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Research Note

Genetic diversity of *Plantago ovata* Forsk. through RAPD markers

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Abstract:
Genetic variability of 15 sets of *Plantago ovata* Forsk. studied using 11 arbitrary oligonucleotide primers. Among the 90 DNA fragments produced 71 fragments were found to be polymorphic. The mean number of polymorphic bands per primer among 15 *Plantago ovata* genotypes was 6.45 . The higher polymorphism (90.00 %) was exhibited by primer OPF-17, while the lower polymorphism (60.00 %) was detected by OPF-2. The genetic similarity matrix from RAPD data for 15 genotypes was calculated based on Jaccard's coefficients of similarity ranged from 0.45 to 0.80. UPGMA cluster analysis reveals that the 15 genotypes were clustered in to three clusters. Genetically distinct genotypes identified using RAPD markers could be potential sources of germplasm for Isabgol improvement.

Key words: *Plantago ovata, genetic diversity, RAPD, sex-morphotypes*

Plantago ovata Forsk. commonly known as "Isabgol" belongs to family Plantaginaceae. It is a native of Mediterranean region and is cultivated for its valuable husk used as medicine. *Plantago* ovata Forsk. is one of the most important medicinal plants in the Unani and Ayurvedic medicine. It belongs to the order plantaginales which consists of only a single family Plantaginaceae. In spite of great importance of Isabgol as a medicinal plant considerably less work has been done so far on genetic. In crop improvement programme, genetic diversity has been considered as an important factor which is also essential pre-requisite for hybridization programme for obtaining high yielding progenies. The inclusion of diverse parents in hybridization programme helps in combining desirable genes which remained in isolation in nature, so as to obtain superior recombination. Application of molecular marker system has significantly advanced the understanding of plant genomes; RAPD has been successfully employed for varied purposes in plant genetics and breeding. In the present study, attempts were made to assess the genetic diversity of *Plantago* using RAPD markers.

Fifteen germplasm lines were raised in Randomize Block Design with two replication. Each treatment was represented by a single row having a row to row distance of 30 cm and plant to plant 10 cm. Young leaves from 45 to 50 days old plants which has just flowered were excised and subjected to genomic DNA extraction The genomic DNA was extracted following the CTAB (Cetyltrimethylethyl Ammonium Bromide) method of Doyle and Doyle (1990) with The genomic DNA samples extracted from each of 15 *Plantago ovata* genotypes were subjected to PCR amplification. Amplification was carried out in a 200 µl thin walled PCR tube containing a 25 µl reaction mix. The 25 µl PCR mix contains, 2.5 µl of 10X Assay buffer, 0.5 µl of 10 mM/µl dNTP mix, 0.5 µl of 3IU/µl Taq polymerase, 2.0 µl of 10 µM primer, 2.0 µl of 10 ng of genomic DNA. Amplification was carried out through PCR in a thermal cycler (Eppendorf) & Biometra along with the control (without template DNA). Reaction mix was gently tapped and spun briefly. The PCR amplification was carried out under following thermal cycling regime: Initial Denaturation of 94° C for 2 minutes, 35 cycles includes three steps, Denaturation (94° C for 30 seconds), Annealing (40° C for 60 seconds), and Extension (72° C for 60 seconds) after Final Extension was given at72° C for 10 minutes.

The amplified product was collected from the thermal cycler and loaded on to 1.4 percent (w/v) agarose gel prepared in 1.0X TBE (pH 8.0). The required volume of 1.0X TBE (pH 8.0) was used as running buffer mixed with EtBr. Whole of the 25 µl PCR amplified product was mixed with 6X agarose gel loading dye of which 10 µl was loaded in well. Along with the samples known molecular weight supermix DNA ladder (Banglore Genei) was also loaded. The band profiles were visualized and documented using gel documentation system Alpha EaseFC4.0.0 (Alpha Innotech Corporation, USA). For each locus the

presence and absence of the band was recorded as 1 and 0.

DNA based molecular markers have proved valuable in crop breeding especially in studies on genetic diversity, genetic purity testing and gene mapping. The commonly used PCR based DNA marker systems are random amplified polymorphic DNA (RAPD), Inter-simple sequence repeats (ISSR), simple sequence repeats (SSR), amplified fragment length polymorphism (AFLP) and many more. The important feature of these markers is that they are devoid of environmental interaction, dominant, co-dominant and allow selection at genotypic level. Not much information is available on molecular marker studies in genus *Plantago.*

The data collected from random amplification of polymorphic DNA with 11 arbitrary oligo-nucleotide primers produced a total 90 DNA fragments. Among these 71 fragments were found to be polymorphic. The mean number of polymorphic bands per primer among 15 *Plantago ovata* genotypes was 6.45. The size of PCR amplified DNA fragments ranged from 114 to 2818 bp. The highest polymorphism (90.00%) was exhibited by primer OPF-17, while the lowest polymorphism (60%) was detected with OPF-2. The average polymorphism detected by the RAPD loci in the present investigation was 78.88% (Table. No. 2). Maximum number of in *Plantago* also molecular diversity has been analyzed earlier by many workers in different species. Wolff *and* Morgan (1998) detected polymorphism for DNA sequences between *P. major* and *P. lanceolata.* RAPD and ISSR variation for chloroplast genome differentiated two sub species of *P. major* (Wolff and Morgan, 1998), Wolff *et al.* (2000) analyzed *P. major* and *P. intermedia* using RAPD and ISSR for O_3 resistance. Squirrell and Wolff (2001) found molecular markers as efficient tool to investigate the evolution of two species *viz.*, *P. major* and *P. intermedia.* Koorevaar *et al.* (2002) found eleven polymorphic loci in *P. coronopus* generated from a GA enriched genomic library. Marie *et al*. (2003) developed microsatellite primers for *P. lanceolata* and *P. major* to detect molecular variation.

Out of 15 genotypes studied in the present investigation JI-150, JI-214, JI216, JI-150, JI-189 produced the higher number of DNA fragments while JI-131 and JI-137 produced lower number of DNA fragments. The genetic similarity matrix [Table.No.5] from RAPD data for 15 genotypes was calculated based on Jaccard's coefficients of similarity and is shown in Table 3. The genetic similarities ranged from 0.45 to 0.80. Average genetic similarity among these 15 genotypes was found to be 0.69. Dendrogram generated by UPGMA cluster analysis based on jaccard's similarity coefficients the genotypes based clustered. The first cluster in the presently constructed dendrogram comprised of cluster in first cluster the genotypes viz., GI-2, JI-107, JI-129, JI-189, JI-214, JI-216, JI-132, JI-192, JI-127, JI-227, JI-206, JI-130 and JI-137 were clustered. In the second cluster the genotype JI-150. In third cluster the genotype JI-131 was clustered. Based on the diversity analysis the genotypes from each cluster can be selected and intercrossed for the *Plantago* improvement programme.

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Table 1. List of preliminary screened different germplasm of *P. ovata*

Germplasm line	Charactertics features
Gujarat Isabgol-2	Medium broad and pale green leaves, medium long spike, more length
Jagudan Isabgol – 189	High yielder
Jagudan Isabgol - 216	Erect type
Jagudan Isabgol – 227	More erect, long spike, synchronize spike
Jagudan Isabgol – 192	Early type
Jagudan Isabgol – 206	Short spike
Jagudan Isabgol – 214	Long spike erect type, tiller more
Jagudan Isabgol – 107	Early type
Jagudan Isabgol – 127	High yielding, more seed, bold seeded
Jagudan Isabgol – 129	More spikes & spike
Jagudan Isabgol – 130	Medium spike length
Jagudan Isabgol – 131	Erect type plants, short spikes
Jagudan Isabgol – 132	More erect, long spikes, synchronous maturity
Jagudan Isabgol - 137	High yielder
Jagudan Isabgol – 150	Tall type

Table. 2 Details of RAPD markers used in the study

Primer Name	Primer sequence	Total amplicons	No of monomorphic amplicons	No of polymorphic amplicons	Percent Polymorphism (%)
OPF-02	GAG GAT CCC T	10	4	6	60.00
OPF-04	GGT GAT CAG G	-	-	-	-
OPF-05	CCG AAT TCC C	12	2	10	83.33
OPF-06	GGG AAT TCG G	8	2	6	75.00
OPF-17	AAG CCG GGA A	10	1	9	90.00
OPG-3	GAG CCC TCC A	8	2	6	75.00
OPG-10	AGG GCC GTC T	9	1	8	88.88
OPG-15	ACT GGG ACT C	7	1	6	85.71
OPG-16	AGC GTC CTC C	10	3	7	70.00
OPH-01	GGT CGG AGA A	8	1	7	87.50
OPH-12	ACG CGC ATG T	8	2	6	75.00

Table.3. The genetic similarities based on pooled RAPD data on *P. ovata* lines

	GI-2	JI-214	JI-216	JI-150	JI-192	JI-206	JI-107	JI-132	JI-189	JI-129	JI-130	JI-127	JI-227	JI-137
JI-214	0.66													
JI-216	0.70	0.74												
JI-150	0.60	0.58	0.67											
JI-192	0.62	0.75	0.66	0.59										
JI-206	0.63	0.75	0.67	0.67	0.71									
JI-107	0.63	0.67	0.64	0.58	0.63	0.69								
JI-132	0.55	0.71	0.72	0.48	0.60	0.64	0.55							
JI-189	0.62	0.66	0.63	0.69	0.61	0.60	0.71	0.60						
JI-129	0.67	0.70	0.62	0.53	0.58	0.67	0.80	0.65	0.69					
JI-130	0.68	0.68	0.60	0.63	0.76	0.80	0.68	0.54	0.62	0.61				
JI-127	0.64	0.71	0.63	0.62	0.76	0.71	0.68	0.59	0.67	0.68	0.69			
JI-227	0.66	0.72	0.61	0.54	0.74	0.72	0.63	0.52	0.59	0.64	0.77	0.76		
JI-137	0.53	0.57	0.51	0.53	0.58	0.65	0.63	0.50	0.58	0.63	0.64	0.70	0.71	
JI-131	0.55	0.50	0.49	0.51	0.48	0.55	0.55	0.45	0.54	0.47	0.60	0.48	0.49	0.59

Fig. No.1. Dendrogram based on euclidean distance matrix showing genetic relationship among *Plantago*