



Phenotypic and RAPD diversity among 80 germplasm accessions of the medicinal plant isabgol (*Plantago ovata*, Plantaginaceae)

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ABSTRACT. *Plantago ovata*, popularly known as isabgol, has great commercial and medicinal importance due to thin rosy white membranous seed husk. Isabgol seeds and husks have emollient, demulcent and laxative properties. We used both biometric and molecular techniques to assess the genetic variability and relatedness of 80 germplasm accessions of *Plantago* spp (*P. ovata*, *P. lanceolata*, and *P. major*) collected both from India and abroad. The range of D² values (2.01-4890.73) indicated a very high degree of divergence among the accessions. Based on the degree of divergence, 80 accessions/genotypes were grouped into seven clusters. Thirty-six accessions were analyzed through RAPD profiling for similarity and genetic distances, using 20 random primers. Intraspecific differences in all three species were smaller [range for *P. ovata* (2-17%), *P. lanceolata* (3-15%), *P. major* (2-11%)] than interspecific

diversity. These highly divergent lines could be used to produce superior hybrids.

Key words: *Plantago*; Genetic diversity; Degree of divergence; RAPD markers

INTRODUCTION

The genus *Plantago* (family - Plantaginaceae) comprises 200 species, ten of which occur in India (Anonymous, 1969). *Plantago ovata* is one of these plants, valued for its seeds and husks, which have been used in indigenous medicine for many centuries all over the world. Seeds are believed to have cooling, demulcent effects and to be useful in inflammatory and biliary disorders of the digestive organs; they are also applied as a poultice for rheumatic and gouty swelling, and thought to be beneficial in the treatment of dysentery and irritation of the intestinal tract. The husk from the seed has the property of absorbing and retaining water, and hence, it works as an anti-diarrheal drug. The plant, of west Asian origin, was introduced into India during the Muslim settlement in the middle Ages.

India currently ranks first in the production and trade of *Plantago ovata* in the world market. Each year, India produces about 45,000 metric tons of isabgol seeds and 15,000 metric tons of seed husk from nearly 50,000 ha of cultivated land in India, where most production occurs in the States of Gujarat, Madhya Pradesh, Uttar Pradesh, Bihar, Haryana, and part of Rajasthan (especially in the Malwa tract and the northern belt). Some 90% of the seeds and husks are exported, making isabgol a major foreign exchange earner for India. Although *Plantago psyllium* (cultivated earlier in France), *P. major*, *P. lanceolata*, *P. pumilla*, *P. coronopus*, *P. argentia*, and *P. lagopus* are also grown in small quantities for mucilage, these species are only of small importance in the pharmaceutical industries.

In this study, genetic variability and the random amplified polymorphic DNA (RAPD) technique were used for the assessment of diversity for various traits in available germplasm collections assembled from different places in India and from abroad, as a prelude to crop improvement.

MATERIAL AND METHODS

Plant materials

The materials for the present investigation comprised eighty indigenous and exotic accessions/collections of diverse origin. Sixty-nine was from India, including sixty-one from Uttar Pradesh, five from Gujarat and one each from Uttaranchal, Himanchal Pradesh and Maharashtra. Eleven were exotic, including three from Malaysia, two from the USA and one each from Pakistan, France, Hungary, Poland, Baluchistan and Bulgaria, of *Plantago* belonging to three species, namely *Plantago ovata*, *P. lanceolata*, and *P. major* (Table 1).

Thirty-six accessions were selected on both a geographical and morphological basis (Figure 1) to study the genetic divergence using RAPD markers (Table 1).

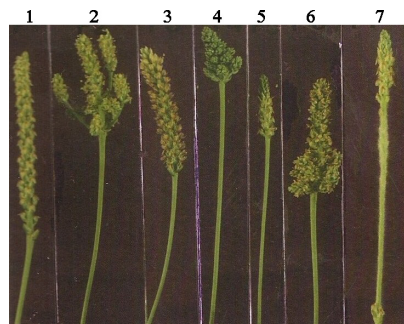


Figure 1. Morphological variations in panicles among the *Plantago ovata* accessions. 1 = Normal; 2 = Branched; 3 = Feathery; 4 = Club shaped; 5 = Normal (check); 6 = Club; 7 = Cottony long.

Table 1. Geographical distribution and type of panicle of eighty accessions of *Plantago*.

S. No.	Accession No.	Type of panicle	Origin/Places of collection	S. No.	Accession No.	Type of panicle	Origin/Place of collection
1	PO 1*	Normal	Bulgaria	41	PM 41*	Normal	Uttar Pradesh (Lucknow), India
2	PO 2*	Normal	USA	42	PL 42	Normal	Uttar Pradesh (Lucknow), India
3	PO 3*	Normal	USA	43	PM 43	Normal	Uttar Pradesh (Lucknow), India
4	PO 4*	Normal	Pakistan	44	PL 44	Normal	Uttar Pradesh (Lucknow), India
5	PO 5*	Normal	Maharashtra, India	45	PL 45*	Normal	Uttar Pradesh (Lucknow), India
6	PO 6*	Normal	Baluchistan	46	PL 46	Normal	Uttar Pradesh (Lucknow), India
7	PO 7*	Normal	Uttaranchal (Pantnagar), India	47	PL 47	Normal	Uttar Pradesh (Lucknow), India
8	PO 8*	Normal	Himanchal Pradesh, India	48	PM 48*	Normal	Uttar Pradesh (Lucknow), India
9	PO 9	Normal	Gujarat, India	49	PL 49	Normal	Uttar Pradesh (Lucknow), India
10	PO 10	Normal	Uttar Pradesh (Lucknow), India	50	PL 50	Normal	Uttar Pradesh (Lucknow), India
11	PO 11	Normal	Gujarat (Sidhpur), India	51	PL 51	Normal	Malaysia
12	PO 12	Normal	Uttar Pradesh (Lucknow), India	52	PL 52*	Normal	Malaysia
13	PO 13*	Normal	Uttar Pradesh (Lucknow), India	53	PL 53*	Normal	Malaysia
14	PO 14	Normal	Uttar Pradesh (Lucknow), India	54	PL 54	Normal	Uttar Pradesh (Lucknow), India
15	PO 15*	Normal	Uttar Pradesh (Lucknow), India	55	PO 55	Club	Uttar Pradesh (Lucknow), India
16	PO 16*	Normal	Uttar Pradesh (Lucknow), India	56	PO 56*	Club	Uttar Pradesh (Lucknow), India
17	PO 17	Normal	Uttar Pradesh (Lucknow), India	57	PO 57	Club	Uttar Pradesh (Lucknow), India
18	PO 18	Normal	Uttar Pradesh (Lucknow), India	58	PO 58	Club	Uttar Pradesh (Lucknow), India
19	PO 19*	Normal	Gujarat (Mehsana), India	59	PO 59	Club	Uttar Pradesh (Lucknow), India
20	PO 20	Normal	Uttar Pradesh (Lucknow), India	60	PO 60*	Club	Uttar Pradesh (Lucknow), India
21	PO 21	Normal	Uttar Pradesh (Lucknow), India	61	PO 61	Club	Uttar Pradesh (Lucknow), India
22	PO 22	Normal	Uttar Pradesh (Lucknow), India	62	PO 62	Feathery	Uttar Pradesh (Lucknow), India
23	PO 23	Normal	Uttar Pradesh (Lucknow), India	63	PO 63	Feathery	Uttar Pradesh (Lucknow), India
24	PO 24	Normal	Uttar Pradesh (Lucknow), India	64	PO 64*	Club	Uttar Pradesh (Lucknow), India
25	PO 25*	Normal	Uttar Pradesh (Lucknow), India	65	PO 65	Club	Uttar Pradesh (Lucknow), India
26	PO 26*	Normal	Gujarat, India	66	PO 66*	Feathery	Uttar Pradesh (Lucknow), India
27	PO 27	Normal	Uttar Pradesh (Lucknow), India	67	PO 67*	Feathery	Uttar Pradesh (Lucknow), India
28	PO 28	Normal	Uttar Pradesh (Lucknow), India	68	PO 68	Club	Uttar Pradesh (Lucknow), India
29	PO 29*	Cottony long	Uttar Pradesh (Lucknow), India	69	PO 69*	Club	Uttar Pradesh (Lucknow), India
30	PL 30*	Normal	Poland	70	PO 70	Club	Uttar Pradesh (Lucknow), India
31	PM 31*	Normal	France	71	PO 71	Club	Uttar Pradesh (Lucknow), India
32	PO 32*	Normal	Uttar Pradesh (Lucknow), India	72	PO 72*	Branched	Uttar Pradesh (Lucknow), India
33	PO 33*	Normal	Uttar Pradesh (Lucknow), India	73	PO 73*	Branched	Uttar Pradesh (Lucknow), India
34	PO 34	Normal	Uttar Pradesh (Lucknow), India	74	PO 74*	Normal	Uttar Pradesh (Lucknow), India
35	PO 35	Normal	Gujarat, India	75	PO 75	Feathery	Uttar Pradesh (Lucknow), India
36	PO 36	Normal	Uttar Pradesh (Lucknow), India	76	PO 76	Club	Uttar Pradesh (Lucknow), India
37	PO 37	Normal	Uttar Pradesh (Lucknow), India	77	PO 77	Branched	Uttar Pradesh (Lucknow), India
38	PO 38	Normal	Uttar Pradesh (Lucknow), India	78	PO78(check)*	Normal	Uttar Pradesh (Lucknow), India
39	PL 39*	Normal	Hungary	79	PO 79	Normal	Uttar Pradesh (Lucknow), India
40	PL 40*	Normal	Uttar Pradesh (Lucknow), India	80	PO 80	Normal	Uttar Pradesh (Lucknow), India

PO = *Plantago ovata*; PM = *Plantago major*; PL = *Plantago lanceolata*. Note: Accessions indicated with asterisks were selected to study genetic divergence using RAPD markers.

Methods

The performance of eighty accessions/genotypes was recorded in a randomized block design replicated twice in two environments. Plot size for each treatment was one row 3 m in length with 10 x 30 cm plant to plant and row to row spacing. The crop was fertilized with 60 kg N, 30 kg P₂O₅ and 30 kg K₂O per hectare and irrigated four times during the crop season. The plants were harvested in the month of April. Data were recorded on five randomly selected competitive plants in each line for the ten morphometric traits, plant height (cm), branches per plant, panicles/plant, peduncle length (cm), panicle length (cm), days to maturity, seed yield (g/plant), husk yield (g/plant) and swelling factor.

Swelling factor = volume of soaked-water (mL) uptake / amount of seeds (g).

Mean data were subjected to statistical analysis for genetic divergence (D²) and canonical analysis according to Mahalanobis (1936) and Rao (1952) among the different accessions/collections using a computer program package developed at the Data Processing Division of the CIMAP, Lucknow, based on the research of Singh and Choudhury (1979).

DNA extraction and polymerase chain reaction amplification

DNA was isolated from leaf tissue of samples according to the protocol described by Khanuja et al. (1999) and amplified by polymerase chain reactions. The reactions were carried out in a 25- μ L volume. The reaction tube contained 25 ng DNA, 0.6 units Taq DNA polymerase, 0.25 μ L of each dNTPs (10 mM), 1.5 mM MgCl₂ and 5 pmoL decanucleotide primers. The amplifications were carried out using the DNA Engine Thermal Cycler (MJ Research, USA). The amplified products were loaded on a 1.2% agarose gel containing 0.5 μ g/mL ethidium bromide and photographed using a Polaroid system. Custom-made decanucleotide primers (MAP01 to MAP20) were used for the analysis. The sequences of the primers MAP01 to MAP20 were AAATCGGAGC, GTCCTACTCG, GTCCTTAGCG, TGCGCGATCG, AACGTACGCG, GCACGCCGGA, CACCCTGCGC, CTATCGCCGC, CGGGATCCGC, GCGAATTCCG, CCCTGCAGGC, CCAAGCTTGC, GTGCAATGAG, AGGATACGTG, AAGATAGCGG, GGATCTGAAC, TTGTCTCAGG, CATCCCGAAC, GGA CTCCACG, AGCCTGACGC, respectively.

Data analysis

Well-marked amplified fragments/bands were counted for the analysis. The similarity matrix obtained after multivariate analysis using Nei and Li's coefficient (1979) and the observed similarity coefficients were used to generate a tree for cluster analysis, using the UPGMA method (unweighted pair group method with arithmetic average).

RESULTS

The range of D² values (2.01-4890.73) indicated a very high degree of divergence among the accessions. Based on the degree of divergence, 80 accessions/genotypes were grouped into 7 clusters (Figure 2), such that the genotypes within the clusters had smaller D² values between themselves than those between clusters. Thus, intra-cluster distance for each

of the clusters was observed to be less than the inter-cluster distances in all the cases. Cluster I, i.e., the biggest cluster, included 57 genotypes followed by cluster II (13), cluster III (5) and cluster IV (2). Each of the remaining 3 clusters comprised solitary individuals (Table 2).

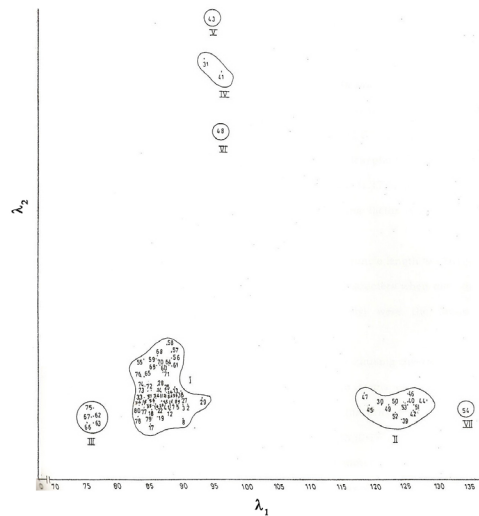


Figure 2. Spatial distribution of 80 accessions of *Plantago*.

Table 2. Accessions included in the different clusters of *Plantago*.

Clusters	Number	Accessions included in clusters
I	57	PO 1, PO 2, PO 3, PO 4, PO 5, PO 6, PO 7, PO 8, PO 9, PO 10, PO 11, PO 12, PO 13, PO 14, PO 15, PO16, PO 17, PO 18, PO 19, PO 20, PO 21, PO 22, PO 23, PO 24, PO 25, PO 26, PO 27, PO 28, PO 29, PO 32, PO 33, PO 34, PO 35, PO 36, PO 37, PO 38, PO 55, PO 56, PO 57, PO 58, PO 59, PO 60, PO 61, PO 64, PO 65, PO 68, PO 69, PO 70, PO 71, PO 72, PO 73, PO 74, PO 76, PO 77, PO 78, PO 79, PO 80
II	13	PL 30, PL 39, PL 40, PL 42, PL 44, PL 45, PL 46, PL 47, PL 49, PL 50, PL 51, PL 52, PL 53
III	5	PO 62, PO 63, PO 66, PO 67, PO 75
IV	2	PM 31, PM 41
V	1	PM 43
VI	1	PM 48
VII	1	PL 54

The highest intra-cluster divergence (78.43 for cluster I) was invariably smaller than the lowest inter-cluster divergence between cluster IV and cluster V (122.66), thus authenticating the clustering patterns formed in this study. Intra-cluster divergence ranged from 12.93 (cluster III) to 78.43 (cluster I), whereas inter-cluster divergence ranged from 122.66 to 5372.48 between clusters IV and V and between clusters V and VII, respectively. The average inter-cluster divergence (D^2) was highest for cluster VII (3176.86) followed by cluster V (3152.12). It was lowest for cluster VI (1969.85). Other clusters were moderately divergent from other clusters, on average, 2039.55 to 2753.15 (Table 3).

Table 3. Intra- and inter-cluster divergence (D^2) among seven clusters of *Plantago*.

Clusters	I	II	III	IV	V	VI	VII	Av (D^2)
I	78.43	1431.63	245.32	2714.95	3655.89	1887.49	2302.00	2039.55
II		56.81	2350.25	3634.48	4636.04	2673.46	171.83	2482.95
III			12.93	3433.39	4375.14	2739.28	3375.52	2753.15
IV				39.42	122.66	314.25	4385.30	2434.17
V					0.0	750.61	5372.48	3152.12
VI						0.0	3454.02	1969.85
VII							0.0	3176.86

Av (D^2) = average D^2 values.

Canonical analysis of genetic differentiation

The divergence among eighty accessions of *Plantago* (*P. ovata*, *P. lanceolata*, and *P. major*) was measured and confirmed in terms of spatial distribution also by canonical analysis. Since the proportionate contribution by the first two canonical roots λ_1 (57.95%) and λ_2 (34.36%) was more than 90%, the two-dimensional representation (λ_1 - λ_2 chart) was found adequate. The distribution of each individual against λ_1 and λ_2 coordinating axes is presented in Figure 2.

A very high divergence among the three species of *Plantago* was clearly shown by the spatial distribution. Accessions PO 62, PO 63, PO 66, PO 67, and PO 75 of *P. ovata* formed a separate cluster III and were also morphologically very different from the accessions of cluster I of *P. ovata*; however, accessions PO 55, PO 56, PO 57, PO 58, PO 59, PO 60, PO 61, PO 64, PO 65, PO 68, PO 69, PO 70, PO 71, and PO 76 were closer than the other members of cluster I and formed a separate group within the cluster, thus differentiated from the other accessions. Accession PO 29 was also farthest from all the other members of cluster I and occupied a separate space. Accessions of *P. lanceolata* were divided into two clusters, i.e., cluster II and cluster VII. Accessions PL 47 and PL 45 were farther from the other members of cluster II, whereas cluster VII had only one accession PL 54. Three clusters were formed by four accessions of *P. major* in which PM 43 and PM 48 were highly divergent and formed solitary clusters, whereas accessions PM 31 and PM 41 were included in a single cluster.

Role of traits at different levels of differentiation

At the inter-cluster level, panicles/plant (115.25%), peduncle length (99.76%), and panicle length (77.16%) were the highest contributing characters when days to flower and maturity (8.42 and 8.64%, respectively) were the lowest contributing characters (Table 4). The importance of different traits causing divergence at the primary and secondary axes of differentiation in terms of coefficients associated with Z_1 and Z_2 vectors were days to maturity (0.522), plant height (0.49), peduncle length (0.290), and panicle length (0.177) at the primary axis (Z_1 vector) and panicles/plant (0.683), days to maturity, (0.279), plant height (0.231), and days to flowering (0.196) at the secondary axis (Z_2 vector). Above traits were the important contributors to the total divergence, respectively (Table 4).

Table 4. Mean (X̄), ranges, Z₁ and Z₂ vectors and coefficient of variations (CV) of clusters for ten economic traits of *Plantago*.

Clusters	Statistical parameters	Days to flower (50%)	Plant height (cm)	Branches/plant	Panicles/plant	Peduncle length (cm)	Panicle length (cm)	Days to maturity	Seed yield (g)	Husk yield (g)	Swelling factor (mL/g)
I	X̄ Range	85.77 79.00-90.00	35.39 30.93-41.81	6.53 4.49-9.25	73.81 43.42-121.24	22.40 19.06-24.96	4.23 2.51-8.47	147.53 141.75-154.75	20.26 8.92-48.00	5.06 2.23-12.00	13.25 11.10-15.15
II	X̄ Range	92.98 91.25-94.75	65.45 57.04-71.45	2.33 1.50-2.99	15.84 9.66-22.67	54.29 44.84-62.74	7.41 5.92-8.54	167.96 159.25-170.75	13.63 8.23-17.86	3.41 2.05-4.46	7.17 5.80-10.48
III	X̄ Range	77.15 76.00-78.50	29.36 27.56-30.54	6.26 5.49-7.16	74.51 65.42-81.00	19.16 18.30-19.95	3.56 2.97-4.02	135.00 133.25-136.50	16.56 15.89-17.61	4.14 3.97-4.40	13.04 12.15-13.73
IV	X̄ Range	98.63 98.25-99.00	61.30 59.75-62.84	5.54 5.11-5.96	372.90 365.04-380.75	2.91 2.74-3.07	0.93 0.92-0.93	172.13 171.75-172.50	14.86 12.96-16.75	3.72 3.24-4.19	9.20 7.35-11.05
V	X̄ Range	99.50 100.00	64.06 57.86	5.88 5.83	434.58 260.21	2.83 3.30	0.88 1.09	173.75 173.25	24.00 18.31	6.00 4.58	9.13 11.20
VI	X̄ Range	92.75 8.42	82.18 29.63	3.12 30.18	22.09 115.25	69.29 99.76	8.60 77.16	168.75 8.64	13.16 20.99	3.29 20.88	7.00 23.95
VII	X̄ CV (%) Z ₁ Z ₂	0.190 0.196	0.490 0.231	-0.271 -0.033	-0.100 0.683	0.290 -0.462	0.177 -0.325	0.522 0.279	0.040 0.162	0.160 0.050	-0.470 0.120

Study the genetic divergence through RAPD markers

In order to study the diversity at DNA level, twenty arbitrary primers were used for RAPD analysis. Fourteen primers responded by producing detectable bands (Figure 3). A total of 102 bands were scored against 36 genotypes across the three species of *Plantago*. Of 102 bands, 89 (87.25%) were found to be polymorphic, while 5 (4.9%) were monomorphic and 8 (7.8%) were unique. When the scorable bands were examined in different species of *Plantago*, the *P. ovate* species showed a total of 57 bands among 27 genotypes/accessions. Of these 57 bands, 32 (56.14%) were polymorphic, 23 (40.35%) were monomorphic and 2 (3.8%) were unique. In *P. lanceolata*, there were a total of 50 bands distributed within 6 accessions, of which 9 (18%) were polymorphic, 35 (70%) were monomorphic and 6 (12%) were unique. Among the 3 accessions of *P. major*, a total of 46 bands could be detected, of which 4 (8.7%) were polymorphic, 33 (71.74%) were monomorphic and 5 (10.86%) were unique.

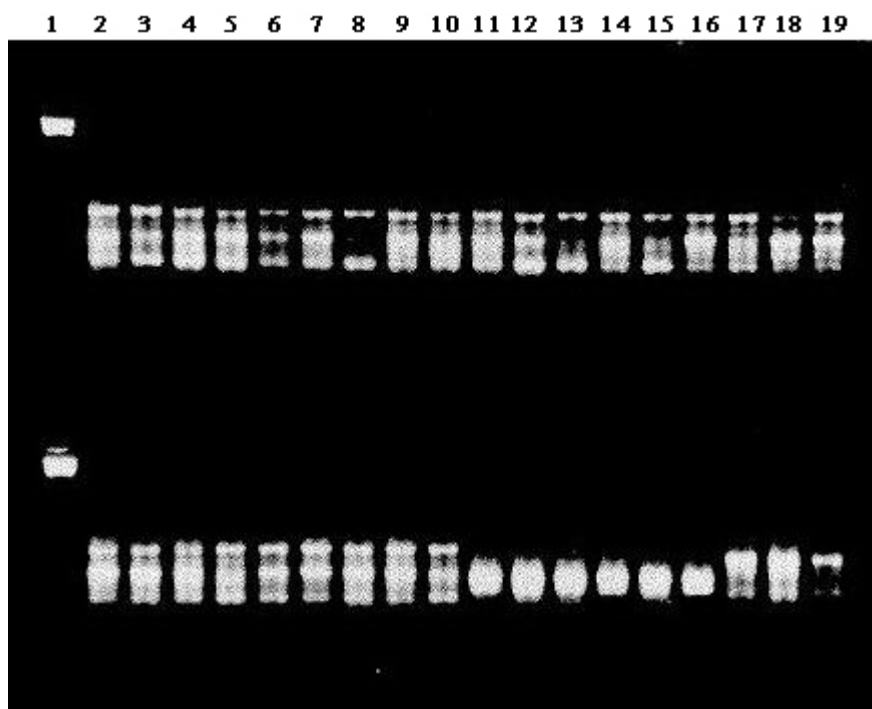


Figure 3. RAPD amplification of 36 accessions of *Plantago* with primer MAP06. **1st Row:** Lane 1 = Molecular marker λ DNA digested with *Hind*III; Lane 2 to Lane 9 = PO 1 to PO 8; Lane 10 = PO 13; Lane 11 = PO 15; Lane 12 = PO 16; Lane 13 = PO 19; Lane 14 = PO 25; Lane 15 = PO 26; Lane 16 = PO 29; Lane 17 = PO 32; Lane 18 = PO 33; Lane 19 = PO 56. **2nd Row:** Lane 1 = Molecular marker; Lane 2 = PO 60; Lane 3 = PO 64; Lane 4 = PO 66; Lane 5 = PO 67; Lane 6 = PO 69; Lane 7 = PO 72; Lane 8 = PO 73; Lane 9 = PO 74; Lane 10 = PO 78; Lane 11 = PL 30; Lane 12 = PL 39; Lane 13 = PL 40; Lane 14 = PL 45; Lane 15 = PL 52; Lane 16 = PL 53; Lane 17 = PM 31; Lane 18 = PM 41; Lane 19 = PM 48. PO = *Plantago ovata*; PL = *Plantago lanceolata*; PM = *Plantago major*.

Cluster analysis based on RAPD

To analyze the genetic distances within different accessions/genotypes of a species and among all the three species of *Plantago*, the similarity coefficients (Nei and Li, 1979) were used to generate a tree for cluster analysis using the UPGMA method. The resulting dendrogram (Figure 4) showed a differentiation into three major clusters, representing the three species of *Plantago*, namely *P. ovata*, *P. lanceolata*, and *P. major*. The three major clusters were observed in the phenogram. The first major cluster was represented by *P. ovata*. The diversity among the accessions of *P. ovata* was in the range of 2 to 17%. Again, within the cluster of *P. ovata*, accessions PO 60 out-grouped completely and were the most diverse accessions among all of them. The next sub-cluster was constituted by the accessions PO 2, PO 6 and PO 19, which were acquired from the USA, Baluchistan and India (Gujarat), respectively. The other large sub-cluster included all other accessions with varying degree of similarity among them. The accessions PO 1 and PO 8 were observed sharing the highest level of similarity among themselves, but they belong to Bulgaria and India (Himanchal Pradesh), respectively. They clustered with PO 25 and PO 5, which were from India (U.P. and Maharashtra, respectively). In the next subculture, PO 4 belongs to Pakistan yet it clustered with PO 29, PO 26, and PO 67, which are Indian (U.P.) accessions. Accessions PO 4 and PO 29 were the most similar in this sub-cluster, whereas PO 26 and PO 67 were separated from these two and also between themselves. Another sub-cluster of accessions PO 72 and PO 73, which were from U.P. (Lucknow), clustered together, while PO 7 and PO 15, belonging to different states, namely Uttaranchal (Pantnagar) and U.P. (Lucknow), respectively, also clustered together. The other sub-cluster included PO 3, PO 4, PO 78, and PO 16, in which PO 3 was obtained from the USA and other accessions from India (U.P.). The accession PO 13 was separated from all other accessions belonging to U.P. (Lucknow). The next large sub-cluster included PO 32, PO 33, PO 64, PO 56, and PO 69, in which PO 32 out-grouped from the rest. PO 33 and PO 66, PO 56 and PO 69 shared the highest level of similarity, whereas all the accessions in this sub-cluster belonged to U.P. (Lucknow).

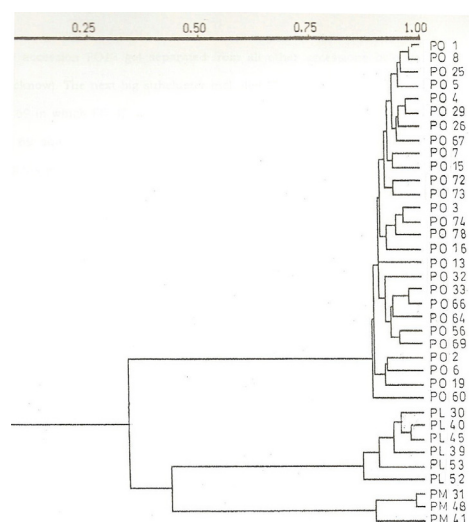


Figure 4. Similarity among *Plantago* accessions.

Diversity was higher within the accessions of *P. lanceolata* compared to the accessions of *P. ovata*. The major subgroup with maximum similarity was observed between PL 40 and PL 45, which represents India. The French accession PL 30 out-grouped from the cluster, followed by PL 39 (Hungary), PL 52 and PL 53 (Malaysia).

Of the three accessions of *P. major*, PM 31 and PM 48 were the most similar, sharing maximum similarity in the genome, yet they were collected from Poland and India, whereas PM 41 (India, U.P.) formed a distinct subgroup, which is a diverse accession.

Species-specific markers

Species-specific markers among the three species of *Plantago* used in this experiment were found to differentiate *P. ovata* from *P. lanceolata* and *P. major*, and in another way: *P. lanceolata* from *P. ovata* and *P. major* from *P. lanceolata* and *P. ovata*, with the primers MAP02, MAP04, MAP05, MAP06, MAP09, MAP10, MAP12, and MAP19. The unique fragment sizes generated in *Plantago* for species-specific markers are presented in Table 5.

Table 5. Unique fragment sizes generated in *Plantago* for species-specific markers.

Primers	No. of bands			Molecular weight (bp)		
	<i>P. ovata</i>	<i>P. lanceolata</i>	<i>P. major</i>	<i>P. ovata</i>	<i>P. lanceolata</i>	<i>P. major</i>
MAP02	2	0	2	1000, 79.43	-	630.95, 316.23
MAP04	2	0	0	1122.01, 407.38	-	-
MAP05	1	1	2	251.18	316.23	794.33, 398.11
MAP06	2	2	2	794.33, 398.11	562.34	
					316.23	630.96, 501.19
MAP09	1	1	0	478.63	549.54	-
MAP10	1	1	1	316.23	301.99	239.88
MAP12	2	1	1	251.19, 158.48	1000	223.87
MAP19	2	2	1	1995.26, 398.11	1000, 316.23	562.34

DISCUSSION

The average D^2 ranged from 1969.85 to 3176.86, which indicates remarkable genetic divergence among the genetic accessions of isabgol. High morphological variation was also noted among the isabgol accessions for plant types and shape and size of panicles. As such, a considerable degree of divergence was found for all the traits in terms of D^2 values, ranging from 2.01-4890.73. The cluster to cluster composition revealed that cluster I contained the accessions of isabgol from Uttar Pradesh, Maharashtra, Uttranchal, Himanchal Pradesh, Gujarat among the states of India, as well as from different countries, namely Bulgaria, USA, Pakistan, and Baluchistan. Similarly, cluster II included accessions from Poland, Hungary, Malaysia and India (U.P.), cluster III from Uttar Pradesh, and cluster IV from France and India (U.P.) and clusters V, VI and VII all from U.P. (India). The three genotypes, namely PM 43 of cluster V, PM 48 of cluster VI and PL 54 of cluster VII, were unique with each forming distinct clusters. Notably, all *P. ovata* accessions occurred in only two clusters, I and III (though cluster I can still be bifurcated), as did *P. lanceolata* accessions in V and VII. However, *P. major* with only 4 accessions formed 3 clusters IV-VI. Thus, *P. major* seems to have very high variability fol-

lowed by *P. lanceolata* and *P. ovata*. The mean inter-cluster D^2 values indicated the highest divergence between cluster V and VII ($D^2 = 5372.48$).

At the inter-cluster level, panicles/plant was the highest contributor and ranked first towards the total divergence (115.25%). Days to 50% flowering was the least contributor towards the total divergence (8.42%, ranked 10th) (Table 4). The clusters V, VI and VII each with solitary and diverse accessions were distinct for a number of economic characters. For instance, the accession in cluster V was characterized by the highest mean values for panicles/plant (434.58) and days to maturity (173.75 days) followed by seed yield (24.00 g) and husk yield (6.00 g) (Table 4). Analysis of cluster composition revealed that the largest grouping comprised accessions collected from U.P., Maharashtra, Uttaranchal, Himanchal Pradesh and Gujarat among the states of India and Bulgaria, USA, Pakistan and Baluchistan. The smallest clusters were IV, V and VII, having one accession each belonging to France and India.

Accessions from U.P. (India) appeared to be highly divergent where they were represented in all clusters. The distribution pattern of accessions of diverse origin in a single cluster indicates that genetic diversity observed within isabgol genetic stocks was not related to geographical origin. Noted differences in plant characters probably occurred over time due to the migration of alleles among genetic stocks, cross-pollination/or spontaneous mutations and/or free movement of plant materials from location to location. Thus, isabgol genotypes to be used for crop improvement/hybridization should be selected on the basis of quantified degree of divergence as opposed to geographical origin. The genotypes of cluster III and cluster I in *P. ovata*, of cluster II and cluster VII in *P. lanceolata* and of cluster IV, cluster V and cluster VI in *P. major* were highly divergent and may be used in hybridization programmes.

Genetic relationship among the accessions of *Plantago* analyzed with RAPD markers

The RAPD technique is being used for the analysis of diversity and defining the 'core collection' through identification of duplicates within the large germplasm collections (Virk et al., 1995). This technique is widely used for the identification of cultivars, phylogenetic relationship and, rational designing of breeding programs (Powell et al., 1996). A high level of polymorphism was observed on the specific level, but within species a low level of polymorphism was obtained. The number of unique bands was higher in *P. lanceolata* and *P. major*, as compared to *P. ovata*. No single primer was found that distinguishes all the accessions of a species, whereas MAP01, MAP10 and MAP16 gave maximum divergence within the species. The dendrogram shows a clear-cut variation among the species, as three major clusters were formed. It was observed that *P. major* and *P. lanceolata* were more similar as compared to *P. ovata*, which was out-grouped in the phylogenetic tree. From the RAPD study, the accessions could be differentiated, but intra-specific differences in all three species were much less (range for *P. ovata*, 2-17%; for *P. lanceolata*, 3-15%, and for *P. major*, 2-11%) in comparison to inter-specific diversity. The grouping of accessions did not correlate with their geographical distribution as well as morphological variation. Within *P. ovata*, the accession number PO 29 (cottony long), PO 67 and PO 66 (feathery) were morphologically very distinct, but it could not be clearly detected by RAPD. PO 4 with simple inflorescence clustered with PO 29 (cottony long), PO 26 (simple) and PO 67 (feathery), similarly PO 33 (simple) and PO 66 (feathery) were clustered together; however, PO 72 and PO 73 having a branched panicle grouped together (Figure 1). Of the four plants having a club-shaped panicle, three shared the same

group having one feathery and one simple type of inflorescence. Besides that, the species-specific monomorphic bands of different sizes were identified for *P. ovata*, *P. lanceolata* and *P. major*, which were used as markers for differentiating these three species from each other. Similar results have also been reported in *Capsicum* by Thull et al. (2008). Thus, the molecular analyses carried out using 36 accessions of *Plantago* were very efficient in detecting diversity among populations and between different species. Genotypes, both geographically and morphologically distinct, were not found to be appreciably distant genetically, but the genetic similarities obtained from the analysis can be used for the selection of parents to generate mapping populations and selecting parents for breeding purposes.

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