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Genomic Relationship Among 25 Species of *Mammillaria* Haw. as Revealed by Isozyme and Protein Polymorphism

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Abstract

Buffer soluble protein and five isozymes were analyzed to assess the inter specific relationship among 25 species of the genus *Mammillaria* Haw. A total of 102 types of proteins were resolved, out of which eighty-six types were found to be polymorphic and only two were unique. A total of 248 bands (isoforms) were detected for 5 isozymes, among them only 4 were found to be monomorphic and 35 were exclusive. Mantel 'Z' statistics revealed wide variations in the correlation among different enzymes. The correlation value 'r' was the highest in case of esterase with pooled data of all the five enzymes. The dendrogram constructed on the basis of pooled data (protein and allozyme) divided the species into two major clusters containing 14 and 11 members respectively. The species *M. matudae* and *M. bella* were found to be the most closely related while *M. decipience* and *M. camptroticha* were distantly apart. The present study gave an indication of usefulness of the isozyme and protein markers for genetic discrimination between different species of *Mammillaria*.

Key words: Isozyme, Cactus, *Mammillaria*, Protein, Genomic relations, Chemical characterization

Introduction

Mammillaria Haw. is one of the largest genera in the family Cactaceae. It is second only to *Opuntia* in size and to none in popularity. Due to its beautiful shape, it is used as an ornamental houseplant with beautiful flowers. There are about 200 species (Slaba 1992) and only 62 cultivated/naturalized species were reported from India (Panda and Das 1995). The genus has a vast geographical distribution. From Mexico it extends north into the USA, east to the West Indies and south into northern South America and also with odd species occurring in the Central America, Colombia and Venezuela.

Interpretations by different authors of classifications and cactus names are more or less in agreement as far as the larger groupings are concerned. But at the lower levels of plant units, such as genus and species major differences of opinion exist (Schuster, 1990). This can be clearly observed if one studies the past history of the works done by different taxonomists Marsden (1957), Weniger (1969), Rowley (1978), Hunt (1987), Pilbeam (1987), Lamb (1991) and Slaba (1992)] to further classify the genus *Mammillaria*. These taxonomists tried to subdivide *Mammillaria* on the basis of morphological characters like stem, tubercles, spines, flowers and fruit characters, etc. and there exist many discrepancies in their views.

For the purpose of conservation and to carry out successful breeding programmes, proper identification of the plant is of the prime importance, for which an accurate, reliable and more authentic system of classification is required. Biochemical markers and molecular markers are the powerful tools available today for discerning biosystematic, biogeographic and phylogenetic relationships. These markers are more advanced and have many advantages over the conventional markers. Visual descriptions of morphological characters of a plant require long time and it can be affected by environmental and soil conditions and the number of useful characters in some species are also limited. Allozyme and protein characterization can provide reliable information to establish distinctness over and above morpho-agronomic characteristics (Chang et al. 2003).

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Therefore, morphological markers need the support of biochemical and molecular informations to clarify taxonomic structures. Molecular markers are presently the marker of choice for characterising plant species (Henry 2001) but in cacti the presence of high quantity of polysaccharides in the form of mucillage has made it difficult to isolate good quality of DNA (Scott and Playford 1996) and also interference with Taq DNA polymerase activity (Fang et al. 1992) prevented the researchers to work with molecular markers and molecular markers are also costly in comparison with the biochemical markers. Besides, isozyme markers have been utilised successfully for discrimination between species and also to characterize interspecific hybrids (Desborough and Peloquin 1966, 1968, 1971; Gao and Hong 2000; Kumar et al. 2001). So, in the present investigation biochemical markers like isozyme and protein polymorphism have been utilized for characterization of 25 species of *Mammillaria*.

Materials and Methods

Plant materials

For extraction of total soluble proteins and isozyme, meristematic zone of the fresh young vegetative buds were collected on ice bucket. The tissues were washed thoroughly after removing the spines. The fresh tissue was pulverized with 2% of insoluble polyvinyl pyrrolidone (PVPP) in presence of liquid nitrogen.

Isolation of total soluble protein

Soluble protein was isolated in a buffer system suggested by Sengupta and Chattopadhyaya (2000). Five hundred mg of tissue were ground to fine powder with 10 mg (2%) of insoluble PVP (PVP was added to avoid contamination by any phenolic compounds, Geburek et al. 1987) in a cold mortar with repeated addition of liquid nitrogen to avoid thawing. The powdered tissue was then homogenized in 0.5 ml of cold extraction buffer (0.2 M Tris Cl, pH 7.6). The homogenate was transferred to an eppendorf tube (1.7 ml). Again the mortar was washed with 0.5 ml of extraction buffer and the washing was collected to avoid any loss of protein. The homogenate was centrifuged at 10,000 rpm for 10 min. at 4℃ in a cooling centrifuge (Remi, India). The supernatant was pipetted out and used for qualitative and quantitative analysis of total soluble protein. The protein content was measured following the protocol of Bradford (1976) using known concentration of Bovine Serum Albumin (BSA) as reference concentration.

Extraction of Isozymes

To avoid phenolic compounds 20 mg of PVP was added to 500 mg of tissue (Geburek et al 1987) and ground to fine powder in presence of liquid nitrogen in a cold mortar. The powder was then homogenized with 0.5ml cold extraction buffer containing 0.05 M Tris Cl, pH 7.2, 6mM ascorbic acid, 6mM Cystein-Cl and 0.5 M Sucrose (Rychter and Lewak 1969).

Poly Acrylamide Gel Electrophoresis (PAGE)

SDS-PAGE

A denaturing polyacrylamide gel containing 15% running and 4% stacking gel was prepared. Gel composition, preparation and composition of the running buffer were similar to those as described by Dadlani and Varier (1993). 30 μg of each protein sample was mixed with equal volume of 2 X sample loading buffer (Dadlani and Varier 1993), boiled at 100℃ for 5 minutes in water bath and immediately transferred into icebox. The samples were loaded in the wells of the gel after thoroughly cleaning the wells using running buffer (0.1% SDS, 25 mM Tris-Glycine, pH 8.3). Electrophoresis was performed in a vertical gel electrophoresis (Protean IIXi, BioRad, USA) at 1.5 mA per well constant current till the tracking dye entered the separating gel and then the current was increased to 2 mA per well till the tracking dye reached the bottom of the gel. The temperature of the whole system was maintained at 5℃ using a cold water circulator.

After electrophoresis the gel was washed twice with distilled water and fixed in 12.5% trichloroacetic acid (TCA) for overnight. Then the gel was removed from TCA and washed thoroughly to remove excess SDS. The gel was stained overnight in a staining solution containing 0.05% of Coommassie Brilliant Blue R250 (BioRad, USA) as per the methodology of Charambach et al. (1967). Distaining was done in 10% TCA solution and the gel was photographed, recorded using Gel scanner (GS-710 Calibrated Imaging Densitometer, BIO RAD, USA) and then fixed in 7% acetic acid for longer storage.

Native- PAGE

Electrophoretic resolution for isozymes was carried out in native PAGE as per the methodology described by Dadlani and Varier (1993). The gels were stained in different staining solutions for five different enzymes (ACP, EST, GOT, MDH and PRX) using the methodology as described by Cardy et al. (1981).

Data analysis

After staining, the gels were photographed and data were recorded in binomial matrix form i.e. presence and absence of band was denoted as '1' and '0' respectively. The relative migration (R_m) value was calculated according to the formula described by Eeswara and Peiris (2001):

$$R_m = \frac{\text{Distance migrated by the band from the cathode edge}}{\text{Distance migrated by the tracking dye}}$$

All the bands were taken into consideration to avoid over/underestimation of genomic relations. After scoring, the data were analyzed for similarity matrix (Jaccard 1908) and UPGMA cluster analysis (SAHN) and Mantel correlation (Mantel 1967) using the standard computer package NTSYS-PC 2.02e (Rohlf 1997).

Results

Buffer soluble proteins and five isozymes namely acid phosphatase (ACP), esterase (EST), glutamate oxaloacetate transaminase (GOT) {also known as aspartate amino transferase (AAT)}, malate dehydrogenase (MDH), peroxidase (PRX) were used as biochemical markers to assess the genetic relations among 25 species of *Mammillaria* (listed in Table 1). The number of proteins and isoforms of different enzymes varied so much so that it was difficult for comparison. The details of band resolution are described below.

Twenty-five species of *Mammillaria* were subjected to the studies on the polymorphism in buffer soluble proteins using SDS-PAGE. A total of 102 types of proteins were observed with the Rm (relative migration) values ranging from 0.11 (65 kDa) to 0.99 (3 kDa) (Fig. 1e). Eighty-six types of proteins were found to be polymorphic whereas only two were found to be unique (Table 1). The types of proteins varied from species to species, 54 in M. knippeliana and M. winterae to 74 in *M. glassii*. The similarity matrix values revealed high degree of similarity among different species of *Mammillaria* values ranging from 0.46 to 0.87 (Table 2). The cluster diagram based on the similarity coefficient among 25 species of *Mammillaria* revealed two distinct groups with 8 species in one group and the rest 17 in another group (Figure not shown).

A total of 5 enzymes (4 isozymes are represented in the Fig.1a-d) resulted in the resolution of 248 bands (isoforms) out of which only 4 were found to be monomorphic and 33 were species specific. The esterase ranked first among all the isozymes when the number of bands (95) resolved and the glutamate oxaloacetate transaminase showed the lowest number of bands (22) (Table 1). In individual species the highest number of bands (93) was resolved in *M. albicoma* and *M. pectinifera* and that of lowest (38) was found in *M. camptotricha*. Total 33 isoforms were obtained for acid phophatase (Table 1) with the highest number in the species *M. pectinifera* (15) and that was lowest in 4 species, namely *M. karwinskiana*, *M. longimamma*, *M. baumii* and *M. bocasana* (3). Two bands with the Rm values 0.44 and 0.55 were shared by maximum number (14) of species. A total number of 9 isoforms were observed to be species specific (Table 1). The similarity matrix of the combined data showed the maximum similarity (0.5106) between *M. zuccariniana* and *M. compressa* and the lowest similarity (0.1296) between *M. tonalensis* and *M. camptotricha* (Table 3). Similar results were reflected when the dendrogram was constructed using SAHN clustering. The dendrogram showed two major clusters; cluster-I represented by 16 species and the rest 9 species were grouped under cluster-II. The cluster-I was further subdivided into two sub clusters, IA presenting 14 species and the other (IB) having two species. Cluster II was further divided into two sub-clusters, IIA, containing two species and the rest 7 species were grouped under IIB (Fig not shown).

The pooled data of proteins and isozymes showed a total

Table 1. Details of biochemical markers (proteins and enzymes)

Bochemical markers		Total no. of bands resolved	Range of Rm values	Total no. of polymorphic bands	Total no. of monomorphic bands	No. of bands resolved in a single species
Protein		102	0.11-0.99	86 (84.31%)	16 (15.69%)	2 (1.96%)
Enzymes	ACP	33	0.10-0.83	33 (100%)	0.0 (0%)	9 (27.27%)
	EST	95	0.03-0.99	95 (100%)	0.0 (0%)	7 (7.37%)
	GOT	22	0.36-0.65	20 (90.91%)	2 (9.09%)	5 (22.73%)
	MDH	54	0.04-0.86	53 (98.15%)	1 (1.85%)	6 (11.11%)
	PRX	44	0.07-0.78	43 (97.73%)	1 (2.27%)	6 (13.64%)
Total		**350**	**0.03-0.99**	**330 (94.29%)**	**20 (5.71%)**	**35 (10%)**

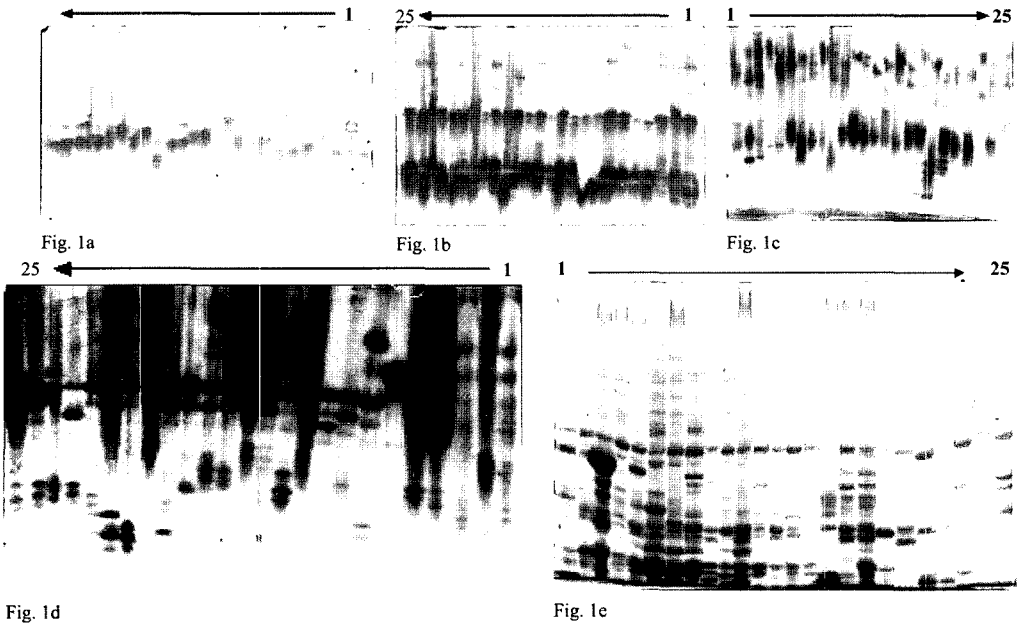

Figure 1. Isozymes and buffer soluble protein profiles (Fig Ia -Ie = Glutamate oxaloacetate transaminase, Malate dehydrogenase, Esterase, Peroxidase and Protein respectively) in 25 species of *Mammillaria.* (Lane 1 to 25 = *M. sheldonii*, *M. fraileana*, *M. tonalensis*, *M. Knippeliana*, *M. karwinskiana*, *M. longimamma*, *M. baumii*, *M. decipiens*, *M. camptotricha*, *M. bella*, *M. mammillaris*, *M. winterae*, *M. zuccariniana*, *M. compressa*, *M. albicoma*, *M. zeilmanniana*, *M. plumosa*, *M. hahniana*, *M. glassii*, *M. pectinifera*, *M. bocasana*, *M. bombycina*, *M. elongata*, *M. pennispinosa* and *M. matudae* respectively).

of 350 bands, out of which, 330 (94.29%) were polymorphic and only 35 (10%) were unique (Table 1). The similarity values ranging from 0.3224 to 0.6797 revealed a fair degree of similarities among different species of *Mammillaria.* The dendrogram based on similarity coefficient showed two distinct clusters. In cluster I, 14 species and in cluster II, 11 species were grouped. Both the clusters II and I were subdivided into two sub-clusters, IA, IB and IIA, IIB respectively (Fig. 2).

Mantel 'Z' statistics revealed wide variations in the correlation among different enzymes. The 'r' value was the highest in case of esterase with pooled data of all the five enzymes. The correlation of the similarity matrices of the total biochemical markers (proteins + enzymes) with that of the pooled data of all enzymes was very good (0.8) and the same with the protein was equally comparable. (0.7).

Discussions

The limitations of morphological markers have resulted in the deployment of biochemical markers like isozymes and proteins (Hunter and Markert 1957). It was proposed that isozymes (Scandalios 1969) and total protein banding patterns (Desborough and Peloquin 1968; Stegemann and Loeschke 1977) could be used as genetic markers.

In the present study considering the protein polymorphism it was observed that the closely related species as described by morphological characters were grouped together sharing same sub-clusters. It was interesting to note that though *M. knippeliana* and *M. sheldonii* belong to different series (Hunt 1987), they shared highest similarity i.e. 0.87 using protein markers in contrary to other markers. The protein-banding pattern changes according to specific gene action and the genetic set up of each species making it useful for studying the genetic variability among the species. This confirmed the earlier reports of Yaakor and De wet (1975) in seed proteins from cultivated races of *Sorghum* (Fullington et al. 1983).

The specific isozyme pattern was observed only in five species. This result contradicted the earlier report of Oleary and Boyle (1998, 1999, 2000) who studied the diversity in easter cactus (*Hatiora* sp.). This variation might be due to the selection of species chosen from widely differing genera. However, their report on the GOT allozyme pattern shared by at least two species was confirmed by the present work. A reasonable number of polymorphic isoforms (43 out of 44) of peroxidase enzyme indicated it' s usefulness for studying inter specific variations in *Mammillaria.* The present investigation supported the earlier report of Gray et al. (1973), Lee and Fairbrothers (1973), Payne and Fairbrothers (1976) and Scogin (1969) who reported presence of many Isozymes in esterases and peroxidases in other plant species

Table 2. Genetic similarity among 25 species of *Mammillaria* detected by protein polymorphism (the underlined numerals represent the highest and lowest value)

	M.sheldonii	*M.fraileana*	*M.tonalensis*	*M.knippeliana*	*M.karwinskiana*	*M.longimamma*	*M.baumii*	*M.decipiens*	*M.camptotricha*	*M.bella*	*M.mammillaris*	*M.winterae*	*M.zuccariniana*	*M.compressa*	*M.albicoma*	*M.zeilmanniana*	*M.plumosa*	*M.hahniana*	*M.glassii*	*M.pectinifera*	*M.bocasana*	*M.bombycina*	*M.elongata*	*M.pennispinosa*	*M.matudae*
M.sheldonii	1.00																								
M.fraileana	0.86	1.00																							
M.tonalensis	0.86	0.80	1.00																						
M.knippeliana	**0.87**	0.80	0.83	1.00																					
M.karwinskiana	0.70	0.69	0.74	0.71	1.00																				
M.longimamma	0.68	0.63	0.68	0.69	0.67	1.00																			
M.baumii	0.59	0.55	0.64	0.56	0.69	0.65	1.00																		
M.decipiens	0.67	0.63	0.69	0.66	0.68	0.69	0.69	1.00																	
M.camptotricha	0.70	0.70	0.68	0.70	0.65	0.72	0.64	0.86	1.00																
M.bella	0.57	0.59	0.57	0.60	0.63	0.61	0.54	0.61	0.64	1.00															
M.mammillaris	0.60	0.59	0.62	0.61	0.63	0.61	0.62	0.64	0.60	0.60	1.00														
M.winterae	0.60	0.58	0.60	0.61	0.64	0.67	0.56	0.62	0.56	0.70	0.68	1.00													
M.zuccariniana	0.67	0.64	0.65	0.64	0.60	0.60	0.59	0.64	0.65	0.61	0.66	0.64	1.00												
M.compressa	0.63	0.61	0.59	0.58	0.63	0.63	0.58	0.63	0.66	0.64	0.60	0.65	0.77	1.00											
M.albicoma	0.60	0.59	0.54	0.59	0.57	0.57	0.54	0.58	0.60	0.58	0.52	0.59	0.59	0.60	1.00										
M.zeilmanniana	0.68	0.66	0.62	0.61	0.65	0.64	0.58	0.74	0.73	0.62	0.61	0.63	0.70	0.73	0.72	1.00									
M.plumosa	0.59	0.59	0.64	0.61	0.69	0.59	0.66	0.71	0.64	0.66	0.60	0.61	0.59	0.56	0.62	0.64	1.00								
M.hahniana	0.58	0.54	0.57	0.53	0.52	0.50	0.55	0.62	0.57	0.54	0.53	0.55	0.62	0.56	0.59	0.59	0.63	1.00							
M.glassii	0.69	0.63	0.71	0.64	0.62	0.65	0.65	0.72	0.69	0.55	0.64	0.58	0.68	0.65	0.64	0.72	0.67	0.68	1.00						
M.pectinifera	0.59	0.60	0.61	0.56	0.56	0.56	0.54	0.62	0.57	**0.46**	0.52	0.48	0.53	0.48	0.52	0.57	0.63	0.64	0.66	1.00					
M.bocasana	0.64	0.59	0.66	0.61	0.61	0.65	0.56	0.71	0.66	0.62	0.60	0.63	0.61	0.62	0.62	0.71	0.62	0.67	0.73	0.69	1.00				
M.bombycina	0.62	0.58	0.66	0.61	0.61	0.66	0.59	0.72	0.66	0.58	0.63	0.63	0.61	0.56	0.61	0.71	0.70	0.61	0.71	0.65	0.82	1.00			
M.elongata	0.56	0.52	0.57	0.57	0.53	0.62	0.48	0.62	0.61	0.54	0.55	0.57	0.58	0.54	0.61	0.64	0.59	0.60	0.67	0.62	0.75	0.77	1.00		
M.pennispinosa	0.55	0.51	0.58	0.54	0.55	0.59	0.47	0.54	0.52	0.53	0.52	0.56	0.57	0.55	0.61	0.61	0.52	0.59	0.60	0.55	0.71	0.72	0.77	1.00	
M.matudae	0.59	0.59	0.57	0.60	0.54	0.59	0.46	0.57	0.59	0.57	0.53	0.54	0.55	0.51	0.62	0.62	0.58	0.58	0.61	0.53	0.68	0.67	0.66	0.67	1.00

thus making them useful for systematic studies.

While the isozyme patterns of ACP along with other enzymes were used for biochemical characterization in the cactus species *Cereus peruvianus* (Mangolin et al. 1997), this was the first report for *Mammillaria*. Esterase isozyme revealed high degree of polymorphism in twenty-five species of *Mammillaria* studied.

All the five enzymes revealed many isozymes with polymorphism depicting wide interspecific variation among the 25 species of *Mammillaria*. Different enzymes showed different isozymes ranging from 95 for esterase to only 22 for GOT. The banding pattern in different species also varied widely (Table 3) revealing a wide variation in their genetic set up. This result was also reflected in the similarity matrix where the similarity value varied from 0.1296 to 0.5106 again indicating moderate similarity among them. Similar results were also reflected in the cluster analysis. Though only two major clusters were obtained, the intra-cluster variations were too many indicating major differences in their genetic set up. Similar observations were reported by Turner (1969) who reported high degree of inter as well as intra population variations. Hamrick and Godt (1989, 1996) collated published data from allozyme studies and concluded that, in general, widespread species have high levels of variation. Variation at the population and species level and distribution were described in different ways (Wright 1951; Nei 1973; Hartl and Clark 1989). This result confirmed the earlier report of Mangolin et al. (1997), Oleary and Boyle (1998, 1999, 2000), Machado et al. (2000), MartinezPalacios et al. (1999) and Nassar et al. (2001) who studied allozyme diversities in other cacti species. When the individual efficiency of all the enzymes were compared with the pooled data, it was observed that esterase showed the highest efficiency as evident from the high 'r' value ('r' = 0.75) obtained from Mantel 'Z' statistics. This supports the earlier findings by different researchers (Gray et al. 1973; Lee and Fairbrother 1973; Payne and Fairbrother 1976; Scogin 1969). The esterase isozyme pattern was also reported to be superior over other enzymes for testing the purity of cultivars (Choer et al. 1999).

Though the present investigation is a preliminary report of studies on genetic diversity in *Mammillaria* using isozyme and protein markers but from the present result it was evident that they can be quite usefull if combined with

Table 3. Genetic similarity among 25 species of *Mammillaria* detected by isozyme polymorphism of all the five enzymes (acid phosphatase, esterase, glutamate oxaloacetate transaminase, malatedehydrogenase, peroxidase) (the underlined numerals represent the highest and the lowest value).

	M.sheldonii	*M.fraileana*	*M.tonalensis*	*M.knippeliana*	*M.karwinskiana*	*M.longimamma*	*M.baumii*	*M.decipiens*	*M.camptotricha*	*M.bella*	*M.mammillaris*	*M.winterae*	*M.zuccariniana*	*M.compressa*	*M.albicoma*	*M.zeilmanniana*	*M.plumosa*	*M.hahniana*	*M.glassii*	*M.pectinifera*	*M.bocasana*	*M.bombycina*	*M.elongata*	*M.pennispinosa*	*M.matudae*
M.sheldonii	1.00																								
M.fraileana	0.46	1.00																							
M.tonalensis	0.44	0.50	1.00																						
M.knippeliana	0.31	0.22	0.20	1.00																					
M.karwinskiana	0.30	0.27	0.28	0.48	1.00																				
M.longimamma	0.27	0.34	0.29	0.24	0.34	1.00																			
M.baumii	0.35	0.44	0.34	0.27	0.37	0.43	1.00																		
M.decipiens	0.22	0.20	0.18	0.27	0.23	0.22	0.17	1.00																	
M.camptotricha	0.22	0.21	0.13	0.26	0.22	0.20	0.18	0.46	1.00																
M.bella	0.26	0.30	0.27	0.26	0.29	0.32	0.22	0.18	0.25	1.00															
M.mammillaris	0.35	0.31	0.29	0.29	0.27	0.28	0.26	0.33	0.33	0.39	1.00														
M.winterae	0.22	0.26	0.24	0.30	0.27	0.30	0.22	0.35	0.29	0.38	0.44	1.00													
M.zuccariniana	0.30	0.30	0.27	0.27	0.24	0.25	0.24	0.29	0.22	0.40	0.42	0.40	1.00												
M.compressa	0.27	0.32	0.25	0.33	0.30	0.32	0.25	0.32	0.30	0.36	0.43	0.42	0.51	1.00											
M.albicoma	0.31	0.36	0.28	0.28	0.24	0.32	0.34	0.20	0.24	0.30	0.31	0.27	0.35	0.39	1.00										
M.zeilmanniana	0.27	0.26	0.26	0.23	0.22	0.21	0.25	0.15	0.15	0.18	0.23	0.19	0.24	0.26	0.30	1.00									
M.plumosa	0.28	0.29	0.21	0.24	0.23	0.26	0.33	0.25	0.19	0.21	0.25	0.25	0.23	0.26	0.35	0.31	1.00								
M.hahniana	0.27	0.26	0.21	0.29	0.31	0.30	0.32	0.22	0.21	0.28	0.24	0.30	0.30	0.36	0.36	0.26	0.34	1.00							
M.glassii	0.34	0.29	0.26	0.24	0.21	0.32	0.31	0.20	0.20	0.28	0.26	0.24	0.32	0.30	0.36	0.31	0.30	0.29	1.00						
M.pectinifera	0.25	0.32	0.26	0.32	0.24	0.30	0.34	0.21	0.20	0.28	0.29	0.25	0.33	0.36	0.39	0.28	0.31	0.26	0.43	1.00					
M.bocasana	0.21	0.21	0.22	0.30	0.22	0.27	0.21	0.20	0.18	0.24	0.28	0.23	0.22	0.26	0.26	0.27	0.25	0.23	0.34	0.33	1.00				
M.bombycina	0.23	0.25	0.26	0.28	0.27	0.22	0.18	0.16	0.14	0.25	0.23	0.26	0.21	0.31	0.25	0.26	0.23	0.23	0.28	0.26	0.37	1.00			
M.elongata	0.27	0.33	0.32	0.22	0.25	0.27	0.31	0.18	0.14	0.28	0.25	0.28	0.29	0.29	0.35	0.27	0.30	0.30	0.34	0.32	0.31	0.30	1.00		
M.pennispinosa	0.31	0.31	0.31	0.25	0.25	0.26	0.28	0.23	0.17	0.20	0.34	0.29	0.25	0.30	0.35	0.28	0.32	0.28	0.35	0.34	0.34	0.34	0.35	1.00	

morphological markers however, better result can be obtained using more specifc markers like molecular markers.

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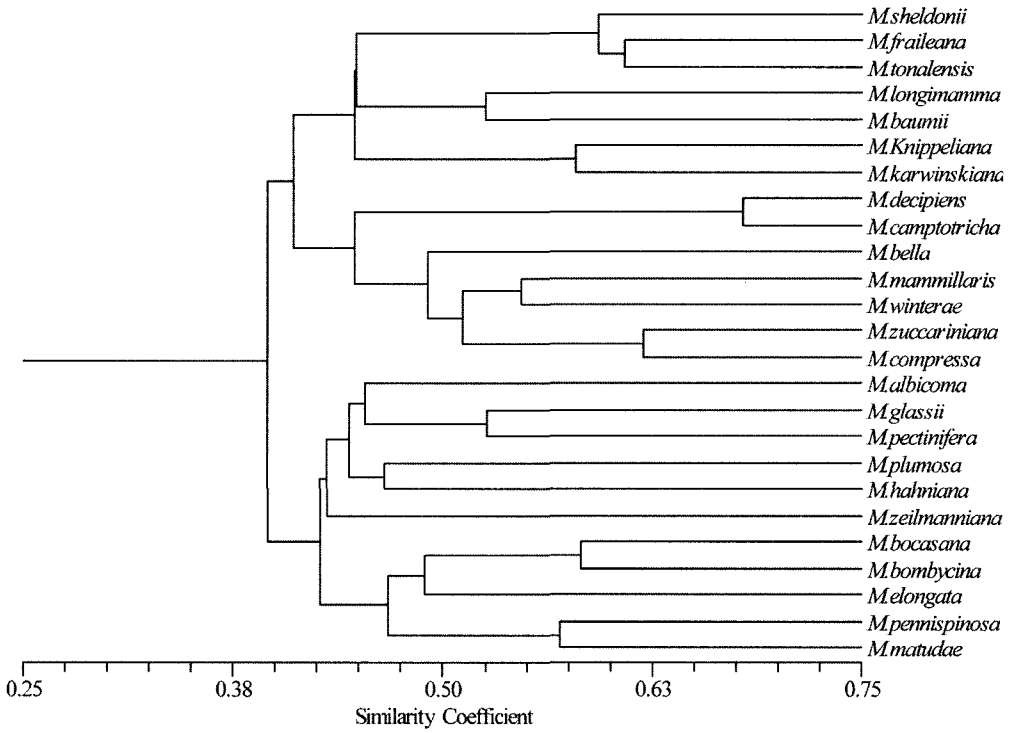

Figure 2. Dendrogram based on protein and isozyme profiles.

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