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Short communication

Genetic diversity analysis in *Morinda tomentosa* collected from Gujarat using RAPD markers

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ABSTRACT

Twelve Random Amplified Polymorphic DNA (RAPD) primers were used to evaluate the genetic diversity of 31 *Morinda tomentosa* genotypes collected from different villages of Gujarat. An average of 92.4% polymorphism was observed and all the genotypes which could be distinguished using 12 RAPD primers. The numbers of RAPD bands generated per primer varied from 5 to 10 with an average of 6.6 bands per primer. The Jaccard's similarity coefficients ranged from 0.32 to 0.92 with an average of 0.65. Further, UPGMA clustering grouped all the 31 *Morinda* genotypes into one main cluster. Dudhwa and Richia have been identified as distinct genotypes as they stood separate in the dendrogram. Average Nei's diversity value and Shannon's information index was 0.32 ± 0.17 and 0.48 ± 0.22, respectively suggesting the existence of significant diversity of *Morinda tomentosa* in Gujarat.

Key words: *Morinda tomentosa*, genetic diversity, RAPD.

Morinda tomentosa (Family: Rubiaceae; common name: *Maddimara*) is abundant in the secondary forest, along roads and forest edges (Nguyen and Nguyen, 1). It is found in India, Sri Lanka, China, Thailand, Philippines and West Sumatra. It is distributed throughout Northern India and Deccan Penninsula. Seeds are dark brown without prominent air-sac and roughly triangular in shape. Its fruits are dark green till maturity, non-glabrous and are eaten. Root bark is used for making red dye. Roots, leaves, fruits and bark are also used in different preparations in medicines (Parihar and Bohra, 2). Molecular characterization of such an important species would be useful in the identification of diverse genotypes and estimating the overall diversity in a particular area for selecting the representative genotypes for conservation and utilization. In Gujarat, wide range of variability in *M. tomentosa* exists in the form of trees and shrubs. Thirty one genotypes were collected from different villages for diversity analysis using molecular markers. PCR based molecular markers provide very effective and reliable tool for assessing the genetic variation among plant genotypes. There is only one report in which, RAPD and ISSR markers were used for diversity analysis among three *Morinda* species collected from Andaman and Nicobar Islands, Tamil Nadu and Karnataka (Singh *et al*., 3). Still RAPD markers had not been used before for diversity analysis in *Morinda tomentosa* genotypes collected from Gujarat. The present study, therefore, was undertaken.

Leaves of 31 *Morinda tomentosa* genotypes were collected from different villages (Table 1) of Gujarat, cleaned with water, wrapped in aluminium foil and dipped in liquid nitrogen and kept at -80°C until DNA extraction. DNA was extracted from bulk leaf samples using CTAB method (Saghai-Maroof *et al*., 4). Polyvinyl pyrrolidone (PVP) was also added to CTAB buffer before keeping the ground leaf material at 65°C. DNA quantification was done using NANODROP 1000 (Thermo Scientific) spectrophotometer. Stock DNA was stored at -20°C and 50 ng working DNA solution was prepared for RAPD profiling using 12 RAPD primers (Table 2). The PCR reaction mixture consisted of *Taq* DNA polymerase (1 µ/µl), 10X PCR buffer, dNTPs mix (10 mM), 25 mM $MgCl_2$, 5 µM RAPD primers and 50 ng/µl genomic DNA. PCR amplification was carried out with 100 ng of genomic DNA, 3.0 mM $MgCl_2$, 1U *Taq* DNA polymerase, 1X PCR buffer without $MgCl_2$, 0.6 µM RAPD primer and 0.2 mM dNTP mix. The volume was made up to 25 µl with sterile distilled water. Thermocycling conditions used for PCR were as : denaturation at 94°C for 5 min.; forty cycles of denaturation at 94°C for 1 min., primer annealing at 37°C for 1 min. and primer extension at 72°C for 1 min. and final extension step at 72°C for 10 min. PCR products were run on 1.6% agarose gel and photographs were taken on SYNGENE G:Box Chemi XT4 gel documentation unit.

RAPD bands were scored as present (1) or absent (0) and Jaccard's similarity coefficient (JSC) for pairwise comparisons based on the proportion of shared bands was used to construct dendrogram using UPGMA (Unweighted Pair Group Method of Arithmetic Means) algorithm (Rohlf, 5). Gene diversity

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Table 1. List of 31 *Morinda tomentosa* genotypes used for RAPD profiling.

Genotype (Village)	Sample type	Sampling type	Habitat
Thasara	Individual plant	selective	Disturbed roadside
Sawalia	Individual plant	selective	Disturbed roadside
Dr Ka movda	Individual plant	selective	Disturbed roadside
Panch pathar 1	Individual plant	selective	Disturbed roadside
Panch pathar 2	Individual plant	selective	Disturbed roadside
Pannia 1	Individual plant	selective	Disturbed roadside
Pannia 2	Individual plant	selective	Disturbed roadside
Vezalpur field	Individual plant	selective	Disturbed roadside
Vezalpur road side	Individual plant	selective	Disturbed roadside
Dang 1	Individual plant	selective	Disturbed roadside
Dang 2	Individual plant	selective	Disturbed roadside
Richia	Individual plant	selective	Disturbed roadside
Panchkhobala 1	Individual plant	selective	Disturbed roadside
Pavagarh 1	Individual plant	selective	Protected forest area
Pavagarh 2	Individual plant	selective	Protected forest area
Pavagarh 3	Individual plant	selective	Protected forest area
Pavagarh 4	Individual plant	selective	Protected forest area
Paroli 1	Individual plant	selective	Disturbed roadside
Paroli 2	Individual plant	selective	Disturbed roadside
Rameshwara 1	Individual plant	selective	Protected forest area
Rameshwara 2	Individual plant	selective	Protected forest area
Dudhwa	Individual plant	selective	Disturbed roadside
Moholia	Individual plant	selective	Disturbed roadside
Simalia	Individual plant	selective	Disturbed roadside
Ranipura	Individual plant	selective	Disturbed roadside
Acchala	Individual plant	selective	Disturbed roadside
Ruparel 1	Individual plant	selective	Disturbed roadside
Panchkhobala 2	Individual plant	selective	Disturbed roadside
Ruparel 2	Individual plant	selective	Disturbed roadside
Manchi	Individual plant	selective	Disturbed roadside
Popatpura	Individual plant	selective	Disturbed roadside

statistics were estimated using POPGENE version 1.31 (Yeh *et al*., 6).

Twelve RAPD primers were used for RAPD genotyping of 31 *Morinda tomentosa* genotypes. The total number of bands observed among the *Morinda* genotypes based on RAPD analysis with 12 polymorphic primers were 79. The number of scorable bands produced per primer varied from 5 (OPE03, OPE07, OPL02 and OPJ20) to 10 (OPV16) with an average of 6.6 bands per primer. The total number of polymorphic bands and the average percentage of polymorphism was 73 and 92.4%, respectively. The polymorphism rate reported in our study is quite high as compared to earlier study (Singh *et al*., 3), which shows the effectiveness of the RAPD markers selected for this study and also the diversity level of the genotypes used in this study.

Seventy nine RAPD bands were used for calculating Jaccard's similarity coefficient. The Jaccard's similarity coefficients ranged from 0.32 to 0.92 with an average of 0.65. Maximum similarity was observed between Dang 1 and Vezalpur roadside and the lowest similarity was observed between Dudhwa and Pannia 1. The UPGMA dendrogram (Fig. 1) based on these data clustered the 31 *Morinda* genotypes within one major group and Dudhwa and

Table 2. Characteristics of the bands generated in 31 *Morinda tomentosa* genotypes using RAPD primers.

Primer	TNB	NPB	P (%)	BS (bp)	na	ne	h	I
OPX01	8	8	100.00	250-1750	2.00	1.41	0.26	0.41
OPE07	5	4	80.00	350-850	1.80	1.38	0.23	0.36
OPE03	5	4	80.00	250-1000	1.80	1.59	0.33	0.48
OPE15	6	6	100.00	300-1700	2.00	1.56	0.33	0.50
OPV16	10	10	100.00	275-900	2.00	1.67	0.38	0.55
OPF01	6	5	83.33	375-1000	1.83	1.47	0.28	0.43
OPE17	6	5	83.33	100-900	1.83	1.29	0.19	0.32
OPM03	9	8	88.89	400-2000	1.89	1.46	0.28	0.43
OPE18	7	7	100.00	375-750	2.00	1.80	0.43	0.62
OPL02	5	4	80.00	300-800	1.80	1.61	0.34	0.49
OPF02	7	7	100.00	250-1500	2.00	1.85	0.45	0.64
OPJ20	5	5	100.00	325-650	2.00	1.56	0.32	0.48
Average	6.6	6.1	92.41		1.92	1.56	0.32	0.48
SD					0.27	0.34	0.17	0.22

TNB = Total number of bands; NPB = Number of polymorphic bands; P (%) = % Polymorphism; BS = Band size (base pairs); na = Observed number of alleles; ne = Effective number of alleles; h = Nei's gene diversity; I = Shannon's information index

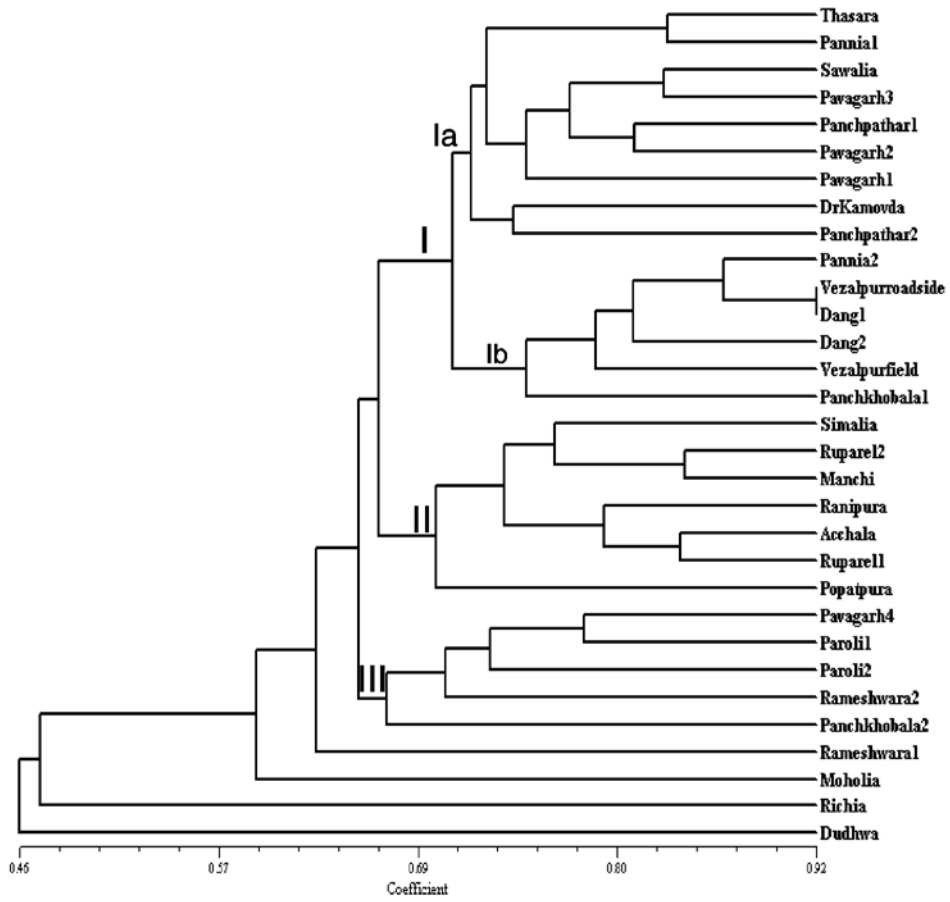

Fig. 1. Dendrogram based on RAPD data of 31 *Morinda tomentosa* genotypes collected from Gujarat.

Richia stood separately in the dendrogram. Within one major group there were three clusters, *viz*. I (Ia and Ib), II and III. Out of four Pavagarh genotypes included in this study, three were present in cluster Ia. Panch Pathara 1 and Panch Pathara 2 were also placed in cluster Ia. Both the Dang 1 and Dang 2 genotypes and Vezalpur roadside and Vezalpur field genotypes were grouped in cluster Ib. Ruparael 1 and Ruparael 2 genotypes were part of the cluster II with genotype from Popatpura as its outlier. Similarly, Paroli 1 and Paroli 2 were closely grouped in cluster III. Rameshwara 1 and Rameshwara 2 were placed as outliers of cluster III. UPGMA clustering revealed the grouping of genotypes collected from same village in one cluster with some exceptions.

Genetic diversity statistics for the *Morinda tomentosa* genotypes is presented in Table 2. Primers OPF02 followed by OPE18 revealed higher effective number of alleles, Nei's diversity and Shannon's information index. The mean diversity value for all the 31 genotypes was 0.32 ± 0.17 and mean Shannon's information was 0.48 ± 0.22. These values suggested significant diversity of *Morinda tomentosa* in Gujarat and more extensive exploration and collections should be done to capture the existing variability.

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