with the grouping obtained from principal co-ordinate analysis (Fig. 4) and it was observed that the grouping remains more or less similar as obtained by the cluster analysis.

The comparison of similarity matrices using Mantel 'Z' statistics (Mantel, 1967) suggested that 10 primers (r = 0.90) as well as 15 primers (r = 0.95) were well comparable with the pooled data of 22 primers.

Discussion

In the present study, RAPD analysis was used to study the phylogenetic relationship among *Mammillarias*, which is extremely important to perform studies on genetic diversity and to develop efficient breeding methods. The RAPD technique has been successfully used in a variety of taxonomic and genetic relatedness studies (Bardacki, 2001; Harris, 1999) and was found by us to be suitable for the use among *Mammillarias* because of its ability to reproducibly generate polymorphic markers. The need for biosystematic inference and genetic conservation for efficient breeding methods of *Mammillaria* creates an incentive for the determination of the genetic variability present in it. The results presented here demonstrate the utility of using RAPD markers to characterize genetic relatedness and diversity among the *Mammillaria* species. The first step towards this direction was to isolate a good quality DNA which was difficult in case of cacti due to a very high amount of mucilage in the plant tissues. The main problem was the binding of water in the extraction buffer by mucilage present in the cacti tissue, producing a gel-like mixture, which prevented all further processing (Mondragon-Jacobo *et al*., 2000). The mucilage present in cacti was described as a water-soluble, pectin-like polysaccharide (Cardenas *et al*., 1997) with high water binding capacity (Loik and Nobel, 1991; Nobel *et al*., 1992). The ability of the Cactaceae to retain water

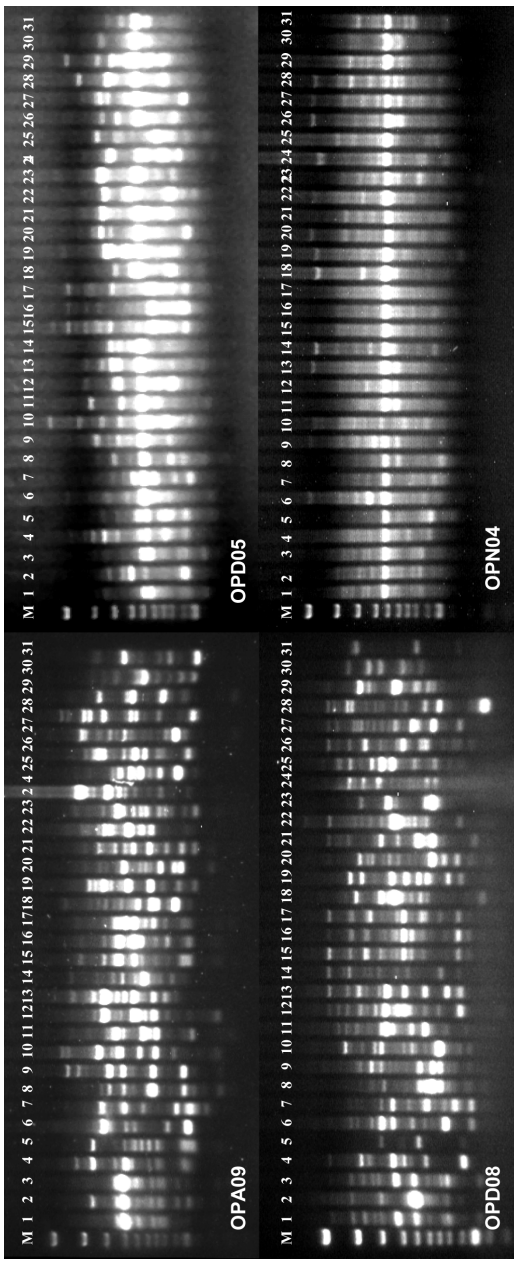

Fig. 2. RAPD banding patterns in 31 species of *Mammillaria* as revealed by the primers OPA09, OPD05, OPD08, and OPN04. M, gene ruler 100 bp ladder plus (MBI Fermantus, Lithuania); lanes 1 to 31, *M. sheldonii, M. fraileana, M. tonalensis, M. knippeliana, M. karwinskiana, M. longimamma, M. baumii, M. decipiens, M. camptotricha, M. matudae, M. elongata, M. spinosissima, M. bella, M. mammillaris, M. winterae, M. zuccariniana, M. compressa, M. albicoma, M. carmenae, M. zeilmanniana, M. pennispinosa, M. plumosa, M. pottsii, M. herrerae, M. saboae, M. hahniana, M. glassii, M. pectinifera, M. bombycina, M. humboldtii*, *M. bocasana*.

under unfavorable climatic conditions is partially due to this property (Nobel, 1988). As the mucilage content varies from species to species, different methods of DNA isolation were tried and modified as per the suitability.

In the present investigation different methods of DNA isolation including some specifically reported for isolation of cacti DNA (Mondragon-Jacobo *et al*., 2000; de la Cruz *et al.,* 1997) were tried. The method of Tel-Zur *et al.* (1999) can be effectively used in cacti species, which can grow independently without rootstock, but most of the *Mammillaria* are cultured as grafts on rootstocks of different genera/species. Barnwell *et al.* (1998) increased the CTAB content in a stepwise manner for specific precipitation of DNA avoiding co-precipitation of mucilage. De la Cruz *et al.* (1997) used a higher CTAB content (4%) for removal of polysaccharides. In the present investigation none of the methods worked properly in the species *M. herrerae* and *M. humboldtii.* For these two species the protocol was modified which resulted in good quality and quantity of DNA (Fig. 1) for further analysis. Out of the 25 primers used in the present investigation 3 primers (OPA06, OPD06, and OPN01) did not give amplified bands. Similar types of observations were reported earlier in different crop plants (Sosinski and Douches, 1996). The other 22 primers resulted in satisfactory amplification patterns with 100% polymorphism. The banding pattern as well as the total number of bands amplified in different species varied widely (from 63 to 195) indicating profound interspecific variation existing among the species. This result supported the usefulness of RAPDs for studying the genetic diversity as well as genetic relationship among the species of *Mammillaria*. RAPD was also reported earlier to be a very powerful technique for fingerprinting of different cacti species (Meregalli *et al*., 2000; Gutman *et al*., 2001).

Finding of wide genetic distances (Table II) reveals that there is relatively high genetic variation among the 31 species of *Mammillaria.* The considerable polymorphism detected in this study also illustrated that it is possible to find genetic divergence among *Mammillaria.* As per the similarity matrix, maximum similarity was found between *M. winterae* and *M. zuccariniana*, which belonged to one series, Macrothelae, and the minimum similarity was obtained between *M. karwinskiana* and *M. mammillaris*, which belonged to two different series, Polyedre and Macrothelae, respectively

Table II. Similarity co-efficient among 31 species of *Mammillaria* detected by RAPD polymorphisms using 22 primers (the bold numerals are the lowest and the highest value).

	M. sheldonii	*M. fraileana*	*M. tonalensis*	*M. knippeliana*	*M. karwinskiana*	*M. longimamma*	*M. baumii*	*M. decipiens*	*M. camptotricha*	*M. matudae*	*M. elongata*	*M. spinosissima*	*M. bella*	*M. mammillaris*	*M. winterae*	*M. zuccariniana*	*M. compressa*	*M. albicoma*	*M. carmenae*	*M. zeilmanniana*	*M. pennispinosa*	*M. plumosa*	*M. pottsii*	*M. herrerae*	*M. saboae*	*M. hahniana*	*M. glassii*	*M. pectinifera*	*M. bombycina*	*M. humboldtii*	*M. bocasana*
M. sheldonii	1.00																														
M. frailana	0.34	1.00																													
M. tonalensis	0.54	0.30	1.00																												
M. knippeliana	0.23	0.20	0.28	1.00																											
M. karwinskiana	0.17	0.17	0.22	0.36	1.00																										
M. longimamma	0.23	0.19	0.25	0.16	0.18	1.00																									
M. baumii	0.23	0.17	0.24	0.17	0.16	0.26	1.00																								
M. decipiens	0.17	0.17	0.16	0.17	0.20	0.19	0.19	1.00																							
M. camptotricha	0.16	0.18	0.17	0.20	0.19	0.20	0.20	0.42	1.00																						
M. matudae	0.23	0.19	0.23	0.22	0.16	0.21	0.19	0.18	0.22	1.00																					
M. elongata	0.19	0.15	0.18	0.21	0.23	0.17	0.20	0.27	0.26	0.22	1.00																				
M. spinosissima	0.23	0.22	0.19	0.22	0.16	0.15	0.20	0.21	0.22	0.22	0.25	1.00																			
M. bella	0.19	0.20	0.17	0.22	0.17	0.14	0.20	0.20	0.18	0.20	0.21	0.41	1.00																		
M. mammillaris	0.13	0.16	0.14	0.18	**0.11**	0.13	0.14	0.18	0.19	0.21	0.17	0.18	0.16	1.00																	
M. winterae	0.21	0.20	0.21	0.24	0.17	0.18	0.15	0.20	0.22	0.22	0.20	0.22	0.22	0.22	1.00																
M. zuccariniana	0.21	0.16	0.20	0.23	0.18	0.16	0.17	0.19	0.17	0.20	0.19	0.23	0.19	0.18	**0.62**	1.00															
M. compressa	0.24	0.18	0.23	0.22	0.18	0.19	0.17	0.21	0.20	0.24	0.20	0.23	0.23	0.21	0.37	0.35	1.00														
M. albicoma	0.21	0.16	0.24	0.20	0.21	0.19	0.18	0.21	0.20	0.22	0.21	0.22	0.22	0.18	0.20	0.17	0.24	1.00													
M. carmenae	0.15	0.20	0.18	0.21	0.17	0.13	0.18	0.17	0.19	0.20	0.17	0.20	0.20	0.19	0.18	0.16	0.17	0.20	1.00												
M. zeilmanniana	0.17	0.18	0.21	0.25	0.19	0.17	0.19	0.21	0.21	0.22	0.22	0.20	0.21	0.19	0.19	0.16	0.20	0.21	0.24	1.00											
M. pennispinosa	0.20	0.24	0.22	0.21	0.17	0.20	0.22	0.21	0.23	0.24	0.23	0.22	0.21	0.15	0.24	0.22	0.25	0.21	0.25	0.29	1.00										
M. plumosa	0.18	0.21	0.20	0.28	0.19	0.20	0.20	0.19	0.21	0.24	0.18	0.21	0.19	0.20	0.23	0.20	0.22	0.24	0.21	0.25	0.26	1.00									
M. pottsii	0.17	0.19	0.18	0.20	0.17	0.22	0.17	0.20	0.23	0.16	0.20	0.21	0.20	0.18	0.20	0.17	0.21	0.18	0.20	0.26	0.29	0.19	1.00								
M. herrerae	0.21	0.20	0.21	0.20	0.17	0.22	0.21	0.18	0.23	0.21	0.19	0.19	0.19	0.17	0.20	0.19	0.21	0.19	0.19	0.22	0.26	0.26	0.19	1.00							
M. saboae	0.20	0.22	0.22	0.21	0.20	0.15	0.17	0.21	0.22	0.27	0.21	0.20	0.18	0.19	0.22	0.19	0.18	0.23	0.21	0.23	0.21	0.23	0.23	0.25	1.00						
M. hahniana	0.17	0.20	0.22	0.25	0.19	0.19	0.22	0.20	0.19	0.26	0.22	0.23	0.25	0.22	0.26	0.25	0.26	0.24	0.19	0.23	0.25	0.22	0.23	0.22	0.25	1.00					
M. glassii	0.23	0.22	0.21	0.21	0.18	0.18	0.22	0.22	0.19	0.25	0.25	0.25	0.26	0.15	0.22	0.19	0.22	0.20	0.18	0.24	0.28	0.20	0.24	0.21	0.22	0.27	1.00				
M. pectinifera	0.20	0.20	0.18	0.26	0.17	0.17	0.17	0.17	0.18	0.26	0.20	0.20	0.21	0.22	0.22	0.20	0.23	0.23	0.19	0.23	0.25	0.21	0.18	0.19	0.24	0.23	0.29	1.00			
M. bombycina	0.21	0.19	0.19	0.19	0.17	0.19	0.17	0.23	0.23	0.21	0.20	0.22	0.19	0.18	0.18	0.15	0.21	0.21	0.19	0.20	0.22	0.21	0.23	0.17	0.21	0.20	0.25	0.21	1.00		
M. humboldtii	0.15	0.17	0.15	0.12	0.18	0.20	0.16	0.16	0.18	0.18	0.15	0.16	0.12	0.22	0.20	0.20	0.19	0.19	0.14	0.15	0.20	0.18	0.21	0.17	0.18	0.22	0.15	0.18	0.21	1.00	
M. bocasana	0.12	0.16	0.13	0.14	0.16	0.17	0.15	0.15	0.14	0.20	0.12	0.14	0.15	0.14	0.13	0.13	0.13	0.14	0.18	0.16	0.19	0.16	0.19	0.14	0.16	0.14	0.17	0.16	0.23	0.17	1.00

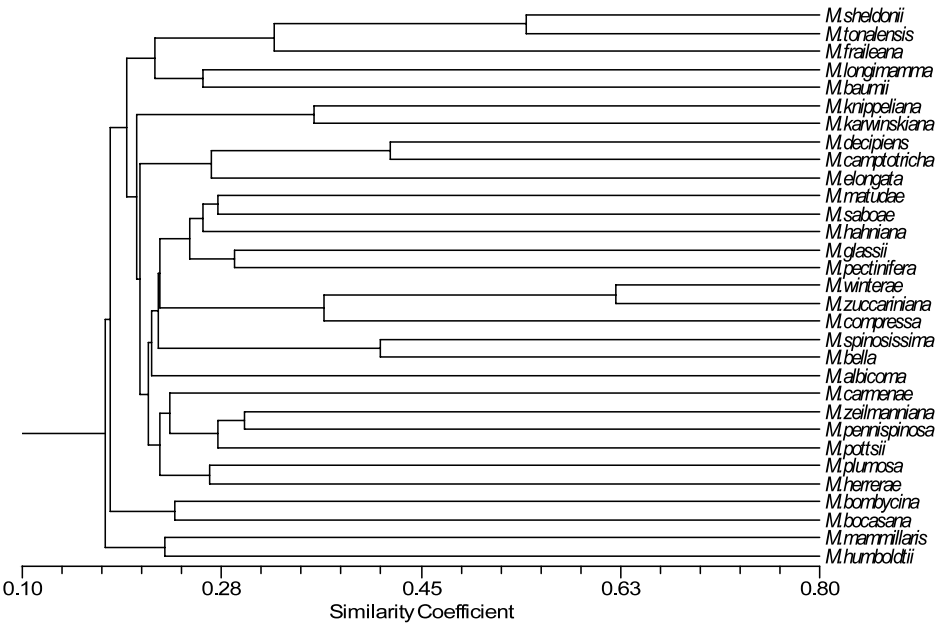

Fig. 3. Dendrogram showing genetic relationship among 31 species of *Mammillaria* as obtained from the UPGMA cluster analysis calculated on the basis of banding pattern in RAPD markers using 22 primers.

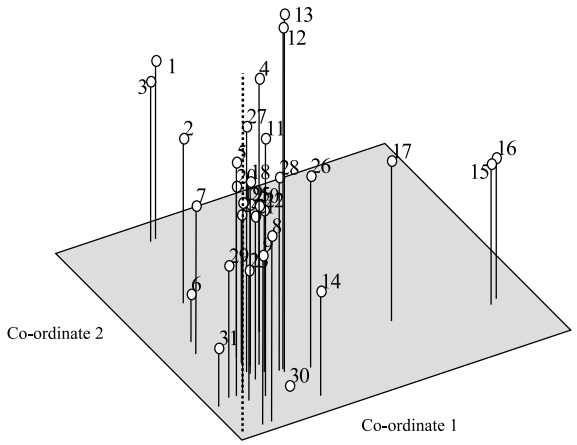

Fig. 4. Relationship among 31 species of *Mammillaria* by principal co-ordinate analysis. 1 to 31, *M. sheldonii, M. fraileana, M. tonalensis, M. knippeliana, M. karwinskiana, M. longimamma, M. baumii, M. decipiens, M. camptotricha, M. matudae, M. elongata, M. spinosissima, M. bella, M. mammillaris, M. winterae, M. zuccariniana, M. compressa, M. albicoma, M. carmenae, M. zeilmanniana, M. pennispinosa, M. plumosa, M. pottsii, M. herrerae, M. saboae, M. hahniana, M. glassii, M. pectinifera, M. bombycina, M. humboldtii*, *M. bocasana*. (The dotted line indicates the 'Z' axis.)

(Marsden, 1957; Hunt, 1987). The dendrogram formed many sub-clusters and in most of the cases the closely related species, as described by authors earlier (Marsden, 1957; Pilbeam, 1987; Hunt, 1987) on the basis of morphological markers, shared the same node justifying their taxonomical status. But some species like *M. mammillaris* and *M. humboldtii* formed an entirely separate cluster. Some variability could, however, be due to the different environmental and soil conditions experienced by the species that are not expected to affect DNA marker patterns. Again, the morphological characters were the result of interactions between environment and gene and hence are not always stable, whereas RAPD takes into account the variability present in the DNA. Further, this difference in grouping might be due to the low number of species studied. Similar observation was earlier reported by lruela *et al.* (2002) in *Cicer*, Wolff and Morgan-Richards (1998) in *Plantago* and Pipe *et al.* (1995) in *Ophiostoma*. The present observation also supports their views. The inter-relationship among different species of *Mammillaria* was further confirmed using principal co-ordinate analysis (Fig. 4). The grouping showed a similar trend as observed by SAHN clustering. In other plant spe-

cies Han *et al*. (2000) and Archak *et al.* (2002) made similar observations. Mantel 'Z' statistics suggested that though 10 primers ($r = 0.90$) were adequate enough for successful fingerprinting of *Mammillaria*, the best results could be obtained using 15 primers ($r = 0.95$). A similar result was reported earlier by Ray Choudhury *et al.* (2001) in rice.

The present report, though a preliminary one on the usefulness of RAPD markers for the classification of *Mammillaria*, shows the usefulness for classification of cacti. However, further studies addressing this point directly are required before any robust hypothesis can be formulated. We believe that the approaches used in this work will make a valuable contribution to the discussions related to the conservation of cacti genetic resources, in order to decide which needs to be conserved, it is first necessary to be able to measure the genetic relatedness that exists.

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Archak S., Karihaloo J. L., and Jain A. (2002), RAPD markers reveal narrowing genetic base of Indian tomato cultivars. Curr. Sci. **82**, 1139–1143.

Attanayaka D. P. S. T. G., Herath S. P., Gamage A. K., and Karuanayake E. H. (2000), Identification of RRIC 100 series and two germplasm clones of rubber (*Hevea brasiliensis*) using random amplified polymorphic DNA (RAPD) technique. J. Plantation Crops **28**, 160–163.

Bardacki F. (2001), Random amplified polymorphic DNA (RAPD) markers. Turk. J. Biol. **25**, 185–196.

Barnwell P., Blanchard A. N., Bryant J. A., Smirnoff N., and Weir A. F. (1998), Isolation of DNA from the highly mucilagenous succulent plant *Sedum telephium*. Plant Mol. Biol. Reporter **16**, 133–138.

Cardenas A. l., Higuera-Ciapara, and Goycoolea F. M. (1997), Rheology and aggregation of cactus (*Opuntia ficus-indica*) mucilage solutions. J. PACD **2**, 152–159.

de la Cruz M., Ramirez F., and Herbabdez H. (1997), DNA isolation and amplification from cacti. Plant Mol. Biol. Reporter **15**, 319–325.

Doyle J. J. and Doyle J. L. (1990), Isolation of plant DNA from fresh tissue. Focus **12**, 13–15.

Gutman F., BarZvi D., Nerd A., and Mizrahi Y. (2001), Molecular typing of *Cereus peruvianus* clones and their genetic relationships with other *Cereus* species evaluated by RAPD analysis. J. Hort. Sci. Biotech. **76**, 709–713.

Han T. H., Jeu M. D., Eck H. V., and Jacobsen E. (2000), Genetic diversity of Chilean and Brazilian *Alstroemeria* species assessed by AFLP analysis. Heredity **84**, 564–569.

Harris S. A. (1999), RAPDs in systematics – a useful methodology? In: Molecular Systematics and Plant Evolution (Hollingsworth P. M., Bateman R. M., and Gornall R. J., eds.). Taylor and Francis, London, pp. 211–228.

Hunt D. (1987), A New Review of *Mammillaria* Names. British Cactus and Succulent Society, Oxford, UK, pp. 105–128.

Iruela M., Rubio J., Cubero J. l., Gil J., and Millan T. (2002), Phylogenetic analysis in the genus *Cicer* and cultivated chickpea using RAPD and ISSR markers. Theor. Appl. Genet. **104**, 643–651.

Jaccard P. (1908), Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. **44**, 223–270.

Lamb M. B. (1991), Letts Guide to Cacti of the World. Charles Letts and Co., London.

Loik M. E. and Nobel P. S. (1991), Water relations and mucopolysaccharide increases for winter hardy cactus during assimilation to subzero temperatures. Oecologia **88**, 340–346.

Mantel N. A. (1967), The detection of disease clustering and a generalized regression approach. Cancer Res. **27**, 209–220.

Marsden C. (1957), *Mammillaria*. Cleaver-Hume Press, London.

Meregalli M., Metzing D., Kiesling R., and Caramiello R. (2000), Systematics of the *Gymnocalycium paraguayense-fleischerianum* Group: Morphological and Biomolecular Data. 25. IOS Congress, Zurich, Switzerland, 19–24 March.

Mondragon-Jacobo C., Doudareva N., and Bordelon B. P. (2000), DNA extraction from several cacti. Hort. Sci. **35**, 1124–1126.

Nobel P. S. (1988), Environmental Biology of Agaves and Cacti. Cambridge University Press, Cambridge.

Nobel P. S., Cavalier J., and Andrade J. L. (1992), Mucilage in cacti: Its apoplastic capacitance, associated solutes and influence on tissue water relations. J. Exp. Biol. **43**, 641–648.

Panda P. C. and Das P. (1995), Genetic resources of cacti and other succculents. In: Advances in Horticulture, Vol. 12, Ornamental Plants (Chadha K. L. and Bhat-

tacharjee S. K., eds.). Malhotra Publishing House, New Delhi, India, pp. 243–266.
Pilbeam J. (1987), Cacti for the Connoisseur: A guide for Growers and Collectors. B.T. Batsford, London.
Pipe N. D., Buck K. W., and Brasier C. M. (1995), Genomic fingerprinting supports the separation of *Ophiostoma piceae* into two species. Mycol. Res. **99**, 1182–1186.
Ray Choudhury P., Kohli S., Srinivasan K., Mohapatra T., and Sharma R. P. (2001), Identification and classification of aromatic rices based on DNA fingerprinting. Euphytica **118**, 243–251.
Riha J. and Subik R. (1992), The Illustrated Encyclopedia of Cacti and Other Succulents (Beckett G. and Beckett K. A., eds.). The Promotional Reprint Co. Ltd., London.
Rohlf F. J. (1997), NTSYS-pc numerical taxonomy and multivariate analysis system. Version 2.02e. Exeter Software, Setauket, New York.
Rowley J. (1978), The Illustrated Encyclopedia of Succulents. Leisure Books, UK, p. 256.
Rowley G. D. (1985), *Caryopphyllidae*. In: Flowering Plants of the World (Heywood V. H., ed). Croom Helm Publishers, England, pp. 63–65.
Schuster D. (1990), The World of Cacti. Facts on File Ltd., Oxford, UK.
Slaba R. (1992), The Illustrated Guide to Cacti. Chancellor Press, London.
Sneath P. H. A. and Sokal R. R. (1973), Numerical Taxonomy. Freeman, San Francisco, California.
Sosinski B. and Douches D. S. (1996), Use of PCR based DNA amplification to fingerprint North American potato cultivars. Hort. Sci. **31**, 130–133.
Tel-Zur N., Abbo S., Myslabodski D., and Mizrahi Y. (1999), Modified CTAB procedure for DNA isolation from epiphytic cacti of the genera *Hylocereus* and *Selenicereus* (Cactaceae). Plant Mol. Biol. Rep. **17**, 249–254.
Wang X., Felker P., Burrow M. D., and Paterson A. H. (1998), Comparison of RAPD marker patterns to morphological and physiological data in the classification of *Opuntia* accessions. J. PACD **3**, 3–14.
Weniger D. (1969), Cacti of the South West. University of Texas Press, London.
Williams J. G. K., Kubelik A. R., Livak K. J., Rafalski J. A., and Tingey S. V. (1990), DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. **18**, 6531–6535.
Wolff K. and Morgan-Richards M. (1998), PCR markers distinguish *Plantago major* subspecies. Theor. Appl. Genet. **96**, 282–296.