Plant Biotechnology 27, 297–303 (2010)

Original Paper

Evaluation of genetic relationships in *Plantago* species using Random Amplified Polymorphic DNA (RAPD) markers

Sanghamitra Samantaray[1,*], Urvik M. Dhagat[2], Satyabrata Maiti[1]

[1] Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand-387310, Gujarat, India; [2] P.G. Department of Home Science, V.V. Nagar, Anand-388001

[*]E-mail: smitralok@rediffmail.com Tel: +91-952692-271602 Fax: +91-952692-271601

Received November 13, 2009; accepted April 30, 2010 (Edited by C. Matsukura)

Abstract Studies were undertaken to assess genetic relationships in seven species of *Plantago* and to evaluate the genetic variance within populations of *P. ovata* (Forsk.), *P. indica* (L.), *P. arenaria* (Waldst.), *P. psyllium* (Linn.), *P. lanceolata* (Linn.), *P. serraria* (Linn.) and *P. coronopus* (Linn.) by using random amplified polymorphic DNA (RAPD) markers. A total of 629 distinct DNA fragments ranging from 0.25 to >3.0 kbp were amplified using 75 selected random decamer primers. The cluster analysis indicated that the seven species of *Plantago* formed three major clusters: the first one consisted of three species and the second and third one represented by two species only. A maximum similarity of 85% was observed in *P. arenaria* and *P. psyllium*. *Plantago indica* shared up to 5% similarity with *P. ovata*. The wide genetic distance was observed within populations of different *Plantago* species. Thus, these RAPD markers have the potential for conservation of identified clones and evaluation of genetic relatedness among the species. This is also helpful in breeding programme and provides a major input into conservation biology.

Key words: Medicinal plant, Plantaginaceae, RAPD.

The *Plantago* is one of the genera in family *Plantagenaceae*, a large genus of herbs or sub-herbs distributed mostly in the temperate region and a few in tropics. About ten species are recorded in India of which *P. ovata* is important for its mucilage content of the seed mainly used in medicine and industry (Ebrahimzadeh Mabood et al. 1998; Koocheki et al. 2007). It was also used to maintain remission in ulcerative colitis (Fernandez et al. 1999). *Plantago coronopus*, *P. lanceolata*, *P. ovata*, *P. psyllium* were used by humans against cancer (Duke 1985; Hartwell 1982). Besides, the presence of luteolin-7-*O*-β-glucoside, a major flavonoid present in the leaves of *P. serraria*, *P. psyllium*, *P. coronopus* and *P. lanceolata* were able to strongly inhibit the proliferation of human cancer cell lines (Galvez et al. 2003). *Plantago arenaria* has been used as a safe and effective laxative for thousands of years in Western herbal medicine and also the dried seeds and the seed husks are demulecent, emollient and purgative. It can also be used in the treatment of diarrhea and by helping to soften the stool they reduce the irritation of haemorrhoids. It also helps to remove toxins from the body and can be used to reduce autotoxicity (Chevallier 1996). The seeds of *P. indica* is known in commerce as Spanish or French psyllium (Trease and Evans 1978) of which the high mucilage is used as a bulk laxative (Leung 1980). Genetic variation in *Plantago* species is of prime importance for the successful breeding of improved cultivars/varieties with added value and durable resistant against diseases.

For the purpose of conservation and to carry out successful breeding programmes, proper identification of the plant is of prime importance, for which an accurate, reliable and more authentic system of classification is required. Conventionally, identification and classification of plant groups are solely based on similarity and dissimilarities in morphological feature, more importantly, the floral character which were considered to be consistent. As already established, morphological character were considered to the interaction between the environment and the genotype and also can change by climatic and edaphic factors. Molecular techniques are very much useful not only to identify the genotypes for authentication, but also in assessing and exploiting the genetic variability (Whitkus et al. 1994). Besides, the technology of DNA markers are considered to be the most suitable means for estimating genetic diversity because of their abundant polymorphism and the fact that they are independent of environment (Gepts 1993). Moreover, it would also be useful for the developing a strategy for *ex situ* conservation of plant genetic resources in addition to the

Abbreviations: CTAB, cetyltrimethyl ammonium bromide; PCR, polymerase chain reaction; RAPD-random amplified polymorphic DNA; TE, Tris-EDTA buffer

This article can be found at http://www.jspcmb.jp/

relative genetic diversity within *Plantago* species. DNA fingerprinting of all the genetic resources of the medicinal plants is necessary to generate a molecular database as well as to utilize the information in a systematic manner.

PCR-based molecular markers have been widely used in many plant species for cultivar identification, phylogenetic analysis, population studies and genetic linkage mapping (Williams et al. 1990). Of the different markers, the RAPDs can also be used assessing genetic variability of species of natural populations (Lashermes et al. 1993; Wilkie et al. 1993) and in the identification of genotypes or cultivars (Schnell et al. 1995; Wilde et al. 1992). Genetic diversity in five cultivars of *P. ovata* was estimated using molecular markers earlier (Pal and Raychaudhuri 2004). Similarly, Vahabi et al. (2008) used molecular and morphological markers for the evaluation of genetic diversity between *P. ovata*. Though Wolff and Morgan-Richards (1998) used RAPD markers for discrimination of *Plantago major* sub species, however, no work has been reported in the seven species of Plantago used for this study. Therefore, in this communication, for the first time, we report the genetic relationships in seven species of *Plantago* using RAPD markers.

Materials and methods

Plant material

Seven species of *Plantago (P. indica* L., *P. arenaria* Waldst., *P. psyllium* L., *P. lanceolata* L., *P. serraria* L., *P. coronopus* L. and *P. ovata* Forsk.) were studied. All seven species were collected from the field (Breeding Block) of Directorate of Medicinal and Aromatic Plants Research (Formerly National Research Centre of Medicinal and Aromatic Plants), Boriavi, Anand, Gujarat, India. Leaves from ten different plants of the same species were collected at random and leaf samples of each species were pooled together and then genomic DNA was isolated; all the 10 samples showed similar banding patterns tested randomly with different decamers used for this study.

Genomic DNA isolation

DNA was extracted from fresh leaves derived from seven species of *Plantago* by the cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle 1990). Fresh leaves (~3 g) were ground to a powder in liquid nitrogen using a mortar and pestle. The fine powder was resuspended in 10 ml preheated DNA extraction buffer [2% CTAB, 1.4 M NaCl, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0 and 0.2% (v/v) β-mercaptoethanol (v/v)]. The mixtures were subsequently incubated at 65°C for 1 h. This was followed by addition of an equal volume of chloroform: isoamyl alcohol (24 : 1) and centrifuged (Sorvall Super T21, Kendro, Germany) at 10,000 rpm for 20 min. After centrifugation, DNA was precipitated from the aqueous phase by mixing with an equal volume of isopropanol. The crude DNA pellet was resuspended in 1 ml TE buffer (10 mM Tris, 0.1 M EDTA buffer, pH; 8.0). Subsequently, it was treated with 1 ml @50 μg RNaseA (Quaigen, USA) and incubated for 1 h at 37°C followed by washing with chloroform : iso-amyl-alcohol (24 : 1; v/v). After centrifugation, the upper aqueous phase was separated and then 1/10 volume of 3M sodium acetate (pH; 4.8) was added after which DNA precipitated with addition of 2.5 volume of pre-chilled absolute ethanol. The DNA was washed in 70% ethanol, air dried and resuspended in TE buffer ($T_{10}E_1$). Quality and quantity were checked by running the dissolved DNA in 0.8% agarose gel along side uncut λ; DNA of known concentration. The resuspended DNA was then diluted in TE buffer to concentration 30 ng for use in PCR amplification.

RAPD analysis

A set of 100 random decamer oligonucleotides (A, D, J, N and P) from Operon Technologies (Alameda, USA) was used as primers for the amplification of RAPD fragments. Polymerase chain reaction (PCR) with single primer was carried out in a final volume of 25 μl containing 2.5 μl of 10× assay buffer (100 mM Tris-Cl; pH 8.3, 500 mM KCl, 15 mM $MgCl_2$ and 0.1% gelatin), 100 mM of each dNTPs (MBI Ferment Inc. USA) 15 ng of primer, 1.0 unit of Taq DNA polymerase (Bangalore Genei Pvt. Ltd., Bangalore, India) and 30 ng of template DNA. The amplification reaction was performed in a DNA Thermal Cycler (Eppendorf AG, Germany) programmed for 44 cycles: 1st cycle of 5 min at 94°C followed by 43 cycles each of 1 min at 92°C, 1 min at 37°C, 2 min at 72°C. The final step consisted of one cycle of 7 min at 72°C for complete polymerization. The soak temperature was 4°C. After completion of the PCR, 2.5 μl of 6X loading dye (MBI Ferment Inc. USA) was added to the amplified products and were electrophorsed in a 1.5% (m/v) agarose (MBI Ferment Inc. USA) gels with 1× TAE buffer, stained with ethidium bromide and visualised under UV light. Gel photographs were scanned through Gel Doc System (Syngene, Cambridge,U.K) The sizes of the amplification products were estimated by comparing them to standard DNA ladder (Gene Ruler 100 bp DNA ladder plus and 1 Kb DNA ladder; MBI Ferment Inc. USA). All the reactions were repeated three times.

Data analysis

Data were recorded as presence (1) or absence (0) of band products from the examination of photograph. All the bands (polymorphic and monomorphic) were taken into account for calculation of similarity with a view to avoid over/underestimation of the distance (Gheradi et al. 1998). Jaccard's similarity coefficient (Jaccard 1908) was measured and a dendrogram based on similarity coefficients was generated by using the un-weighted pair group method using arithmetic averages (UPGMA) (Sneath and Sokal 1973) and the SHAN clustering. All the analysis were done by using the computer package NTSYS-PC version 2.02e (Rohlf 1997).

Results and discussion

Authentic identification of taxa is necessary for breeders to ensure protection of intellectual property right and also for propagators and consumers. Seven species of *Plantago* were fingerprinted with one hundred arbitrary

Table 1. RAPD data of seven species of *Plantago* using 76 primers.

Primer	Sequence of oligonucleotide (5′–3′)	Size range (bp)	Number of amplified bands	Number of polymorphic bands	Number of Unique bands
OPA 01	CAGGCCCTTC	2900–380	7	7	0
OPA 02	TGCCGAGCTG	2900–620	7	7	3
OPA 04	AATCGGGCTG	2200–480	4	4	3
OPA 05	AGGGGTCTTG	2900–550	7	7	4
OPA 06	GGTCCCTGAC	3000–400	10	10	5
OPA 07	GAAACGGGTG	2700–600	12	12	4
OPA 08	GTGACGTAGG	>3000–320	12	12	7
OPA 09	GGGTAACGCC	2100–290	14	14	7
OPA 10	GTGATCGCAG	2100–420	11	11	5
OPA 11	CAATCGCCGT	1700–300	11	11	5
OPA 12	TCGGCGATAG	2500–250	7	7	4
OPA 13	CAGCACCCAC	1550–880	4	3	3
OPA 14	TCTGTGCTGG	2100–450	11	11	5
OPA 15	TTCCGAACCC	2000–500	7	7	2
OPA 16	AGCCAGCGAA	>3000–950	6	6	2
OPA 17	GACCGCTTGT	2080–640	11	11	7
OPA 18	AGGTGACCGT	2080–420	9	9	5
OPA 19	CAAACGTCGG	1500–580	6	6	1
OPA 20	GTTGCGATCC	2200–480	13	13	4
OPD 02	GGACCCAACC	2200–1080	5	5	1
OPD 07	TTGGCACGGG	>3000–900	3	3	1
OPD 11	AGCGCCATTG	1480–1000	5	5	3
OPD 20	ACCCGGTCAC	1600–800	4	4	2
OPJ 01	CCCGGCATAA	3000–620	9	9	6
OPJ 05	CTCCATGGGG	>3000–800	8	8	5
OPJ 06	TCGTTCCGCA	1500–790	5	5	2
OPJ 07	CCTCTCGACA	1800–600	7	7	4
OPJ 09	TGAGCCTCAC	2200–420	8	8	4
OPJ 10	AAGCCCGAGG	>3000–620	10	10	6
OPJ 11	ACTCCTGCGA	>3000–700	7	7	4
OPJ 12	GTCCCGTGGT	>500–650	10	10	4
OPJ 13	CCACACTACC	2200–500	8	8	5
OPJ 14	CACCCGGATG	2800–550	8	8	4
OPJ 15	TGTAGCAGGG	2000–410	9	9	5
OPJ 16	CTGCTTAGGG	2900–380	9	9	5
OPJ 17	ACGCCAGTTC	2200–1100	6	6	2
OPJ 18	TGGTCGCAGA	2700–400	10	10	5
OPJ 20	AAGCGGCCTC	3000–600	7	7	6
OPN 01	CTCACGTTGG	2500–620	9	9	4
OPN 02	ACCAGGGGCA	2300–400	9	9	5
OPN 03	GGTACTCCCC	2900–380	9	9	5
OPN 04	GACCGACCCA	3000–520	11	11	5
OPN 05	ACTGAACGCC	3000–600	6	6	3
OPN 06	GAGACGCACA	2000–480	7	7	3
OPN 07	CAGCCCAGAG	2900–490	13	13	9
OPN 08	ACCTCAGCTC	1700–600	9	9	4
OPN 09	TGCCGGCTTG	2800–520	12	12	6
OPN 10	ACAACTGGGG	>3000–500	12	12	4
OPN 11	TCGCCGCAAA	2400–650	7	7	2
OPN 12	CACAGACACC	2600–500	7	7	4
OPN 13	AGCGTCACTC	2100–400	9	9	5
OPN 14	TCGTGCGGGT	3000–480	7	7	4
OPN 15	CAGCGACTGT	2900–700	9	9	6
OPN 16	AAGCGACCTG	2800–500	8	8	4
OPN 17	CATTGGGGAG	1500–900	3	3	3
OPN 18	GGTGAGGTCA	3000–400	10	10	4
OPN 19	GTCCGTACTG	1800–500	4	4	1
OPN 20	GGTGCTCCGT	2800–450	7	7	4
OPP 01	GTAGCACTCC	3000–1550	4	4	3
OPP 02	TCGGCACGCA	1520–980	5	5	2
OPP 03	CTGATACGCC	2900–400	10	10	7
OPP 04	GTGTCTCAGG	>3000–600	9	9	4
OPP 05	CCCCGGTAAC	3000–520	8	8	3

Table 1. (Continued).

Primer	Sequence of oligonucleotide (5′–3′)	Size range (bp)	Number of amplified bands	Number of polymorphic bands	Number of Unique bands
OPP 06	GTGGGCTGAC	3000–400	10	10	5
OPP 07	GTCCATGCCA	2100–580	4	4	3
OPP 08	ACATCGCCCA	3000–900	9	9	5
OPP 09	GTGGTCCGCA	2300–650	8	8	3
OPP 10	TCCCGCCTAC	>3000–550	14	14	6
OPP 11	AACGCGTCGG	2900–520	8	8	5
OPP 12	AAGGGCGAGT	2800–480	14	14	7
OPP 13	GGAGTGCCTC	2300–320	7	7	3
OPP 14	CCAGCCGAAC	>3000–550	13	13	7
OPP 15	GGAAGCCAAC	2100–420	9	9	4
OPP 16	CCAAGCTGCC	2800–520	10	10	4
OPP 17	TGACCCGCCT	2100–680	8	8	4
OPP 19	GGGAAGGACA	2000–620	5	5	2
	Total		630	629	312

10-base primers from A, D, J, N and P series out of which 76 primers produced reproducible and scorable bands. Out of total 630 bands amplified, only one band was found to be monomorphic in OPA 13, thereby giving an estimate of profound (>99%) polymorphism (Table 1). The bands with the same mobility were considered as identical fragments, receiving equal values, regardless of the staining intensity. Contrastingly, the polymorphic bands indicate the fragments observed in more than one species with different electrophoretic mobility. Maximum number of the PCR products was in the size range of >200–3000 bp whereas minimum numbers were in the range of 300–2000 bp. The size fragments of ≥300–3000 bp were observed in 21 different primers. Out of 76 primers used, the primers such as OPA 09, OPP 10 and OPP12 generated maximum of total 14 bands which were polymorphic in nature. However, with regard to total number of bands, minimum of 3 bands were amplified with the primer OPD 07 and OPN 17 (Table 1). Our result showed highest number of unique bands with the primer OPN 07; unique band could be detected as the diagnostic band for a particular species. The maximum of 158 fragments was amplified in case of *P. arenaria* and *P. serraria* and the minimum of 145 in case of *P. coronopus* (Table 2). The highest number of 58 unique bands was detected in *P. lanceolata* and 6, the lowest in *P. psyllium*. Maximum similarity was observed among species with the primer OPA 07. The highest number of species-specific unique fragments were amplified on OPA 08, OPA 09, OPP 09 and OPP 11 (Fig 1A–D). Wide genetic variation between species was evident from the high number of polymorphic markers and unique fragments, even though the survey was limited by the small number of species available for the study. Moreover, on an all-species basis, all of the PCR products primed by all primers except one were polymorphic. Further analysis of these RAPD profiles for band similarity indices could clearly differentiate all the species of *Plantago* taken for study. The similarity matrix obtained using Jaccard's similarity coefficient is presented in Table 3. The results of the genetic similarity matrix indicated that the highest similarity (85%) was observed between *P. arenaria* and *P. psyllium* whereas the lowest similarity (5%) was found between *P. indica* and *P. ovata*. The cluster analysis indicated that seven species of *Plantago* formed three major clusters (Figure 2). The first major cluster divided into two minor clusters out of which one minor cluster had two species (*P. arenaria* and *P. psyllium*) having 85% similarity between them. The maximum similarity obtained between them is in close association with their growth habit and taxonomical characters (Figure 3A, B). Another one consisted of a single species (*P. indica*) showing similarity index of about 6% with *P. arenaria* and *P. psyllium*. The second major cluster contained two species (*P. serraria* and *P. coronopus*) with 61% similarity between them as they exhibit similar chromosome number (2n=2X=10) and almost same nuclear DNA content of 3.6 pg in *P. serraria* and 3.5 pg in *P. coronopus* (Sen and Sharma 1990). Sometimes, *P. indica* and *P. arenaria* have been used as synonyms by some authors (Dalal and Sriram 1995, Raychoudhuri and Pramanik 1997). However, our results using dendrogram clearly separated these two species as it is distantly related having similarity of 9% only. Moreover, *P. lanceolata*

Table 2. Banding pattern in seven species of *Plantago.*

Species	Total bands	Polymorphic bands	Monomorphic bands	Unique bands
P. indica	146	145	01	42
P. arenaria	158	157	01	08
P. psyllium	155	154	01	06
P. lanceolata	156	155	01	58
P. serraria	158	157	01	17
P. coronopus	145	144	01	17
P. ovata	151	151	01	50

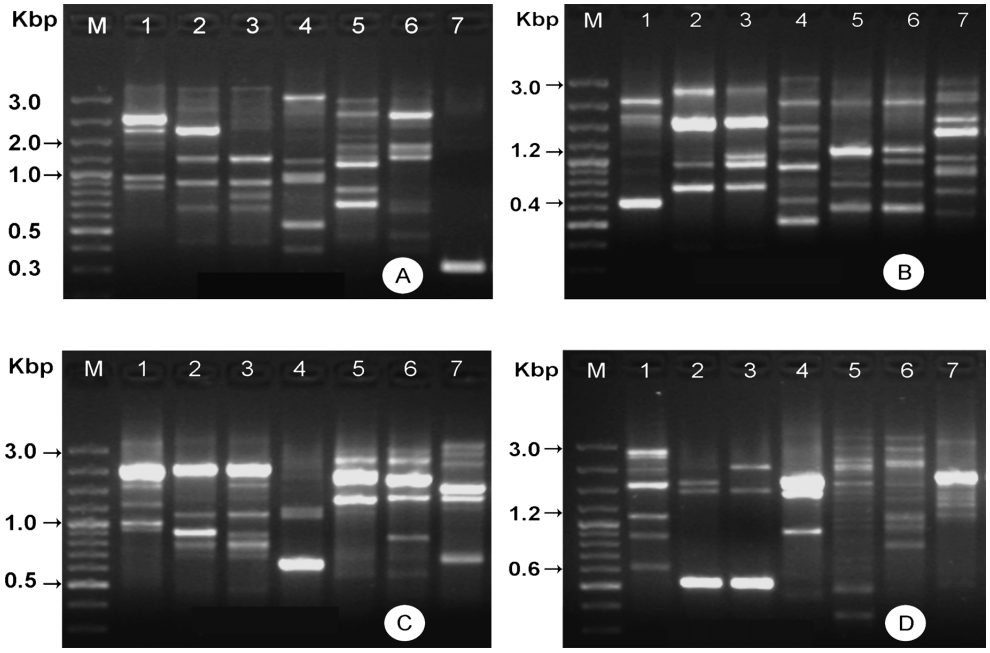

Figure 1. RAPD banding pattern of seven species of *Plantago* as revealed through (A) primer OPA 08, (B) OPA 09, (C) OPP 09 and (D) OPP 11. The lanes represent: M, marker (100 bp ladder plus); 1, *P. indica*; 2, *P. arenaria*; 3, *P. psyllium*; 4, *P. lanceolata*; 5, *P. serraria*; 6, *P. coronopus*; 7, *P. ovata*

Table 3. Similarity matrix (Jaccard's coefficient) of seven *Plantago* species revealed by RAPD markers.

	P. indica	*P. arenaria*	*P. psyllium*	*P. lanceolata*	*P. serraria*	*P. coronopus*	*P. ovata*
P. indica	1.0000						
P. arenaria	0.0893	1.0000					
P. psyllium	0.0780	0.8529	1.0000				
P. lanceolata	0.0667	0.0535	0.0537	1.0000			
P. serraria	0.0554	0.0604	0.0606	0.0606	1.0000		
P. coronopus	0.0616	0.0667	0.0669	0.0521	0.6085	1.0000	
P. ovata	0.0456	0.0692	0.0732	0.0883	0.0655	0.0607	1.0000

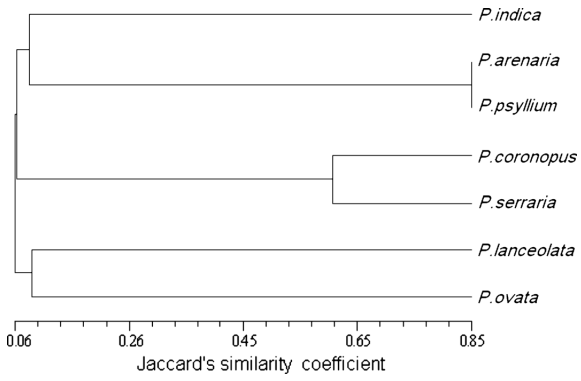

Figure 2. Dendrogram as revealed from RAPD data through SHAN clustering

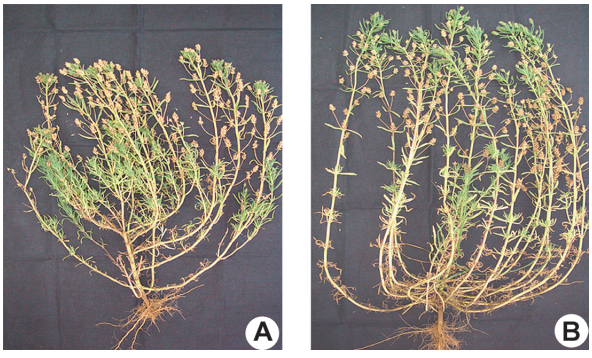

Figure 3. Morphology of plants of (A) *Plantago arenaria* and (B) *Plantago psyllium*

and *P. ovata* formed a separate cluster showing only 8% similarity between them. The dendrogram represents the close distance among five species and large distance with two species according to numerical taxonomy (Sneath and Sokal 1973). The DNA profiling in *Plantago* species clearly showed that it was possible to analyze the RAPD patterns for correlation of their similarity and distance between species by which one could predict the origin of species to a great extent. Since the morphological characters were the result of the expression of the functional genes, however, the RAPD markers took into account not only the functional genes but also the sequences of the DNA which are not expressed.

Recently, RAPDs were successfully used for studying genetic relationships in several medicinal species (Devaiah et al. 2008; Rout 2006; Sarwat et al. 2008; Shi et al. 2008). Two sub-species of *P. major* were also differentiated using RAPD and ISSR markers (Wolff and Morgan-Richards 1998). Simultaneously, Wolff et al. (2000) studied the patterns of molecular genetic variation in *P. major* and *P. intermedia* in relation to ozone resistance.

A molecular relationship of the seven species showed that *P. arenaria* and *P. psyllium* formed a most closely related group followed by the second closely related group of *P. serraria* and *P. coronopus*. However, other three species such as *P. indica*, *P. lanceolata* and *P. ovata* were more distantly related to each other. Similar genetic relationships study was observed in five species of *Plantago* using chloroplast DNA markers (Wollf and Schaall 1992) where they noted that *P. coronopus* was distantly related to *P. lanceolata*. The difference in grouping might be due to the less number of species as well as due to variation in DNA patterns as reflected by RAPDs. Species-specific bands with many primers were observed differentiating all seven species from each other by using 75 decamer random primers. Thus, RAPD markers can be used for differentiating *Plantago* species and is helpful for conservation of germplasm and management of genetic resources (Brown and Kresovich 1996).

In conclusion, RAPD markers provide information on genetic relatedness among the *Plantago* species. An understanding of the level and partitioning of genetic variation within the species would provide an important input in to determining appropriate management strategies.

Acknowledgements

The authors wish to acknowledge the help of the Directorate of Medicinal and Aromatic Plants, Boriavi, Anand for providing necessary facilities. The authors are also thankful to Dr. P. Manivel, Principal Scientist (Plant Breeding), DMAPR for providing the plant materials for study.

References

Brown SM, Kresovich S (1996) Molecular characterization for plant genetic resources conservation. In: Paterson AH (ed) *Genome Mapping in Plants*, Academic Press, London, pp 85–93

Chevallier A (1996) *The Encyclopedia of Medicinal Plants*. Dorling Kindersley, London

Dalal KC, Sri Ram S (1995) Psyllium. In: Chadha KL, Gupta R (eds) *Advances in Horticulture*, Malhotra Publishing House, New Delhi, pp 575–604

Devaiah KM, Venkatasubramanian P (2008) Genetic characterization and authentication of *Embelia ribes* using RAPD-PCR and SCAR marker. *Planta Medica* 74: 194–196

Doyle JJ, Doyle JL (1990) Isolation of plant DNA from fresh tissue. *Focus* 12: 13–15

Duke J A (1985) *Handbook of Medicinal Herbs*. CRC Press, Boca Raton

Ebrahimzadeh MH, Mimasoomi MM, Fakhre Tabatabai SM (1998) Effect of climate and edaphic factors on seed yield of *Plantago ovata*, *Plantago major* and *Plantago psyllium*. *Agric Eco Dev* 6: 125–140

Fernandez BF, Hinojosa JL, Sanchez L, Navarro E, Martinez SJF, et al. (1999) Randomized clinical trial of *Plantago ovata* seeds (dietary fiber) as compared with mesalmine in maintaining remission in ulcerative colitis. Spanish Group for the study of Crohn's disease and ulcerative colitis (GETECCU). *Am J Gastroenterol* 94: 427–433

Galvez M, Martin-Cordero C, Lopez-Lazaro M, Cortes F, Ayuso MJ (2003) Cytotoxic effect of *Plantago* spp. on cancer cell lines. *J Ethnopharma* 88: 125–130

Gepts P (1993) The use of molecular and biochemical markers in crop-evolution studies. In: Hecht MK (ed) *Evolutionary biology*. Plenum Press, New York, pp 51–94

Gherardi M, Mangin B, Goffinet B, Bonnet D, Huguet T (1998) A method to measure genetic distance between allogamous population of alfalfa (*Medicago sativa*) using RAPD molecular markers. *Theor Appl Genet* 96: 406–556

Hartwell JL (1982) *Plants used against cancer: A Survey*. Quarterman Publications, Lawrence, Maryland

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

Koocheki A, Tabrizi L, Nassiri Mahallati M (2007) The effects of irrigation intervals and manure on quantitative and qualitative characteristics of *Plantago ovata* and *Plantago psyllium*. *Asian J Plant Sci* 6: 1229–1234

Lashermes PH, Cros J, Marmey PH, Charrier A (1993) Use of random amplified polymorphic DNA markers to analyze genetic variability and relationships of *Coffea* species. *Crop Evol Genet Res* 40: 91–99

Leung AY (1980) *Encyclopedia of common natural ingredients used in food, drugs and cosmetics*. John Willey and Sons, New York

Pal MD, Raychoudhuri SS (2004) Estimation in genetic variability in *Plantago ovata* cultivars. *Biol Plant* 47: 459–462

Raychaudhuri SS, Pramanik S (1997) Comparative studies on DNA content and superoxide dismutase isozymes of *Plantago ovata*, *P. psyllium, P. indica, P. lanceolata*. *J Med Arom Plant Sci* 19: 964–967

Rohlf FJ (1997) *NTSYS-PC- Numerical taxonomy and Multivariate Analysis System*. Version 2.0, Exeter Software, Setauket, New York

Rout GR (2006) Evaluation of genetic relationship in *Typhonium* species through random amplified polymorphic DNA markers. *Biol Plant* 50: 127–130

Sarwat M, Das S, Srivastava PS (2008) Analysis of genetic diversity through AFLP, SAMPL, ISSR and RAPD markers in *Tribulus terrestris*, a medicinal herb. *Plant Cell Rep* 27: 519–528

Schnell RJ, Ronning CM, Knight RJ (1995) Identification of cultivars and validation of genetic relationship in *Mangifera indica* L. using RAPD markers. *Theor Appl Genet* 90: 269–274

Sen S, Sharma AK (1990) Chromosome complements, nuclear DNA and genetic distance as measures of interrelationship in *Plantago*. *Nucleus* 33: 4–10

Shi W, Yang CF, Chen JM, Guo YH (2008) Genetic variation among wild and cultivated populations of the Chinese medicinal plant, *Coptis chinensis* (Rananculaceae). *Plant Biol* 10: 485–491

Sneath PHA, Sokal R (1973) *Numerical Taxonomy*. WH Freeman and Co., San Francisco, CA

Trease GE, Evans WC (1978) *Pharmacognosy.* Macmillan Publishers Ltd., London

Vahabi AA, Lotif A, Solouki M, Bahrami S (2008) Molecular and morphological markers for the evaluation of diversity between *Plantago ovata* in Iran. *Biotechnology* 7: 702–709

Whitkus R, Doebley J, Wendel JF (1994) Nuclear DNA markers in systematics and evolution. In: Phillips L, Vasil IK (eds) *DNA based markers in Plants*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 116–141

Wilkie SS, Isaac PG, Slater RJ (1993) Random amplified polymorphic DNA (RAPD) markers for genetic analysis in *Allium*. *Theor Appl Genet* 86: 497–504

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphism amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Res* 18: 6531–6535

Wilde J, Waugh R, Powell W (1992) Genetic fingerprinting of Theobroma clones using randomly amplified polymorphic DNA markers. *Theor Appl Genet* 83: 871–877

Wolff K, Morgan-Richards M (1998) PCR markers distinguish *Plantago major* subspecies. *Theor Appl Genet* 96: 282–296

Wolff K, Schaal B (1992) Chloroplast DNA variation within and among five *Plantago* species. *J Evol Biol* 5: 325–344

Wolff K, Morgan-Richards M, Davison A W (2000) Patterns of molecular genetic variation in *Plantago major* and *P. intermedia* in relation to ozone resistance. *New Phytol* 145: 501–509