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Full Length Research Paper

Assessment and characterization of genetic diversity in *Withania somnifera* (L.) Dunal using RAPD and AFLP markers

Bilal Ahmad Mir [1], Sushma Koul[1]*, Arun Kumar[1], M. K. Kaul[1], A. S. Soodan[2] and S. N. Raina[3]

[1]Biodiversity and Applied Botany Department, Indian Institute of Integrative Medicine, Canal Road, Jammu-180001, India.
[2]Department of Botanical and Environmental Sciences, Guru Nanak Dev University, Amritsar, Punjab, India.
[3]Department of Botany, University of Delhi, Delhi, India.

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Genetic diversity of 23 accessions of *Withania somnifera* collected from different geographical regions of India was estimated by employing Random Amplification of Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) markers. Eighteen RAPD primers and six AFLP primer combinations revealed 37.82 and 43.94% polymorphism, respectively, among 163 and 286 genetic loci amplified. The AFLP assay revealed higher levels of polymorphism among the tested *W. somnifera* accessions compared to the RAPD. Mean genetic diversity based on Shannon index ranged from 1.33 (RAPD) to 5.13 (AFLP). Unweighted pair group method based on arithmetic average (UPGMA) analysis was performed on Jaccard's similarity coefficient matrix. The matrix reveals, two main clusters, wild accessions formed one cluster and the cultivated accessions formed the other. The cultivated accessions are well separated from the wild ones at a low similarity value of 0.3, indicating that cultivated and wild accessions are highly distinct. Morphologically cultivated accessions were also quite distinct from the wild ones and the cluster analysis of RAPD and AFLP fingerprints clearly discriminated the five cultivated accessions of *W. somnifera*. A strong correlation was observed between morphology and molecular marker systems. Identification of specific markers to wild as well as cultivated accessions is yet another important finding in the present study. Such genetic diversity is useful in facilitating the development of large number of new varieties through hybridization, transfer of useful genes, thus maximizing the use of such available germplasms as genetic resource materials for breeders. The present input, first of its kind in Ashwagandha, will thus assist the marker assisted crop improvement programme.

Key words: *Withania somnifera*, genetic diversity, RAPD, AFLP, polymorphism, Shannon index.

INTRODUCTION

Withania somnifera (L.) Dunal of the family Solanaceae, known as the Indian ginseng, is a medicinal plant of immense therapeutic value and wide geographic distribution. In India, it grows wild in Madhya Pradesh, Uttar Pradesh Andhra Pradesh, Gujarat, Maharashtra, Rajasthan and Punjab extending to the mountainous regions of Himachal Pradesh and Jammu up to an elevation of 1500m (Singh and Kumar, 1998). According to the classical ayurvedic texts, multiple properties are ascribed to this plants; it is described as a tonic, an anabolic and a nutrient. It has therapeutic use in dysfunctions of the reproductive and nervous system. Also, find use as an aphrodisiac and a sedative, an analgesic and an anti-inflammatory drug useful in arthritis. The plant is known as Medhya Rasayana ("what promotes learning and a good memory"). It is also reported to possess adaptogenic, antipyretic, immunosuppressant, anti-oxidant, immunomodulatory and anti-cancer properties (Uma Devi et al., 1993; Bhattacharya et al., 1987; Scartezzini et al., 2007).

*Corresponding author. E -mail: koul_sushma@yahoo.co.in. Tel: (o) 91-191-2569000-10 Ext. 228 or 09419131891.

Its therapeutic value is ascribed to various bioactive molecules synthesized and accumulated in its leaves and roots. Some of the major constituents include Withanolide A, Withanone and Withaferin A. *W. somnifera* displays a appreciable spectrum of morphological and phytochemical variability (Kumar et al., 2007). Atal and Schwarting (1962) documented five different morphotypes of *W. somnifera* from different populations growing in various regions of India. In view of the great demand in phyto-pharmaceutical industries, commercial cultivation of *W. somnifera* is carried out in the country over an area of about 4000 ha, mainly in Manasa, Madhya Pradesh and in some parts of Rajasthan and Andhra Pradesh. Despite its commercial value, there is a paucity of improved varieties of *W. somnifera* and crop improvement studies need to be taken up vigorously.

For genetic amelioration and effective conservation of the allelic and genotypic variability in a given species, it is imperative to evaluate and catalogue the existing variability. Genetic variability forms the basis for crop improvement and variety development. Precise catalo-guing and characterization of wild genetic resources of this species by molecular DNA markers is therefore urgently required (Negi et al., 2006). In spite of being one of the major medicinal plants in the world, it has received very little attention from geneticists, cytogeneticists and molecular biologists. Systematic morphochemical and molecular characterization of ashwagandha germplasm is required for future programmes of quality improvement.

Different types of marker systems have been used for biodiversity analysis. These include RFLP, SSR, RAPD (Karp et al., 1997) and the AFLP (Vos et al., 1995). These techniques differ in their principles and generate varying amounts of data. The technique of RAPD gained importance due to its simplicity, efficiency, relative ease to perform and non-requirement of sequence information (Karp et al., 1997). However, RAPD technique has been shown to be non-reproducible as it is highly sensitive to experimental conditions (Staub et al., 1996). With the advent of AFLP technique, the problems encountered with both RFLP and RAPD have been overcome. AFLP is currently the method of choice for analysis of germplasm, genetic diversity and phylogeny, gene tagging and the construction of molecular map (Breyne et al., 1997).

In the backdrop of immense therapeutic value of *W. somnifera* and the scarcity of data on genetic diversity, the information on intraspecific relatedness is important for selection of divergent genotypes for breeding and effective conservation and management of its germplasm resources. Furthermore, it would be very interesting to study correlation between the morphological traits and molecular markers. The present study was therefore, undertaken to: (i) assess the genetic diversity present within the W. *somnifera* accessions employing RAPD and AFLP markers in India for its use in collection breeding and germplasm collection and conservation of the Germ-plasm, (ii) identification of diagnostic RAPD and AFLP markers for wild and cultivated accessions and (iii) to understand the degree of congruence between two marker systems employed.

MATERIALS AND METHODS

Plant material and morphological characterization

Twenty three accessions of *W. somnifera* (18 wild and 5 cultivated) used in the present study were collected from different parts of India and maintained in the germplasm repository at Indian Institute of Integrative Medicine (CSIR), Jammu, India. A concise description of the accessions is presented in Table 1. Morphological characters such as plant habit, plant height and shape of leaves, fruiting calyx and berry colour were recorded from fresh material in the field.

DNA extraction

Total genomic DNA was extracted from young and fresh leaves by CTAB method (Doyle and Doyle, 1990).

RAPD analysis

Eighteen randomly selected decamer primers (Operan Technologies, USA) were used for PCR reaction. The reaction mixture of 25 µl volume contained 2 µl 10X assay buffer, 0.2 mM dNTPs (Amersham Pharmacia Biotech, USA), 0.2 mM primers, 0.5 U Taq DNA Polymerase (Bangalore Genie, India), 10-20 ng template DNA and 2.5 mM $MgCl_2$. DNA amplification was performed in Master Cycler Gradient (Ependorf, Germany) programmed to 1 cycle of 5 min at 94°C (denaturation), 1 min at 40°C (annealing) and 2 min at 72°C (extension) followed by 44 cycles of 1 min at 94°C, 1 min at 40°C and 2 min at 72°C ending with 1 cycle of 15 min at 72°C (final extension). The PCR products were resolved on 0.85% agarose gels and photographed in UV light (LKB Pharmacia, USA).

AFLP analysis

About 500 ng of genomic DNA was digested with *Eco*RI and *Mse*I at 37°C for 2h followed by heat treatment at 70°C for 10 min to inactivate the enzymes. The digested DNA was ligated to *Eco*RI and *Mse*I adaptors for 2 h at 20°C. The ligation mixture was then diluted to five fold and selectively pre-amplified (*Eco*RI primer + A, *Mse*I primer + C) during 20 PCR cycles each at 94°C for 30 s, 56°C for 30 s and 72°C for 60 s. Twenty-five fold diluted aliquots of pre-amplified fragments were then selectively amplified with *Eco*RI + 3 and MseI + 3 (primers with 3 selective nucleotides) primers (Invitrogen, Life Technologies, USA). The PCR profile for this amplification reaction was one cycle at 94°C for 30 s and 72°C for 60 s, followed by 12 cycles in which the annealing temperature was progressively lowered by 1°C and finally 20 cycles at 94°C for 30 s, followed by 56°C for 30 s and 72°C for 60 s. the amplified fragments were electrophoresed in 6% denaturing polyacrylamide sequencing gel on a Sequi-Gen (BioRad, USA) sequencing cell. Electrophoresis was carried out for 3 h in 1 × TBE at 75 W at 55°C.

For silver staining gel was fixed in 10% (v/v) acetic acid for 30 min and was rinsed three times in deionised water (2 min per rinse). The gel was then stained for 30 min in a 2 l solution containing 2 g of silver nitrate and 3 ml of 37% formaldehyde (Promega). The stained plate was rinsed with deionised water for 20 s and developed in a prechilled (10°C) developer (2 l) solution containing 60 g of sodium carbonate, 3 ml of 37% formaldehyde and 400 µl aliquot of sodium thiosulphate (10 mg/ml). When bands became

Table 1. *Withania somnifera* accessions used in the present study.

Code	Accession name	Place of Collection
1 [a]	AGB025	Neemuch (MP)
2 [a]	AGB026	Manasa (MP)
3 [a]	AGB036	Pune (MH)
4 [a]	AGB039	Mandsaur (MP)
5 [a]	AGB053	Hyderabad (AP)
6	AGB002	Bikaner (Rajasthan)
7	AGB023	Kullu (HP))
8	AGB040	Jodhpur (Rajasthan)
9	AGB042	Jodhpur (Rajasthan)
10	AGB047	Udaipur (Rajasthan)
11	AGB016	Borivali (Gujarat)
12	AGB018	Lucknow (UP)
13	AGB021	Lucknow (UP)
14	AGB030	Bhopal (MP)
15	AGB048	Udaipur (Rajasthan)
16	AGB006	Jammu (JandK)
17	AGB007	Mudaki (Punjab)
18	AGB015	Dabur Research Foundation, Ghaziabad
19	AGB009	Amritsar (Punjab)
20	AGB049	Delhi
21	AGB056	Bhopal (MP)
22	AGB055	Hyderabad (AP)
23	AGB011	Palampur (HP)

[a]Cultivated accessions.

visible, the gel was immediately transferred to 10% acetic acid solution to stop further reaction. The gel was finally rinsed with distilled water and air dried.

Data analysis

Amplification products were scored for the presence (1) or absence (0) of bands and the binary matrices were assembled for the two markers. The binary matrices constructed for the two DNA markers were subjected to statistical analysis using NTSYS-pc version 2.02 k software (Rohlf, 1992). Jaccard's coefficient was used for calculating pair wise genetic similarities. The dendrograms were constructed by applying unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm. For individual primer/primer combination, number of polymorphic bands, percentage polymorphism and polymorphic information content (PIC) were calculated. Percentage polymorphism (P) was calcula-ted as $P = n_p / n \times 100$; where n_p and n are the number of polymer-phic bands and total bands produced by a primer combination. Polymorphic information content (PIC; the probability of detection of polymorphism by a primer/ primer combination between two randomly drawn genotypes) was calculated as:

$$PIC = 1 - \sum p_i^2$$

where pi is the band frequency of the *i*th allele. Genetic diversity was estimated by Shannon index (Lewontin, 1972).
The Shannon's diversity index was calculated as:

$$H = - \sum pi \ln pi$$

RESULTS

Identification and characterization of germplasm diversity is an essential prerequisite for formulating strategies for plant improvement and conservation of genetic resources. Molecular markers represent a powerful and rapid tool for characterizing diversity within the target species. A wide array of molecular markers is now available. Two of these marker systems, RAPD and AFLP, were employed in the present study for detecting genetic diversity and relationships among 18 wild and 5 cultivated genotypes of *W. somnifera* genotypes.

Morphological characterization

On the basis of the morphological characters viz plant habit, plant height, leaf shape, fruiting calyx and berry colour the five cultivated accessions (AGB025, AGB026, AGB036, AGB039 and AGB053) of *W.* somnifera were found to be distinct from the other group of eighteen wild accessions (AGB002, AGB006, AGB007, AGB009, AGB011, AGB015, AGB016, AGB018, AGB021, AGB023, AGB030, AGB040, AGB042, AGB047, AGB048, AGB049 and AGB055, AGB056) (Table 2). Wild accessions are perennial, tall, bearing acute leaves with entire margin,

Table 2. Morphological differences between wild and cultivated accessions.

Character	Wild accessions	Cultivated accessions
Habit	Perennial	Annual
Plant height (cm)	Tall (120 - 130)	Dwarf (35 - 60)
Leaves	Ovate, sharply acute, entire margin, densely pubescent, veins conspicuous	Ovate, Sub acute, wavy margin minutely stellately pubescent, veins inconspicuous
Fruiting calyx	globular	elongated
Berry	Red	Orange
Seed	Oily to touch	Non-oily

globular fruiting calyx and red coloured berries. On the other hand, the cultivated accessions are annuals, dwarf, with sub-acute leaves with undulate margin, elongated fruiting calyx and orange coloured berries (Table 2).

RAPD analysis

A total of 163 amplification products were scored by 18 RAPD primers across 23 accessions of *W. somnifera* ranging from 6 (OPH02 and OPK04) to 13 products (OPK01). The average frequency of bands per primer was 9.06. The molecular size of bands ranged from 200 bp to 3250 bp. As an example of the representative RAPD profiles of *W. somnifera* accessions with two primers OPA15 and OPA20 are shown in Figure 1. The number of polymorphic bands varied from 1 (OPH02) to 6 (OPK01) with an average frequency of 3.5 per primer. The percentage polymorphism ranged between 16% (OPH02) to 50% (OPB10) with an average percentage polymorphism of 37.82%. The genetic diversity values based on Shannon index ranged between 0.32 (OPH02) to 2.22 (OPA15) with a mean of 1.33 (Table 3). The PIC value, which is a measure to know the usefulness of the primer for diversity analysis, ranged from 0.40 to 0.49 with a mean value of 0.45. Some primers (OPB10, OPK08, OPC20 and OPK16) produced bands that were monomorphic or fixed in wild and cultivated accessions. One marker generated by (OPB10) was present in all the 5 cultivated accessions but absent in all wild samples suggesting that this marker could be a diagnostic marker for cultivated accessions. On the hand three markers generated by (OPK08, OPC20 and OPK16) were specific to wild accessions but absent in cultivated ones suggesting that these markers could be used as diagnostic marker for wild accessions (Table 5).

Pair wise genetic similarities with regard to RAPD marker ranged from 0.70 to 0.93. UPGMA dendrogram were generated based on Jaccard's coefficient. *W. somnifera,* accessions were grouped into two major clusters (cluster I and cluster II) in both the dendrograms irrespective of their geographic distribution (Figure 3a). Cluster I includes only cultivated accessions whereas, cluster II includes only wild accessions although several sub groups were also formed in wild accessions.

AFLP analysis

The six primer combinations resulted in 286 amplification products with an average of 47.6 bands per primer combination. All the primer combinations generated amplification products in the size range of 50 to 400 bp. A typical AFLP profile generated by employing the primer combination EACC-MCAG is shown in Figure 2. The number of amplified products ranged from 32 with primer combination EACT-MCAG to 52 with primer combinations EAGG-MCAGT and EACC-MCAGT. The percentage polymorphism varied from 31.25% (EACT-MCAG) to 59.61% (EAGG-MCAGT) with an average percentage polymorphism of 44.6% per primer combination (Table 4). Primer combination EACC-MCAGT distinguished all the 23 accessions and therefore could be regarded as the best primer combination. Distinct fingerprint profiles were obtained for the wild and cultivated accessions of *W. somnifera* The PIC values ranged from 0.34 (EAAC-MCAGT) to 0.43 (EACT-MCAG) with an average of 0.39. The genetic diversity values ranged between 2.71 (EACT-MCAG) to 8.07 (EACC-MCAGT) with an average of 5.13 (Table 4). This is indicative of large differences among the accessions.

Six-primer combinations resulted in thirty-three bands specific to wild and cultivated genotypes. Of these 30 bands were specific to 18 wild genotypes and three were specific to cultivated ones (Table 5). The number of diagnostic AFLP markers generated ranged from 4 with primer combination EACT-MCAG to 7 with primer combination EAGG-MCAGT (Table 5).

The genetic similarities ranged from 0.88 to 0.96 and 0.78 to 0.96 within cultivated and wild populations respectively. An UPGMA dendrogram was generated based on Jaccard's coefficient (Figure 3b). The five cultivated genotypes are well separated from the wild genotypes with a high bootstrap support. The principal coordinate analysis of the AFLP data (Figure 4), in congruence with dendrogram, revealed distinctness of

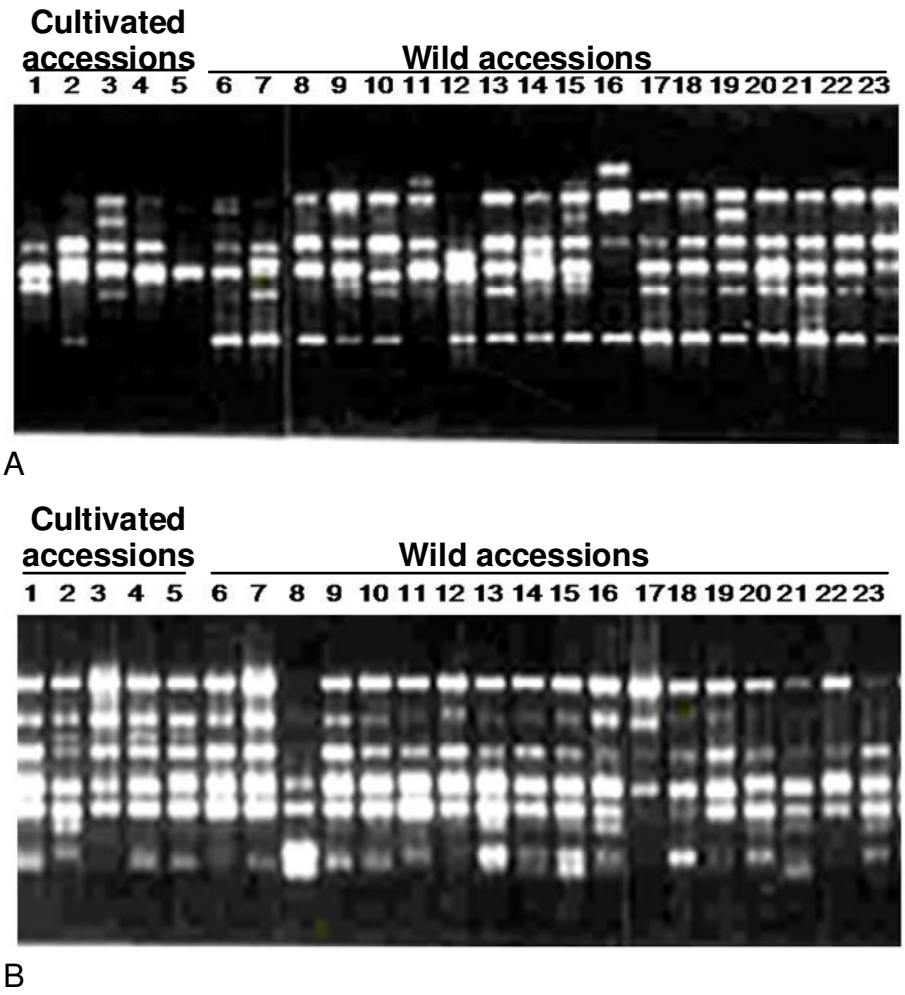

Figure 1. Gel electrophoresis of amplification products obtained with RAPD Primers OPA15 (a) and OPA20 (b) in 23 accessions of *W. somnifera.*

cultivated genotypes from wild ones.

DISCUSSION

Scrutiny of morphological and morphometric data on 23 accessions reveals that cultivated and wild accessions constitute two distinct groups with a very high intra-group and a very low inter-group similarity (Table 2). Thus, it emerges from the present study that the cultivated plants are different from the wild ones not only in their therapeutic properties but also in morphological characters like roots, stems, leaves, flowers, pollen grains, mature fruits and seeds. Even earlier, Kaul et al. (1957) observed that the cultivated type constituted a distinct group and that the level of morphological divergence of cultivated types is large enough to justify their separation into a new species *Withania ashwagandha.* Atal and Schwarting (1962) and Atal et al. (1975) lent support to the proposal by their report that the name *W. somnifera* has been indiscriminately applied to a conglomeration of dissimilar forms and that some of these types deserve to be put in separate varieties or even subspecies. Molecular data collected in the present study seems to reinforce the above proposal on the taxonomic treatment of the material in two important ways.

Firstly, cluster analyses of RAPD and AFLP fingerprints clearly discriminated the five cultivated accessions (AGB025, AGB026, AGB039, AGB053 and AGB055) of *W. somnifera* from the wild ones. Both the marker systems clearly grouped the wild (red berry and high root biomass) and cultivated (yellow berry and low root biomass and high WithaferinA content in leaves) accessions into two separate clusters (Figure 3). Secondly, all the six AFLP primer combinations produced specific banding patterns in cultivated and wild genotypes. A large number of bands (33) distinguished cultivated genotypes from wild genotypes (Table 5). In the UPGMA dendrogram, the five cultivated genotypes branched out from the wild genotypes at a low similarity coefficient of ~

Table 3. Percentage polymorphism (P), Polymorphic information content (PIC) and Shannon index of the RAPD primers.

Primers used	Primer sequence (5' → 3')	Total bands	Polymorphic bands	P (%)	PIC	Shannon index
OPA15	TTCCGAACCC	11	5	45.55	0.47	2.22
OPA20	GTTGCGATCC	10	4	40.00	0.42	1.98
OPA14	TCTGTGCTGG	10	4	40.00	0.47	1.63
OPB10	CTGCTGGCAC	8	4	50.00	0.47	1.69
OPH02	TCGGACGTGA	6	1	16.66	0.49	0.32
OPH12	ACGCGCATGT	7	2	28.57	0.45	1.03
OPH16	GAGCGTCGAA	8	3	37.50	0.48	0.93
OPC03	GGGGGTCTTT	9	4	44.44	0.47	1.24
OPK03	CCAGCTTAGG	11	4	36.36	0.40	1.17
OPK08	GAACACTGGG	11	4	36.36	0.44	1.44
OPK18	CCTAGTCGAG	10	4	40.00	0.40	1.11
OPC13	AAGCCTCGTC	7	3	42.86	0.43	0.84
OPC20	ACTTCGCCAC	10	3	30.00	0.40	1.10
OPK01	CATTCGAGCC	13	6	46.15	0.49	1.95
OPK04	CCGCCCAAAC	6	2	33.33	0.48	0.91
OPK06	CACCTTTCCC	7	2	28.57	0.40	0.72
OPK07	AGCGAGCAAG	9	4	44.44	0.49	1.59
OPK16	GAGCGTCGAA	10	4	40.00	0.44	1.17
Total		163	63			
Average		9.06	3.5	37.82	0.45	1.33

0.3 with high bootstrap support at major nodes. The two major clusters obtained from molecular analysis correlate well with those based on morphological, phytochemical and pharmacognostic attributes (Kaul et al., 1957; Dhalla et al., 1961, present findings). Even earlier AFLP markers have helped to discriminate and establish the tall and the dwarf groups of coconuts as separate varieties (Perera et al., 1998). Similarly, molecular markers especially AFLP has been widely used to discriminate between different accessions of a number of plant species including *Triticum aestivum* L. (Soleimani et al., 2002; Almanza-Pinzon et al., 2003).

The present evidence from molecular data shows that the morphological divergence between the cultivated and the wild types is genetic rather than environmental. Hence, a possible scenario appears to be that some ancestral cultivated population of *W. somnifera* acquired genetic changes that isolated it from the wild species. The pressure of artificial selection under cultivation has resulted in the genetic and morphological divergence of cultivated from the wild types. The predominantly self-pollinated nature of the species seems to have contributed to the fixation of genes and the consequent diversification between wild and cultivated types. The genetic isolation of the two groups is further indicated by experimental evidence as well; artificial hybridization between the two groups have revealed that they are 97% cross incompatibility (Kaul et al., 2005).

Apart from providing molecular evidence to taxonomic separation of the cultivated and wild types, the present study on molecular markers holds relevance on the following counts.

Firstly, it has brought out the pattern and extent of polymorphism in the *W. somnifera* germplasm vis-à-vis geographical distribution. Analyses of the RAPD profiles revealed that the primer namely OPK01 showed maximum (six) polymorphic bands, OPA15 showed 5 while OPA14, OPA20, OPB10, OPC03, OPK03, OPK07, OPK08, OPK16 and OPK18 showed 4 bands each. The average number of polymorphic bands per primer is 3.5 and the percentage of polymorphism ranges from 16 (OPH02) to 50% (OPB10) (Table 3). In case of AFLP, the average number of polymorphic bands per primer combination is 21.1 and the percentage polymorphism ranges from 31.25% (EACT-MCAG) to 59.61% (EAGG-MCAGT) with an average percentage polymorphism of 44.6% per primer combination (Table 4). When data from all primers were pooled, it was seen that 23 accessions (5 cultivated and 18 wild) showed 37.82 and 44.6% polymorphic bands with RAPD and AFLP markers respectively. Apart from percentage of polymorphic bands, Shannon's index was used to estimate polymorphism observed using different markers. The Shannon index values correlate well with the percentage values both in the use of RAPD ($r = 0.742$, $p \leq 0.0001$) and AFLP ($r = 0.876$, $p \leq 0.001$) markers. However, the absolutes values for the Shannon index were significantly higher with the AFLP than the RAPD markers (Tables 3 and 4).

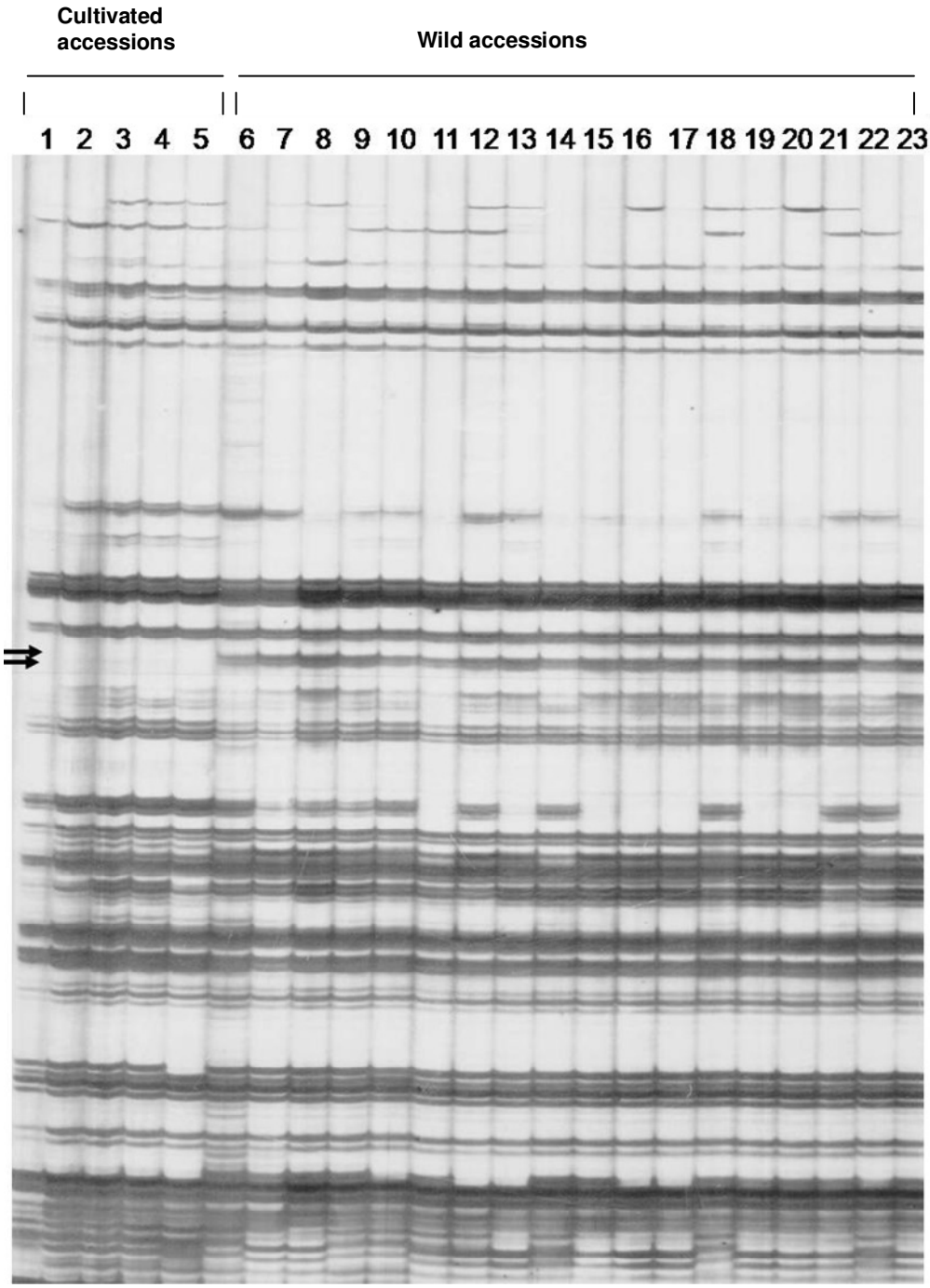

Figure 2. AFLP profile of 23 accessions of *W. somnifera* with primer combination EACC-MCAG. Arrows shows the diagnostic bands in wild accessions.

Shannon index, being sensitive to number of items in the data set, the higher values in case of AFLP seem to be a direct result of large number of bands obtained with the AFLP markers. The average number of bands per primer with AFLP marker was 47.6 compared to only 9.06 in case of RAPD. Similarly, the average number of polymerphic bands was found to be significantly higher with the AFLP (21.1) compared to RAPD (3.5) markers.

The comparatively low percentage of polymorphism found in the present study may have resulted from two possible reasons. First that all the accessions have been cultivated under uniform environmental conditions for past three years. The second reason could be the self-pollinating nature of the species. However, our results show a higher degree of polymorphism than obtained for melon and oat accessions (Garcia-Mas et al., 2000;

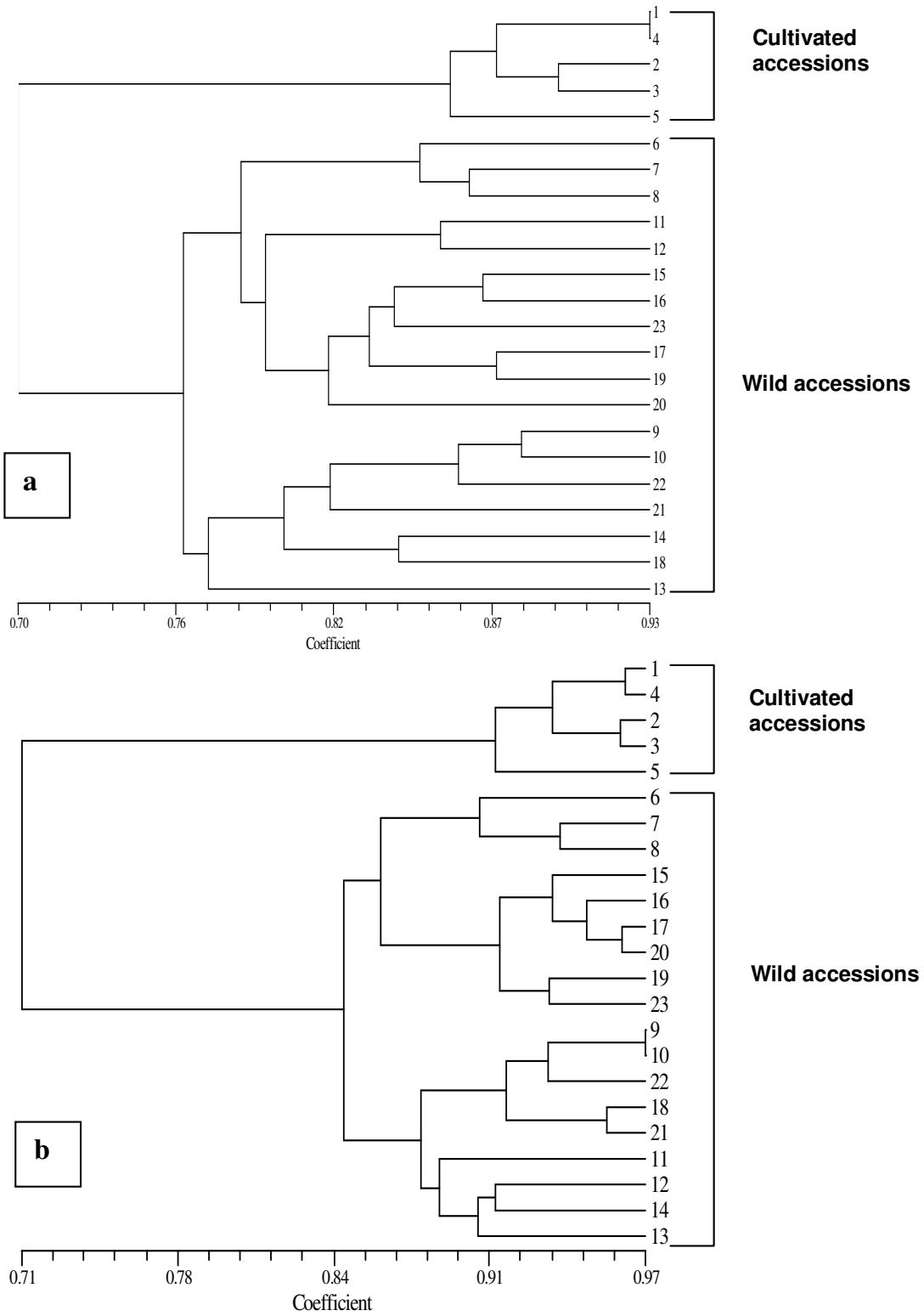

Figure 3. Dendrograms generated using UPGMA of 23 accessions of *W. somnifera* based on RAPD (a) and AFLP (b) data.

Paczos-Grezeda, 2004). However, the quantum of polymorphism is comparatively less than reported by Negi et al. (2006). This could be due to different primer combinations employed in the present study.

Table 4. Percentage polymorphism (P), Polymorphic information content (PIC) and Shannon index of the AFLP primer combinations.

Primer combination	Total bands	Polymorphic bands	P (%)	PIC	Shannon index
EAGG-MCAGT	52	31	59.61	0.36	7.31
EACC-MCAG	57	15	26.32	0.40	3.85
EAGG-MCAG	48	17	35.42	0.37	3.98
EACC-MCAGT	52	30	57.69	0.42	8.07
EAAC-MCAGT	45	24	53.33	0.34	4.86
EACT-MCAG	32	10	31.25	0.43	2.71
Total	286	127			
Average	47.6	21.1	43.94	0.39	5.13

Table 5. Diagnostic RAPD and AFLP markers identified in the present study.

Primer	Number of unique bands	
	Wild accessions	Cultivated accessions
RAPD		
OPB10	0	1
OPC20	1	0
OPK08	1	0
OPK16	1	0
Total	3	1
AFLP		
EAGG-MCAGT	7	0
EACC-MCAG	2	3
EAGG-MCAG	5	0
EACC-MCAGT	6	0
EAAC-MCAGT	6	0
EACT-MCAG	4	0
Total	30	3

A scrutiny of Figure reveals the strength of association among the accessions belonging to the cultivated and the wild clusters. In the cultivated group (cluster 1) accessions AGB025 (Neemuch, Madhya Pradesh) and AGB039 (Mandsaur, Madhya Pradesh) in the sub show more than 99% similarity even though they are collected from places far apart. High similarity values indicate genotypic similarity. Even earlier, genetic similarity of genotypes collected from disjunct geographical regions has been reported (Jain et al., 1994). In the present studies, all the cultivated genotypes collected from different geographical regions of the country showed close resemblance and were grouped into a single sub-population. This association between genotypes from contiguous regions may be the result of similar agro-climatic conditions or due to seed movement and gene flow (Padmesh et al., 1999). In fact, better understanding of the distribution of genetic variation at the intraspecific level is important for the identification of superior genotype(s) for cultivar improvement and to devise strategies for *in situ* and *ex situ* conservation (Bhutta et al., 2006; Basha and Sujatha, 2007). We have shown above that the use of RAPD based fingerprinting is an appropriate method to determine the genetic diversity and relationship between the different accessions. However, our findings indicate that the AFLP technique, with a higher multiplex ratio, is extremely effective in discriminating between closely related types. These results corroborate the earlier studies in which sub-specific level diversity of *Musa acuminata* was established through AFLP markers (Wong et al., 2001).

Our results also touch upon the question of congruence between the profiles obtained from different categories of molecular markers. In the present analysis, the AFLP based dendrograms showed congruence with the RAPD-based dendrogram indicating their parallel behavior and usefulness in the determination of genetic relationships in *W. somnifera*. Such congruence between the various marker systems has also been reported to occur in some other autogamous crop species and inbred lines (Archak

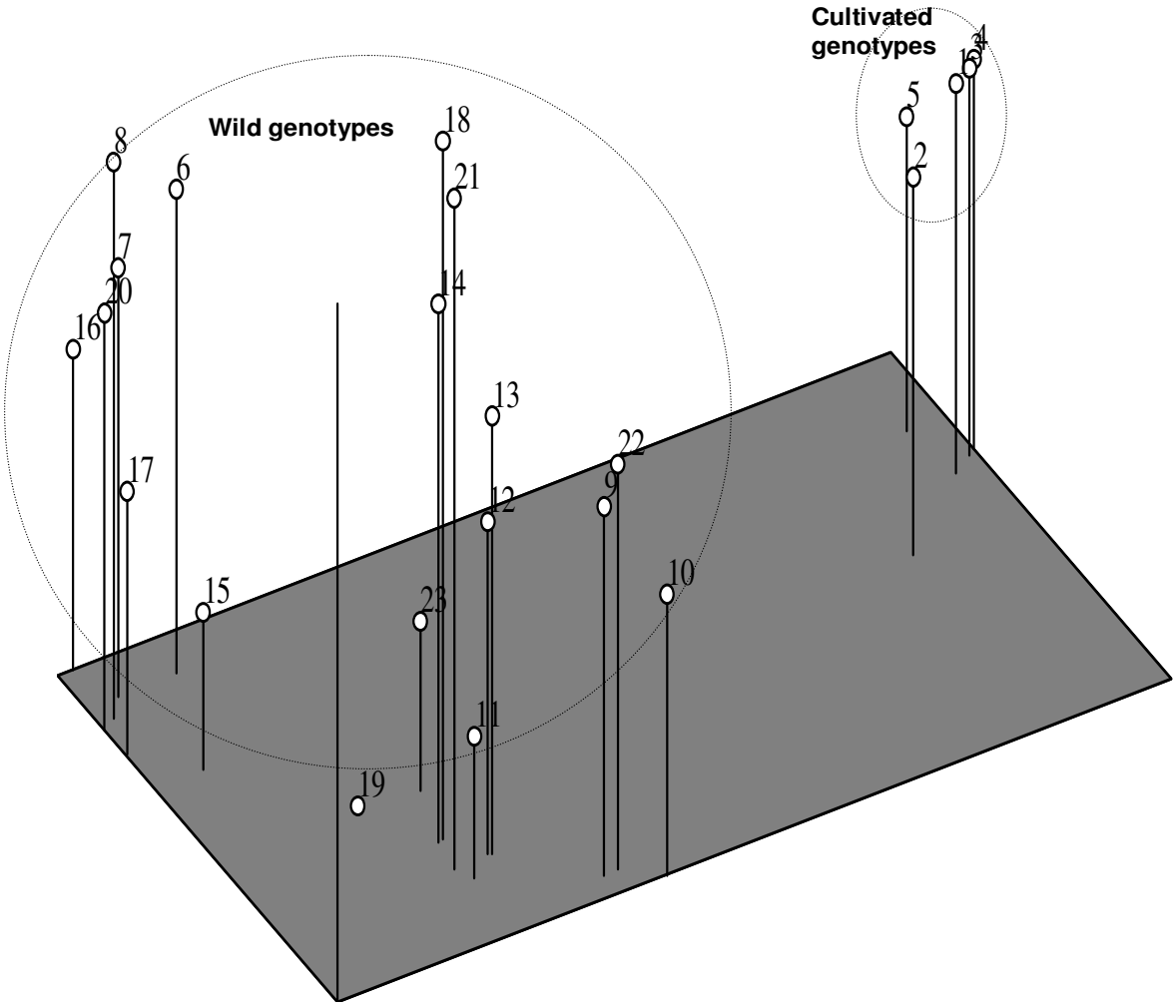

Figure 4. PCO generated using UPGMA (3D view).

et al., 2003). A high degree of correlation between marker systems has also been reported in wheat and safflower (Bohn et al., 1999). However, Powell et al. (1996) observed little correlation between various marker systems in soybean. The incongruity between various marker systems has been reported in some other species as well (Sehgal and Raina, 2005; Degani et al., 2001). According to Powell et al. (1996), the congruity (or incongruity) between various markers is controlled by genome coverage and/or the type of sequence variation recognized by each marker system. Further studies are required before any conclusion can be made with regard to genome coverage of markers *in W. somnifera*.

Lastly, identification of specific markers is another significant finding of the present study. The high number of specific markers identified in wild accession reflects the presence of unique regions in the genome structure of these accessions. In the present study, AFLP has proved to be an efficient tool in the identification of diagnostic or specific markers. Even earlier, the efficacy of AFLP in this regard has been reported *Vitis vinifera, Nicotiana tabacum* and *Carthamus tinctorius* (Vos et al., 1995; Cervera et al., 1998; Campbell et al., 2003; Sehgal and Raina, 2005; Siva Raju et al., 2008). A possible reason for the ability of AFLP to generate higher number of group specific bands could be its wide genome coverage and the wide distribution of the marker loci on the chromosome (Zhu et al., 1998). Such markers hold the promise in devising breeding and conservation strategies apart from their role in the detection of mixtures and duplicates in the germplasm. The primers with high PIC values identified in this study could be of use for rapid evaluation of diversity in the germplasm of *W. somnifera*. Furthermore, the markers if tagged to some gene of interest, for instance, gene for withanolide A/withaferin A production, can serve as an addendum in breeding and selection programmes

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