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Research Article

Genetic divergence analysis in *Plantago* germplasm

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Abstract

Plantago is an important medicinal plant valued for its seeds and husk which are used in indigenous medicine, all over the world. An investigation was carried out to assess the genetic divergence among 49 accessions of *Plantago,* comprising four species. Highly significant differences were noted for all nine economic traits indicating the existence of considerable genetic diversity. The germplasm could be grouped into six clusters and immense diversity among the accessions was indicated by the wide range of D^2 values (2.487 - 7178.147). The cluster pattern was also confirmed by the canonical analysis. Panicles/plant was the highest contributor (25.21%) followed by panicle length (14.84%), whereas swelling factor was the lowest contributor (2.68%) to divergence. Five accessions were identified to be best either in panicle length, seed yield, husk yield or swelling factor. Three accessions were identified for dual preference and one for multiple preferences of economic traits and can be exploited for commercial cultivation.

Key words
Plantago, accessions, canonical analysis, genetic divergence, swelling factor.

Introduction

Plantago comprises approximately 200 species of which 10 occur in India (Anonymous 1969). Among these *Plantago ovata* F. (Isabgol) is valued for its seeds and husk which is used in indigenous and traditional medicine system all over the world (Lal *et al*., 2009). Isabgol husk, the seed epidermis, having muco-polysachride layers is used in stomach disorder such as diarrhea, ulcers, gonorrhea, piles, cough, chronic dysentery and also used for treating constipation and intestinal disorder in ayurvedic medicines (Dalal and Sriram 1995). India ranks first in the production and trade of isabgol making it substantial foreign exchange earner and continues hold on a monopoly in the world market, but is not able to meet the global demand because of low production and productivity. The nearly stagnant yield of isabgol is due to narrow genetic base and use of traditional breeding techniques with little or no understanding of genetic architecture of the plants (Lal and Singh 2009). In the scenario of increasing demand and lack of improved varieties, there is immediate need to develop high-yielding varieties of this crop having better husk quality and to look at suitable locations for growing this crop in other states of the country.

The present study was carried out to identify the accessions of *Plantago* for high seed and husk yield and genetic variability for diverse traits among 49 accessions assembled from different parts of India and abroad. The genetic divergence among them was quantified by multivariate analysis to assess the proximity of accessions with each other, assort them in different clusters/groups and find out highly divergent accessions for further breeding programme.

Materials and methods

Plant materials: The present experiment was carried out on 49 indigenous and exotic accessions of *Plantago,* representing four species: *P. ovata* Forsk., *P. lanceolata* L., *P. arinaria* Waldst., *P. psyllium*.L. (Table1).

Field preparation and allied practices: The accessions were evaluated by growing at Research farm of CSIR- Central Institute of Medicinal and Aromatic Plants, Lucknow, (India) located at 26.5°N latitude and 80.50° E longitude, 120m above mean sea level. The climate was semi-arid to sub-tropical in nature. The minimum and maximum night and day temperatures ranged from 9-12 °C to 16-19 °C, respectively during growth period and from 26-31 °C to 36-40°C during harvesting time respectively. Average rainfall during the growing season was 4-6 mm according to weather data of the Metrological Laboratory of CSIR-CIMAP, Lucknow in two consecutive years, 2014-2015 and 2015-2016 in randomized complete block design with three replications and 0.90 m² plot size.

Statistical analysis: The data were recorded on five randomly selected plants from each accession with respect to nine important characters namely: plant height (cm) = X1, branches/plant = X2, panicles/plant = X3, panicle length (cm) = X4, peduncle length (cm) = X5, inflorescence length (cm) = X6, seed yield (g/plot) = X7, husk yield (g/plot) = X8, and swelling factor (ml/g) = X9. The mean data were collected and subjected to statistical analysis using statistical software ver. 0.3 for D^2 and canonical analysis of genetic divergence as per procedure outlined by

Mahalanobis (1936) and Rao (1952), based on Singh and Chaudhary (1979) and Panse and Sukhatme (1967).

Results and Discussion

Highly significant differences (P<0.01%) were noted for all the nine characters, which signify the existence of genetic variability among the accessions studied. The enormous variation in shape and size of inflorescence was observed in the accessions (Figs. 1). The individual D² values for accessions n(n-1)/2= 1176, where n = 49 (number of accessions) pairs of genotypes were calculated and on the basis of these values all the 49 accessions could be grouped into six clusters such that the genotype within the clusters had lesser D² values among themselves than those belonging to different clusters. Tremendous diversity among the accessions was indicated by a range of D² value, D^2 = 2.487 (for *P. ovata,* GSD 18 and *P. ovata,* GSD 27) to 7178.147 (*P. ovata*, GSD 36 and P. *psyllium*, GSD 49) (Table 2). This indicates that the accession GSD 18 and GSD 27 are close to each other whereas accessions GSD 36 and GSD 49 were divergent (Figs 2 and 3). Though on the basis of average inter cluster D² the accession PP 49 was highly divergent and GSD 34 was less divergent among the collections (Fig 2 and Table 2). The swelling factor, panicle length, husk yield/plot, peduncle length and seed yield/plot on the primary axis (z_1 -vector) and seed yield/plot, plant height, panicle length, peduncle length and inflorescence length on the secondary axis (z_2 -vector) were imperative contributors in order to the total genetic divergence (Table 3).

Among six clusters, the IV, V and -VI were solitary clusters (Fig. 2, table 3). The PP 49, in cluster VI, was characterized by the highest number of panicles/plant (624.22). The collection PA 48, under V, was the tallest showing more plant height (77.77cm), has maximum number of branches/plant, had highest seed yield/plot (58.03gm) and husk yield/plot (19.15 gm). Among *Plantago ovata* accessions, GSD 34 present in cluster IV, was the tallest in plant height (43.00cm), had maximum number of panicles/plant (33.16), had longest panicle length (3.91cm), peduncle length (30.83cm), and inflorescence length (34.74cm). The accessions of other clusters had meager to modest character expression, suggesting more affinity among themselves as compared to cluster IV-VI.

The panicles/plant had the maximum character contribution (25.21%) followed by panicle length (14.84%), branches/plant (13.86%), seed yield (12.33%), husk yield (12.32%), peduncle length (7.17%), inflorescence length (6.88%), plant height (4.68%) and swelling factor was the lowest (2.68%) contributor towards genetic diversity among the character studied (Table 3). Five *P. ovata* accessions, (GSD 36, GSD 38, GSD 42, GSD 45, and GSD 47) were identified as the best for seed yield as well as husk yield, five accessions (GSD 3, GSD 4, GSD 5, GSD 36 and GSD 42) were found to be best for swelling factor, five accessions (GSD 2, GSD 12, GSD 36, GSD 44 and GSD 45) were identified as best for having longer panicle length. Some accessions were identified for dual preferences like GSD 36 and GSD 45 for better seed yield and longer panicle length. The accessions GSD 36 and GSD 42 were found to be better for husk yield and swelling factor. GSD 36 was identified for the multiple preferences viz. for longer panicle length, better seed yield, husk yield and swelling factor (Table 4).

This intra- and inter-cluster divergence indicated that considerable genetic divergence exits among the accessions. Although 22 accessions (44.89%) could be grouped within cluster I followed by 20 (40.81%) within cluster II, four (8.16%) within cluster III and remaining three were the solitary clusters, IV (2.04%), V (2.04%) and VI (2.04 %), were distantly placed form the rest of the clusters being greatly divergent (Table 2 and 5). The clusters, IV, V and –VI were peculiar, having solitary, very diverse accessions namely GSD 34 (IV), PA 48 (V) and PP 49 (VI). The highest genetic distances were among VI and I followed by II, III, V and IV (Fig 2, 3 and Table 5).

It has been revealed that the largest cluster grouping was occupied by the accessions collected from Bulgaria, USA, Pakistan, Maharashtra, Uttaranchal, Himachal Pradesh, Gujarat and Uttar Pradesh (Tables 1 and 5). It is also apparent that there is no relationship between genetic divergence and geographical origin, as genotypes from different localities were grouped into more than one cluster. The accessions from Uttar Pradesh were present in cluster I, II and IV and accessions from Gujarat entered in to I and II. Accession from Bulgaria (GSD 1), USA (GSD 2 and GSD 3), Pakistan (GSD 4 and GSD 6), Maharashtra (GSD 5), Uttaranchal (GSD 7) and Himachal Pradesh (GSD 8) were grouped in the same cluster I where most of the accessions were from Gujarat - GSD 9, GSD 19, GSD 26 and Uttar Pradesh - GSD 17, GSD 18, GSD 20, GSD 21, GSD 22, GSD 23, GSD 24, GSD 27, GSD 29, GSD 30, GSD 31 (Table 6). Similarly the accession of *Plantago lanceolata* from Hungary (PL 35) was grouped with the accessions from Malaysia (PL 10, PL 28 and PL 32) in the cluster IV. This lack of relationship and level of divergence probably occurred due to free movement of accessions from one place to another, spontaneous mutation, genetic drift etc. Our findings are in consonance with the results of (Lal *et al*., 2008; Singh and Lal, 2009 and Sarkar *et al*., 2015). Hence, the selection of genotypes for crop improvement programmes should be based on genetic diversity rather than geographical diversity.

Conclusion

The selection of promising accessions of *Plantago* for quality and yield, based on better seed yield (X7), husk yield (X8), swelling factor (X9) and panicle length (X4), is an efficient criterion. The accessions, GSD 36, GSD 38, GSD 42, GSD 45, and GSD 47 were identified as the best seed yielder, GSD 3, GSD 4, GSD 5, GSD 36 and GSD 42 were identified for the best swelling factor and GSD 2, GSD 12, GSD 36, GSD 44 and GSD 45 were best in longer panicle length based on genetic divergence. Three accessions were identified for dual preferences viz. GSD 36 and GSD 45 for better seed yield and longer panicle length, while GSD 36 and GSD 42 were found to be better for husk yield and swelling factor. The accession GSD 36 was identified for the multiple preferences as longer panicle length (4.31cm), better seed yield (31.26 gm), husks yield (10.31gm) and swelling factor (14.00 ml/gm). Hence, these accessions could be exploited for commercial cultivation.

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Table 1. Accessions/genetic stocks and places of collection/origin of *Plantago* species

Sl. No.	Accession code	Origin/Place of collection	Sl. No.	Accession code	Origin/Place of collection	Sl. No.	Accession code	Origin/Place of collection
1	GSD1	Bulgaria	18	GSD18	U. P. (Lucknow), India	35	PL*35	U. P. (Lucknow), India
2	GSD2	USA	19	GSD19	Gujarat (Mehsana), India	36	GSD18	U. P. (Lucknow), India
3	GSD3	USA	20	GSD20	U. P. (Lucknow), India	37	GSD18	U. P. (Lucknow), India
4	GSD4	Pakistan	21	GSD21	U. P. (Lucknow), India	38	GSD18	U. P. (Lucknow), India
5	GSD5	Maharashtra, India	22	GSD22	U. P. (Lucknow), India	39	GSD18	U. P. (Lucknow), India
6	GSD6	Baluchistan, Pakistan	23	GSD23	U. P. (Lucknow), India	40	GSD18	U. P. (Lucknow), India
7	GSD7	Uttaranchal,India	24	GSD24	U. P. (Lucknow), India	41	GSD18	U. P. (Lucknow), India
8	GSD8	Himanchal Pradesh,India	25	GSD25	U. P. (Lucknow), India	42	GSD18	U. P. (Lucknow), India
9	GSD9	Gujarat,India	26	GSD26	Gujarat, India	43	GSD18	U. P. (Lucknow), India
10	PL*10	Malaysia	27	GSD27	U. P. (Lucknow), India	44	GSD18	U. P. (Lucknow), India
11	GSD11	Gujarat (Sidhpur), India	28	PL *28	Malaysia	45	GSD18	U. P. (Lucknow), India
12	GSD12	U. P. (Lucknow), India	29	GSD29	U. P. (Lucknow), India	46	PA48**	Italy
13	GSD13	U. P. (Lucknow), India	30	GSD30	U. P. (Lucknow), India	47	PPA49***	U. P. (Lucknow), India
14	GSD14	U. P. (Lucknow), India	31	GSD31	U. P. (Lucknow), India			
15	GSD15	U. P. (Lucknow), India	32	PL*32	Malaysia			
16	GSD16	U. P. (Lucknow), India	33	GSD33	U. P. (Lucknow), India			
17	GSD17	U. P. (Lucknow), India	34	GSD34	U. P. (Lucknow), India			

GSD= *Plantago ovata*, PL*= *Plantago lanceolata*, PA**= *Plantago arinaria*, PP***= *Plantago psyllium*

Table 2. Intra- and inter cluster divergence (D^2) in the accessions of *Plantago sp.* among six clusters

Clusters	I	II	III	IV	V	VI	$\bar{D}^2$
I	**109.57** **(10.47)**	153.93 (12.41)	893.98 (29.89)	478.93 (21.88)	3263.17 (57.12)	6237.31 (78.97)	2205.46
II		**95.76** **(9.78)**	714.73 (26.74)	451.12 (21.24)	2795.95 (52.87)	6218.56 (78.86)	2066.85
III			**68.97** **(8.30)**	456.42 (21.36)	2157.20 (46.45)	6174.18 (78.57)	2079.30
IV				**0** **(0)**	2156.26 (46.44)	4894.00 (69.96)	1687.35
V					**0** **(0)**	5033.14 (70.95)	3081.14
VI						**0** **(0)**	5711.44

$\bar{D}^2$-Average D^2 values; D values ($\sqrt{D^2}$) are in parenthesis; intra cluster values in bold fonts.

Table 3. Cluster means, range, character contribution %, rank and other allied genetic parameters of the nine economic traits in *Plantago* germplasm

Cluster with accessions	X1	X2	X3	X4	X5	X6	X7	X8	X9
I (22)	42.00	5.23	27.32	3.27	26.92	30.19	9.35	3.09	12.10
II (20)	42.43	5.12	28.26	3.44	28.83	32.27	18.97	6.25	11.83
III (4)	70.62	1.30	8.83	6.88	58.73	65.61	16.82	5.55	6.93
IV (1)	43.00	4.83	33.16	3.91	30.83	34.74	4.22	1.39	6.00
V (1)	77.77	6.11	119.11	2.62	4.91	7.53	58.03	19.15	2.16
VI (1)	52.89	4.11	624.22	0.79	4.76	5.56	21.98	7.25	1.24
CC$_{\%}$	4.68	13.86	25.21	14.84	7.17	6.88	12.33	12.32	2.68
Ranks	8th	3rd	1st	2nd	6th	7th	4th	5th	9th
Z Vector 1	-0.19	0.069	-0.58	0.21	0.16	0.01	0.085	0.21	0.70
Z Vector 2	0.36	-0.16	-0.47	0.25	0.16	-0.016	0.58	-0.088	-0.43
CCPA$_{\%}$	-28.19	10.13	-85.02	30.98	23.64	2.49	12.33	29.51	104.11
CCPAR	8th	6th	9th	2nd	4th	7th	5th	3rd	1st
CCSA$_{\%}$	194.53	-85.79	-259.01	138.79	86.33	-8.74	317.46	-48.08	-235.51
CCSAR	2nd	7th	9th	3rd	4th	5th	1st	6th	8th
Range of mean	37.28 -77.77	1.00 -6.44	4.11 -624.22	2.52 -8.29	4.76 -60.88	5.56 -68.93	2.23 -58.03	0.74 -19.15	1.23 -14.00

CC%= character contribution (%); CCPAR%= Character Contribution Primary Axis (%); CCSA%= Character Contribution Secondary Axis (%)

Table 4. Mean performances of certain trait specific promising genotypes of isabgol

Selection preference and their accessions		Botanical character			Inflorescence			Yield	Medicinal effect	
		X1	X2	X5	X3	X4	X6	X7	X8	X9
Preferenceto seed yield and husk yield (X7, X8)	GSD36	38.83	4.22	27.65	22.00	4.31	31.96	31.26	10.31	14.00
	GSD42	41.17	5.11	29.57	33.00	3.50	33.07	30.80	10.17	13.93
	GSD38	42.94	5.44	29.38	26.89	3.20	32.58	28.85	9.52	12.03
	GSD45	43.33	5.78	30.91	30.78	3.98	34.88	26.79	8.84	12.00
	GSD47	42.56	5.44	29.00	32.11	2.94	31.94	26.36	8.70	12.07
Preference to swelling Factor (X9)	GSD5	40.78	5.33	29.70	22.78	2.80	32.50	10.44	3.48	14.00
	GSD36	38.83	4.22	27.65	22.00	4.31	31.96	31.26	10.31	14.00
	GSD42	41.17	5.11	29.57	33.00	3.50	33.07	30.80	10.17	13.93
	GSD3	38.83	3.78	29.57	14.56	2.97	32.54	12.91	4.30	13.77
	GSD4	41.17	5.44	29.89	27.67	3.83	33.71	14.73	4.90	13.60
Preference to Panicle length(X4)	GSD12	42.39	6.00	27.35	36.67	4.54	31.89	14.35	4.73	11.03
	GSD2	40.94	5.67	28.18	34.78	4.34	32.52	22.11	7.37	13.00
	GSD36	38.83	4.22	27.65	22.00	4.31	31.96	31.26	10.31	14.00
	GSD44	42.22	5.22	28.94	36.22	4.05	32.99	23.56	7.77	12.57
	GSD45	43.33	5.78	30.91	30.78	3.98	34.88	26.79	8.84	12.00
Dual preferences (X4,X7)	GSD36	38.83	4.22	27.65	22.00	4.31	31.96	31.26	10.31	14.00
	GSD45	43.33	5.78	30.91	30.78	3.98	34.88	26.79	8.84	12.00
Dual preferences (X8,X9)	GSD36	38.83	4.22	27.65	22.00	4.31	31.96	31.26	10.31	14.00
	GSD42	41.17	5.11	29.57	33.00	3.50	33.07	30.80	10.17	13.93
Multiple preferences (X4,X7,X8,X9)	GSD36	38.83	4.22	27.65	22.00	4.31	31.96	31.26	10.31	14.00

Table 5. Distribution of *Plantago* accessions in different clusters according to their geographic origins/locations

Clusters	Geographic location and accessions	Accessions
I (22)	Bulgaria (1)	GSD 1
	USA (2)	GSD 2, GSD 3
	Pakistan (2)	GSD 4, GSD 6
	Maharashtra (1)	GSD 5
	Uttaranchal (1)	GSD 7
	Himanchal Pradesh (1)	GSD 8
	Gujarat (3)	GSD 9, GSD 19, GSD 26
	Uttar Pradesh (11)	GSD 17, GSD 18, GSD 20, GSD 21, GSD 22, GSD 23, GSD 24, GSD 27, GSD 29, GSD 30, GSD 31.
II (20)	Gujarat (1)	GSD 11
	Uttar Pradesh (19)	GSD 12, GSD 13, GSD 14, GSD 15, GSD 16, GSD 25, GSD 33, GSD 36, GSD 37, GSD 38, GSD 39, GSD 40, GSD 41, GSD 42, GSD 43, GSD 44, GSD 45, GSD 46, GSD 47
III (4)	Malaysia (3)	PL 10, PL 28, PL 32
	Hungary (1)	PL 35
IV (1)	Uttar Pradesh (1)	GSD 34
V (1)	Italy (1)	PA 48
VI (1)	Uttar Pradesh (1)	PP 49

GSD= *Plantago ovata*: PL= *Plantago lanceolata*: PA= *Plantago arinaria*: PP= *Plantago psyllium*

Fig 1. (a) ***Plantago arinaria*** **(b)** ***Plantago psyllium*** **(c)** ***Plantago lanceolata*** **(d)** ***Plantago ovata*** **(e,f,g.) range of variability in the inflorescence of P*lantago ovata* accessions.**

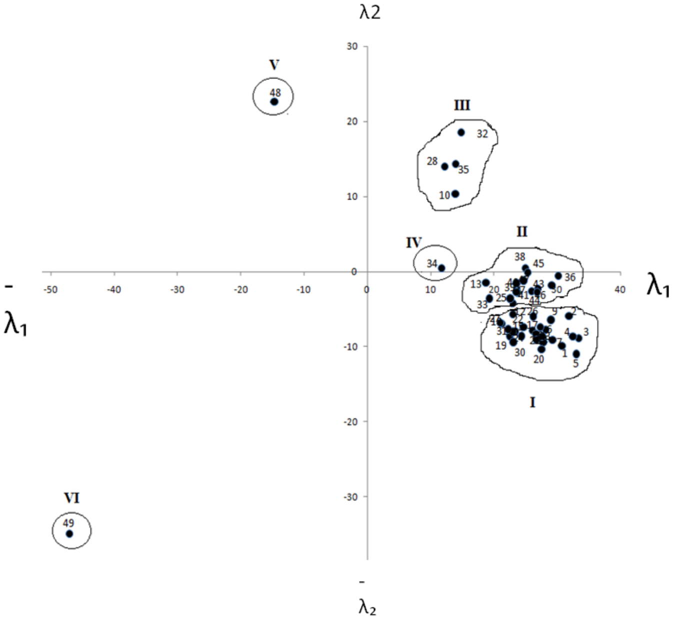

Fig 2. Spatial distribution of 49 accessions of *Plantago* germplasm.

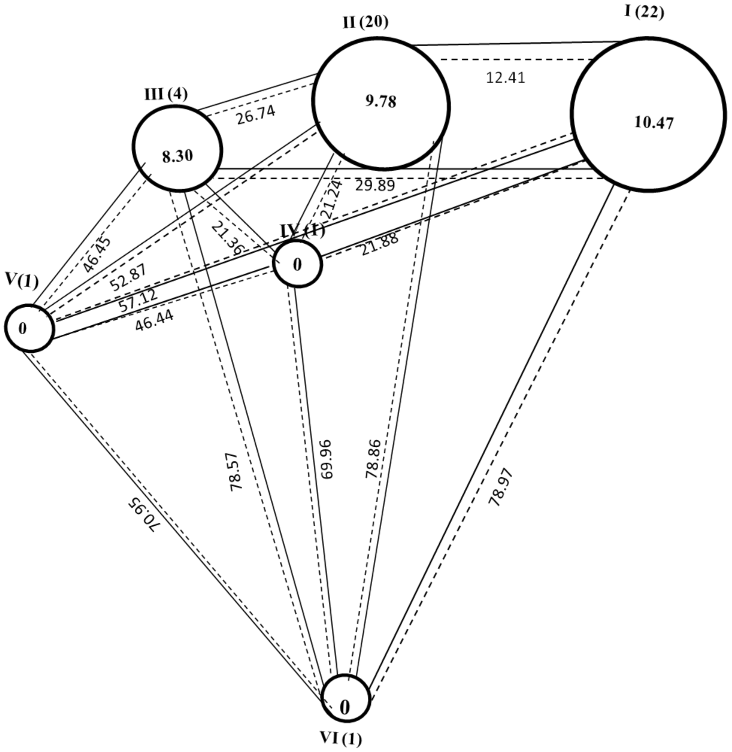

Fig 3. Cluster diagram with number and distances of 49 genotypes in *Plantago* germplasm.