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BRIEF COMMUNICATION

Estimation of genetic variability in *Plantago ovata* cultivars

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Abstract

Five cultivars of *Plantago ovata* Forsk. (medicinal plant) have been developed by different agricultural universities in India. Genetic variability of these cultivars was estimated using RAPD markers. The data were correlated to morphological characters and a dendrogram was obtained from Jaccard's coefficient.

Additional key words: random amplified polymorphic DNA (RAPD), dendrogram.

Isabgul (*Plantago ovata* Forsk.) is an important medicinal plant. It is commercially cultivated in India in the states of Gujarat and Rajasthan. Several institutes and agricultural universities (CIMAP, Lucknow; Gujarat Agricultural University, Anand, *etc.*) have developed improved cultivars of isabgul in India (Maiti and Mandal 2000).

Commercial cultivars released by these institutes respond very well to tissue culture methods. Clonal propagation and somatic embryogenesis of *P. ovata* have been carried out successfully (Pramanik *et al.* 1995, Roy Chowdhury *et al.* 1996, Das *et al.* 2001). However, ifferent cultivars need specific media and culture conditions for *in vitro* regeneration (Das and Raychaudhuri 2001). To estimate genetic variation five cultivars were procured from National Research Center for Medicinal and Aromatic Plants, Boriavi Anand, Gujarat.

DNA was extracted from young seedlings of all these cultivars and subjected to polymorphic chain reaction (PCR) analysis using 14 single random decamer primers, to enumerate random amplified polymorphic DNA (RAPD) markers showing polymorphism (William *et al.* 1990, Edward *et al.* 1991, Betal 2002).

These primers detect polymorphisms in the absence of specific nucleotide sequence information and polymorphism functions as genetic markers (Sonnante *et al.* 1997). A number of scientists have used RAPD markers to study polymorphism in various plants (Machado *et al.* 1996, Staub *et al.* 1996, Sosinski *et al.* 1996, Padmesh *et al.* 1999).

In the present study a detailed statistical analysis was carried out to determine Jaccard's coefficient and dendrograms were prepared depending on this data. Morphological features of seedling and plant yield have been correlated with the affinity of the species showing RAPD analysis (Shoyama *et al.* 1997, Raghunathachari *et al.* 2000).

Dry seeds of five cultivars namely GI 1, GI 2, NIHARIKA, MIB 4 and HI 5 of *Plantago ovata* were obtained from Boriavi Anand, Gujarat. After imbibitions for 18 h in sterile distilled water, the seeds were sterilized in 10 % sodium hypochlorite solution for 20 min and washed for four to five times with sterile distilled water in a laminar flow hood. The sterile seeds were germinated aseptically in sterile glass Petri dishes on wet filter paper. Petri dishes were kept in the dark at first (for two days to induce germination) at temperature of 25 ± 2 °C and a relative humidity of 55 - 60 %. After 8 - 10 d seedlings were taken for extraction of DNA.

DNA was isolated following the method of Edwards *et al.* (1991) using DNA extraction buffer containing 200 mM Tris-HCl, pH 7.5, 200 mM NaCl, 25 mM EDTA, pH 8, and 0.5 % SDS. 7 to 8-d-old seedlings were

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cut with sterile forceps and scissors and placed on to the sterile mortar pestle. Seedlings were crushed gently using DNA extraction buffer. Tissue was macerated and centrifuged for 10 min at 7 826 *g* at room temperature to remove tissue fragments. Equal volume of supernatant was transferred into Ependorff tubes containing phenol:chloroform (1:1) and centrifuged at 7 826 *g* for 5 min and supernatant was washed with equal volume of chloroform. Finally the clear upper aqueous phase was collected carefully and 3 M ammonium acetate and equal volume of isopropanol were added and mixed gently. DNA was spooled with sterile capillary. DNA was then washed with 70 % ethanol and air-dried. Finally the DNA was dissolved in 0.050 cm^3 of sterile triple distilled water.

The DNA isolated was run in 1 % agarose gel to check the quality and was also scanned in spectrophotometer (*Beckman DU-640*, USA). The absorbances were noted at 260 and 280 nm.

RAPD was performed using genomic DNA isolated from five cultivars of *Plantago ovata*. 14 decamer oligonucleotides were used as primers to perform RAPD reactions. In each set, one tube without any genomic DNA (negative control) was also prepared. The RAPD reaction products were resolved in 1.8 % agarose gel at a constant voltage of 60 V for 7 h and photographs were taken under UV-transilluminator after staining with ethidium bromide (Shoyama *et al.* 1997).

The amplification reaction mixture had a final volume of 0.025 cm^3 in each PCR tube containing 10 mM Tris-HCl (pH 9), 50 mM KCl, 100 μM each of dATP, dTTP, dGTP, dCTP, 0.2 μM decamer random primer, 25 ng of genomic DNA, 1.5 cm^3 $MgCl_2$, 3 units of Taq DNA polymerase and 0.015 cm^3 sterile triple distilled water. Finally the PCR tubes were subjected to a denaturation step at 94 °C for 5 min., 45 cycles of 94 °C for 30 s, 35 °C for 30 s, 72 °C for 1 min 30 s and a final elongation at 72 °C for 5 min in a *Perkin Elmer* (Foster City, USA) thermal cycler. Finally the temperature of amplified products was brought down to 4 °C.

In each set of reactions, one tube without any genomic DNA (negative control) was also prepared. The RAPD reaction products were resolved in 1.8 % agarose gel at a constant voltage of 60 V for 7 h. 100 bp ladder was also charged in the last well. Photographs were taken under UV-transilluminator after staining with ethidium bromide.

Seeds of five *P. ovata* cultivars were germinated *in vitro* and after 20 - 25 d 10 to 15 plantlets were transferred to field and grown. Different phenotypic characters like plant height, leaf size, morphology of inflorescence, *etc.*, were studied.

Positions of clearly visible and scorable RAPD bands were transferred into a binary character matrix, with 1 for the presence and 0 for the absence of a band at a particular position. Proximity matrix was directly computed from Jaccard's coefficient using the *Software Package SPSS, version 9.0* (Sonnante *et al.* 1997). A dendrogram was obtained (using average linkage within groups) by hierarchical cluster analysis to establish the affinity and relationship of the 5 cultivars of *P. ovata*.

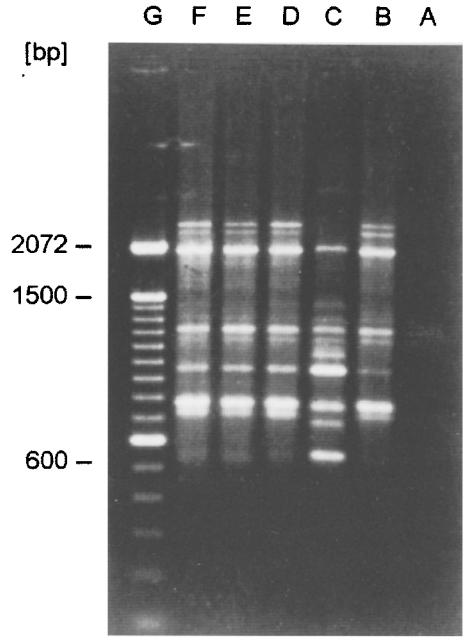

Fig 1. RAPD profiles from genomic DNA of five cultivars of *Plantago ovata* using primer CAGGCCCTTC The lanes represent from right: negative control without any genomic DNA, GI 1, GI 2, NIHARIKA, MIB 4, HI 5 and 100bp ladder (marker).

The phenotypic characters of the five cultivars of *Plantago ovata*. procured from Boriavi Anand, Gujarat, have been recorded (Table 1). Plant height varied from 20 to 29 cm among the five cultivars. Characteristic features of leaf and inflorescence were also variable. Amount of husk was also variable in respect to size and number of inflorescence per plant. Seeds were boat shaped having an ovate outline and pinkish gray to brown in colour.

RAPD analysis of five cultivars of *Plantago ovata*. were performed by 14 decamer primers of GC content 60 - 70 %. Different primers showed different size range of PCR product for a particular cultivar. A total number of 46 bands were obtained with 14 primers of which 39 (84 %) were polymorphic and 7 (16 %) were found to be monomorphic (Fig. 1). Two primers CAGGCCCTTC and AATCGGGCTG were found to be more informative showing 11 bands each. Cluster analysis of the distribution of RAPD bands have been represented as a dendrogram using average linkage (within groups).

Jaccards similarity coefficient ranged from 0.605 - 0.851 (Table 2). Two cultivars namely GI-2 and NIHARIKA were very similar with respect to band position of the RAPD markers. They showed similarity with respect to bands with the 14 different primers used.

Table 1. Characteristics feature of *P. ovata* cultivars. Means ± SD.

Name of cultivars	Developed by	Plant height [cm]	Leaf length [cm]	Leaf morphology	Number of inflorescence	Inflorescence size [cm]	Morphology of inflorescence
GI-1(Gujarat Isabgul-1)	Gujarat Agr. Univ., Anand	23.0 ± 2.5	17.5 ± 2.5	Linear, woolly hairy both side	11 - 17	2.0 ± 1.5	spike, pedicel smooth
GI-2(Gujarat Isabgul-2)	Gujarat Agr. Univ., Anand	25.5 ± 3.0	15.0 ± 4.5	Linear, cuspidate, both side woolly	4 - 5	2.2 ± 1.0	spike, pedicel woolly
NIHARIKA	CIMAP, Lucknow	21.0 ± 1.5	12.5 ± 5.5	Linear, cuspidate, both side woolly, tip acicular	7 - 11	1.7 ± 0.5	spike, pedicel woolly
MIB-4(Jawar Isabgul-4)	KNK Coll. Agr., Mandsour	27.0 ± 2.0	13.0 ± 6.0	Linear, tomentose	8 - 9	1.7 ± 0.5	spike, pedicel woolly
HI-5(HISSAR Isabgul-5)	CCS Agr. Univ., Hissar	23.0 ± 3.5	15.5 ± 7.0	Acicular, hirsute	6 - 10	2.5 ± 0.5	spike, pedicel smooth

Table 2. Proximity matrix.

Case	Matrix file input GI-1	GI-2	NIHARIKA	MIB-4	HI-5
GI-1		0.792	0.771	0.565	0.800
GI-2			0.851	0.609	0.800
NIHARIKA				0.659	0.783
MIB-4					0.605
HI-5					

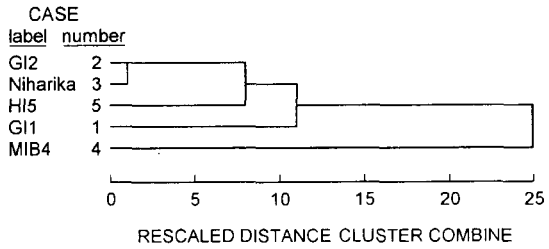

Fig. 2. Dendrogram of 5 cultivars of *P. ovata* based on RAPD bands amplified by 14 arbitrary 10-mer RAPD primers.

The other (HI-5, GI-1 and MIB-4) formed 3 separate clusters.

The dendrogram using average linkage within groups (Fig. 2) allowed 4 main groups to be distinguished. The first group includes GI-2 and NIHARIKA while the remaining three cultivars formed 3 different clusters.

Rout and Das (2002) reported that micropropagated plants of *Plumbago zeylanica* were monomorphic and similar to those of field grown mother plant, which was established by RAPD analysis. Ranade *et al.* 2002, reported RAPD analysis of betel vine cultivars (*Piper betel* L.) Kapoori and Bangla using 10 primers. They observed that Kapoori cultivars were more heterogeneous while Bangla cultivars were mostly similar to each other.

The data on polymorphism revealed by RAPD markers in *Plantago ovata* cultivars was also in agreement with the morphological features studied. GI-2 and NIHARIKA showed close affinity with respect to plant height, leaf size and morphology and seed characteristics (Table 1). The other three cultivars varied distinctly with respect to morphological characters. Reports of intra-specific variability of RAPD markers in different cultivars of *P. ovata* are lacking. The present investigation on RAPD polymorphism coupled with morphological characteristics is useful to deduce the affinity of these five high yielding cultivars cultivated in India. The genotypic characters as evidenced by polymorphic DNA bands could be correlated to some extent to the phenotype (morphology) of two closely associated cultivars namely GI-2 and NIHARIKA.

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