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Assessment of genetic diversity in Isabgol (*Plantago ovata* Forsk.) using random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers for developing crop improvement strategies

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Isabgol (*Plantago ovata* F., family: Plantaginaceae) is one of the most important medicinal plants of South Asia. Two DNA based molecular marker techniques, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR), were used to study the genetic diversity among genotypes of Isabgol. A total of 38 polymorphic primers (22 random and 16 ISSR) were used. Amplification of genomic DNA of 24 genotypes, using RAPD analysis, yielded 208 fragments, of which 98 (47.12%) were polymorphic. The 16 ISSR primers produced 124 bands across 24 genotypes, of which 24 (19.35%) were polymorphic. RAPD markers appeared more informative than ISSR in determining the genetic diversity. The similarity coefficient ranged from 0.77 to 0.97, 0.81 to 1.00 and 0.84 to 0.98 with RAPD, ISSR and combined dendrogram, respectively. This indicates very low level of genetic diversity among genotypes. A poor mantel correlation (*r* = 0.28) was found between both sets of genetic similarity data, suggesting that both sets of markers revealed unrelated estimates of genetic relationships. Therefore, the RAPD and ISSR markers show two genetic grouping of studied Isabgol genotypes. The genotypes RI-168, RI-167, RI-137, PB-62, RI-153, RI-148 and Gumary were spotted as genetically diverse in both sets of markers and could be efficiently utilized in crop improvement programmes.

Key words: *Plantago ovata*, molecular marker, RAPD, ISSR, genetic diversity, medicinal plant.

INTRODUCTION

Isabgol (*Plantago ovata* Forsk., family: Plantaginaceae) is a stemless Ayurvedic herb, used in health care for many centuries in South Asia, and it is now widely used for its medicinal properties all over the world. Seed husk (rosy-white membranous covering of the seed) mainly given as a safe laxative, particularly beneficial in habitual constipation, chronic diarrhea and dysentery (Samantaray et al., 2010). It is also used in lowering blood cholesterol level, ice cream making and cosmetics (Koocheki et al., 2007). In Romania and Bulgaria, leaves of Isabgol are used as a folk remedy to prevent infection on cuts and scratches of its antiseptic properties (Dagar

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Abbreviations: PCR, Polymerase chain reaction; **RAPD,** randomly amplified polymorphic DNA; **ISSR,** inter-simple sequence repeat; **CTAB,** cetyl trimethyl ammonium bromide; **TBE,** tris–borate–ethylenediaminetetraacetic acid; **OUTs,** operational taxonomic units; **SCARs,** sequence characterized amplification regions.

et al., 2006). It is native of Persia, but also widely cultivated throughout India, Pakistan, Mexico, West Asia and Mediterranean Regions. India is the largest producer and exporter of Isabgol in the world (Singh et al., 2009). The productivity of Isabgol is far below the desired levels and India is not able to meet global demand. The limited existing genetic diversity is a major constraint for the improvement of this crop (Dhar et al., 2005). Therefore, it is necessary to analyze or examine the genetic diversity provided by the gene pools and then harnessed for crop improvement.

The concept of molecular marker is an ideal approach for this purpose. They are reliable indicator of genetic diversity because they are neutral to environment influence and reveal differences at the whole genome level (Khurana-Kaul et al., 2012). Among the various molecular marker techniques available, polymerase chain reaction (PCR)-based markers, such as randomly amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) have been most popular because of speed, low cost, does not require prior knowledge of DNA sequence and the use of only minute amounts of DNA template for analysis. RAPD has been the most employed technique in diversity analysis, mapping and genotype identification in number of plant species but low reproducibility is the limit of this technique. ISSR markers overcome the shortcomings of the low reproducibility of RAPD; they produce more reliable and reproducible bands because of the higher annealing temperature and longer sequence of ISSR primers. ISSRs are ideal as markers for genetic mapping and population studies because of their abundance, and the high degree of polymorphism between individuals within a population of closely related genotypes (Singh et al., 2011). Those properties indicate their potential role as good supplements for RAPD-based genome analysis.

So keeping in the view the medicinal value of the plant in trade and commerce, the present study was designed to access the genetic diversity among 24 diverse Isabgol genotypes using RAPD and ISSR markers to identify potentially useful germplasm for crop improvement.

MATERIALS AND METHODS

Plant material and Experimental site

Twenty-four (24) Isabgol genotypes were collected from six different geographical regions of four major Isabgol growing states of India for molecular studies (Table 1). Plants were grown in glasshouse conditions, after 20 days of growth leaves, were cut and frozen in liquid nitrogen for DNA extraction. The present study was conducted at Molecular Biology Laboratory at Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur (India).

DNA Extraction

Leaves were ground in liquid nitrogen to a fine powder with a chilled mortar and pestle. Genomic DNA was extracted using Doyle and Doyle (1990) cetyl trimethyl ammonium bromide (CTAB) method. The quantity and quality of DNA were determined by spectrophotometer and electrophoresis on 0.8 % agarose gel, respectively. DNA samples were diluted to 50 ng μl^{-1} for polymerase chain reaction (PCR) amplification.

RAPD and ISSR-PCR Analysis

RAPD-PCR amplification was performed with 40 random decamer primers obtained from Bangalore Genei Pvt. Ltd., India. Twenty two (22) primers with reproducible and scorable amplifications were chosen for further studies (Table 2). In ISSR-PCR analysis, only 16 primers were selected for further use from 20 ISSR primers obtained from Sigma chemicals Co., USA. PCR amplifications for RAPD and ISSR were performed in 20 µl volume containing 2 µl dNTP (250 µM each dNTP), 1µl primer (30 ng μl^{-1}), 1 µl template DNA (50 ng μl^{-1}), 2.5 µl reaction buffer [(10×) 10 mM Tris-Cl pH 9.0, 50 mM KCl], 0.3 µl Taq DNA polymerase [(5 U μl^{-1}) Bangalore Genei Pvt. Ltd., Bangalore, India], 2 µl $MgCl_2$ (25 mM), and 11.2 µl deionized water. PCR reactions were performed with DNA thermal cycler (Eppendorf AG, Germany). Amplification conditions were as follows: an initial denaturation at 94°C for 4 min followed by 1 min denaturation at 94°C for 40 and 35 cycles for RAPD and ISSR, respectively and 1 min at annealing temperature (37°C for RAPD; for ISSR, it depends upon the primer; Table 3), 2 min polymerization at 72°C and 5 min final extension at 72°C. After completion of amplifications, 2 µl of gel loading dye (MBI Ferment Inc., USA) was added to each sample and 20 µl volume was resolved on 1.5 and 2.0% (w/v) agarose gel for RAPD and ISSR, respectively in 1× Tris–borate–ethylenediaminetetraacetic acid (TBE) buffer, gels were stained with ethidium bromide. The size of amplified DNA fragments was estimated with standard molecular size markers (100 bp DNA ladder and Lambda DNA/*Eco*R I/ *Hind* III double digest; Bangalore Genei Pvt. Ltd., Bangalore, India). The gels were visualized under UV using gel documentation system (BioRad, USA).

Reproducibility of amplification patterns

DNA amplifications with each RAPD and ISSR primers were repeated at least thrice to ensure reproducibility. The bands were considered reproducible and scorable only after observing and comparing them in three separate amplifications for each primer. Clear and intense bands were scored while faint bands against background smear were not considered for further analysis.

Scoring and data analysis

DNA fingerprints were scored for the presence (1) or absence (0) of bands of various molecular weight sizes in the form of binary matrix. Data were analyzed to obtain Jaccard's coefficients among the isolates by using NTSYS-pc version 2.02e (Rohlf, 1998). The SIMQUAL program was used to calculate Jaccard's coefficients (Jaccard, 1908), which are common estimator of genetic identity and were calculated as follows:

$$\text{Jaccard's coefficient} = N_{AB}/(N_{AB} + N_A + N_B)$$

Where, N_{AB} is number of bands shared by samples; N_A is amplified fragments in sample A, N_B amplified fragments in sample B. Similarity matrices based on these indices were calculated. Correlation between the two matrices obtained with two marker types (RAPD and ISSR) was estimated by means of Mantel (1967) test. Coefficient correlation (r) provides one measure of relatedness

Table 1. Detail of Isabgol genotypes collected from different geographical regions of India for the study of genetic diversity

S/ N	*Genotype code*	Genotype	Geographical region	Latitude and longitude
1	G_1	PB-62	Hisar (Haryana)	29° 19' N, 76° 23'E
2	G_2	Gumary	Anand (Gujarat)	22° 32' N, 73° 00'E
3	G_3	GI-4	Dantiwada (Gujarat)	24° 33' N, 72° 36'E
4	G_4	Palampur-2	Dantiwada (Gujarat)	24° 33' N, 72° 36'E
5	G_5	GI-2	Anand (Gujarat)	22° 32' N, 73° 00'E
6	G_6	MIB-125	Mandsaur (Madhya Pradesh)	24° 03' N, 75° 08'E
7	G_7	PB-7	Hisar (Haryana)	29° 19' N, 76° 23'E
8	G_8	RI-89	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
9	G_9	RI-145	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
10	G_{10}	RI-150	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
11	G_{11}	RI-153	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
12	G_{12}	RI-148	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
13	G_{13}	RI-147	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
14	G_{14}	RI-155	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
15	G_{15}	RI-166	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
16	G_{16}	RI-165	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
17	G_{17}	RI-154	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
18	G_{18}	RI-139	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
19	G_{19}	RI-142	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
20	G_{20}	RI-138	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
21	G_{21}	RI-136	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
22	G_{22}	RI-137	Jodhpur (Rajasthan)	26° 18' N, 73° 04'E
23	G_{23}	RI-167	Jaislmer (Rajasthan)	26° 55' N, 70° 57'E
24	G_{24}	RI-168	Jaislmer (Rajasthan)	26° 55' N, 70° 57'E

Table 2. Performance of 22 RAPD primers in the genetic diversity analysis of Isabgol genotypes.

Primer code	Sequence (5'-3')	Size range (bp)	No. of genotypes amplified	Total no. of bands	No. of polymorphic bands	No. of monomorphic bands	Percent polymorphism
C-04	CCGCATCTAC	400-1904	24	7	2	5	28.57
OPA-01	CAGGCCCTTC	450-2300	24	10	4	6	40.00
OPA-02	TGCCGAGCTG	100-2100	24	13	4	9	30.77
OPB-04	GGACTGGAGT	200-2780	24	12	4	8	33.33
OPB-05	TGCGCCCTTC	500-2100	24	9	6	3	66.67
OPB-08	GTCCACACGG	300-2027	24	11	6	5	54.55
OPC-05	GATGACCGCC	250-1750	24	11	5	6	45.45
OPC-09	CTCACCGTCC	400-2200	24	11	3	8	27.27
OPD-02	GGACCCAACC	300-1375	24	5	2	3	40.00
P-10	TCCCGCCTAC	300-1750	24	11	7	4	63.64
Q-05	CCGCGTCTTG	680-3300	24	13	9	4	69.23
Q-07	CCCCGATGGT	300-3530	24	15	8	7	53.33
Q-11	TCTCCGCAAC	700-3300	24	11	7	4	63.64
S-30	GTGATCGCAG	200-947	24	7	5	2	71.43
S-31	CAATCGCCGT	100-1375	24	4	2	2	50.00

Table 2. Contd.

S-34	TCTGTGCTGG	350-1800	24	4	0	4	0.00
S-36	AGCCAGCGAA	300-2027	24	14	14	0	100.00
S-40	GTTGCGATCC	350-831	24	6	2	4	33.33
S-66	GAACGGACTC	300-2200	24	7	1	6	14.29
S-67	GTCCCGACGA	200-1375	24	8	2	6	25.00
S-69	CTCACCGTCC	400-1750	24	14	5	9	35.71
S-70	TGTCTGGGTG	300-1000	24	5	0	5	0.00
Total		100-3530		208	98	110	
Average		335-1987		9.45	4.45	5.0	47.12

Table 3. Performance of 16 ISSR primers and combined (RAPD+ISSR) primers in the genetic diversity analysis of Isabgol genotypes.

Primer code	Sequence (5'-3')	Annealing temperature (°C)	Size range (bp)	Number of genotypes amplified	Total number of bands	Number of polymorphic bands	Number of monomorphic bands	Percent polymorphism
Primer-1	$(AG)_8C$	45.3	670-1584	24	10	8	2	80.00
Primer-2	$(GTG)_3GC$	46.4	400-1375	24	6	0	6	0.00
Primer-3	$(GA)_8T$	44.0	400-1300	24	8	0	8	0.00
Primer-4	$(CT)_8G$	45.7	480-1840	24	5	0	5	0.00
Primer-5	$(GA)_8TG$	45.9	500-2780	24	7	1	6	14.29
Primer-6	$(AG)_8TT$	46.6	450-1690	24	12	1	11	8.33
Primer-7	$(CT)_6G$	32.9	890-1904	24	4	1	3	25.00
Primer-8	$(CT)_6A$	27.0	600-3000	24	6	4	2	66.67
Primer-9	$(GA)_8A$	47.4	550-2200	24	10	1	9	10.00
Primer-10	$(AG)_6T$	27.5	600-1584	24	5	0	5	0.00
Primer-11	$(CT)_8TG$	46.2	630-1584	24	6	0	6	0.00
Primer-12	$(CA)_6AC$	43.7	500-1375	23	9	0	9	0.00
Primer-13	$(CA)_6GT$	40.2	400-1480	24	11	1	10	9.09
Primer-14	$(CA)_6GG$	46.3	470-1904	24	12	7	5	58.33
Primer-15	$(CA)_6AG$	43.4	564-1250	24	6	0	6	0.00
Primer-16	$(GTG)_3GG$	41.6	300-1375	24	7	0	7	0.00
Total			300-3000		124	24	100	
Average			525-1764		7.75	1.5	6.25	19.35
Combined (RAPD+ISSR) primers								
Total			100-3530		332	122	210	
Average			415-1893		8.74	3.21	5.53	36.75

between the two matrices. Similarity matrices were utilized to construct the unweighted pair-group method with arithmetic average (UPGMA) dendrograms.

RESULTS

RAPD band pattern

The PCR amplification using RAPD primers gave rise to reproducible bands across 24 Isabgol genotypes studied (Table 2). The 22 primers produced 208 bands across 24 genotypes, of which 98 were polymorphic, accounting for 47.12% polymorphism. The number of bands ranged from four (S-31 and S-34) to 15 (Q-07) and varied in size from 100 to 3,530 bp. Average number of bands and polymorphic bands per primer were 9.45 and 4.45, respectively. Percentage polymorphism ranged between 0 (S-34 and S-70) and 100 (S-36), with an average of

Table 4. Unique/genotype specific bands as detected by 6 RAPD and 1 ISSR primers in 5 genotypes of Isabgol.

Primer code	Genotype	No. of unique band	Size of band (bp)
RAPD primer			
OPC-05	PB-62	1	1200
P-10	GI-4	1	350
	RI-168	1	300
OPA-02	RI-137	1	2100
	RI-168	1	100
Q-11	Gumary	1	947
	Gumary	1	700
S-67	RI-137	1	1375
OPD-02	Gumary	1	450
	Gumary	1	400
ISSR primer			
Primer-13	PB-62	1	400
Total		11	

47.12%. Ten (10) unique/genotype specific bands (band which is present in a particular genotype and absent in rest of the genotypes) were detected in five genotypes with six RAPD primers (Table 4). The genotype Gumary gave maximum number (four) of unique bands. Figure 1 is the representative of the extent of polymorphism observed among the Isabgol genotypes as revealed by RAPD primer OPA-02. Jaccard's similarity coefficient ranged from 0.77 to 0.97 (Table 5). Phylogenetic tree based on similarity coefficient showed that genotypes RI-168, RI-153, RI-167 and Gumary formed separate operational taxonomic units (OUT's). Remaining genotypes were mainly grouped into four clusters with 88% similarity (Figure 2). Cluster I comprised genotypes PB-62 and RI-148 while cluster II consisted of genotypes RI-147, RI-89 and RI-155. Cluster III consisted of 13 genotypes *viz.* GI-4, Palampur-2, GI-2, PB-7, RI-145, RI-154, RI-139, RI-142, RI-138, RI-136, RI-137, RI-165 and RI-150. Only two genotypes (RI-166 and MIB-125) formed cluster IV.

ISSR band pattern

ISSR primers produced different numbers of DNA fragments, depending upon their simple sequence repeat motifs (Table 3). All the selected 16 primers amplified DNA fragments across the 24 genotypes studied, whereas primer-12 did not show any amplification in genotype RI-137. A total of 124 bands were amplified, of which 24 were polymorphic, with an average of 1.5 polymorphic bands per primer. The number of bands varying from four (Primer-7) to 12 (Primer-14), with size ranging from 300 to 3,000 bp. Percent polymorphism ranged from 0 (Primer-2, 3, 4, 10, 11, 12, 15 and 16) to 80 (Primer-1), with an average percentage polymorphism of 19.35. One unique band was detected in genotype PB-62 with primer-13. Figure 3 is the representative of the extent of polymorphism observed among the Isabgol genotypes as revealed by ISSR primer-14. A dendrogram based on UPGMA analysis with ISSR data is shown in Figure 4. Jaccard's similarity coefficient ranged from 0.81 to 1.00 (Table 6). Phylogenetic tree showed that genotypes PB-62, RI-168, RI-148 and RI-137 formed separate OUT's. Remaining genotypes formed single cluster with maximum 98% genetic similarity (similarity coefficient 0.98).

Combined (RAPD and ISSR) Data

Combining data from both markers, a total of 332 DNA bands were produced, of which 122 (36.75 %) were polymorphic with an average of 3.21 polymorphic bands per primer. By combining (RAPD + ISSR) data, the Jaccard's similarity coefficient ranged from 0.84 to 0.98 (Table 7). Phylogenetic tree based on similarity coefficient showed that genotypes PB-62, Gumary, RI-137, RI-167, RI-153, RI-148 and RI-168 formed separate OUT's (Figure 5). Remaining genotypes were mainly grouped into two clusters with 87% similarity. Cluster I comprised genotypes GI-4, Palampur-2, GI-2, PB-7, RI-145, RI-150, RI-165, RI-154, RI-139, RI-138, RI-136, RI-142 and MIB-125, while cluster II consisted of genotypes RI-147, RI-89, RI-155 and RI-166. In combined (RAPD + ISSR) dendrogram, only some clusters showed similar grouping pattern with individual marker dendrograms. The matrices for two markers, RAPD and ISSR, were also compared by using Mantel's (1967) test. The correlation values between the matrices based on RAPD

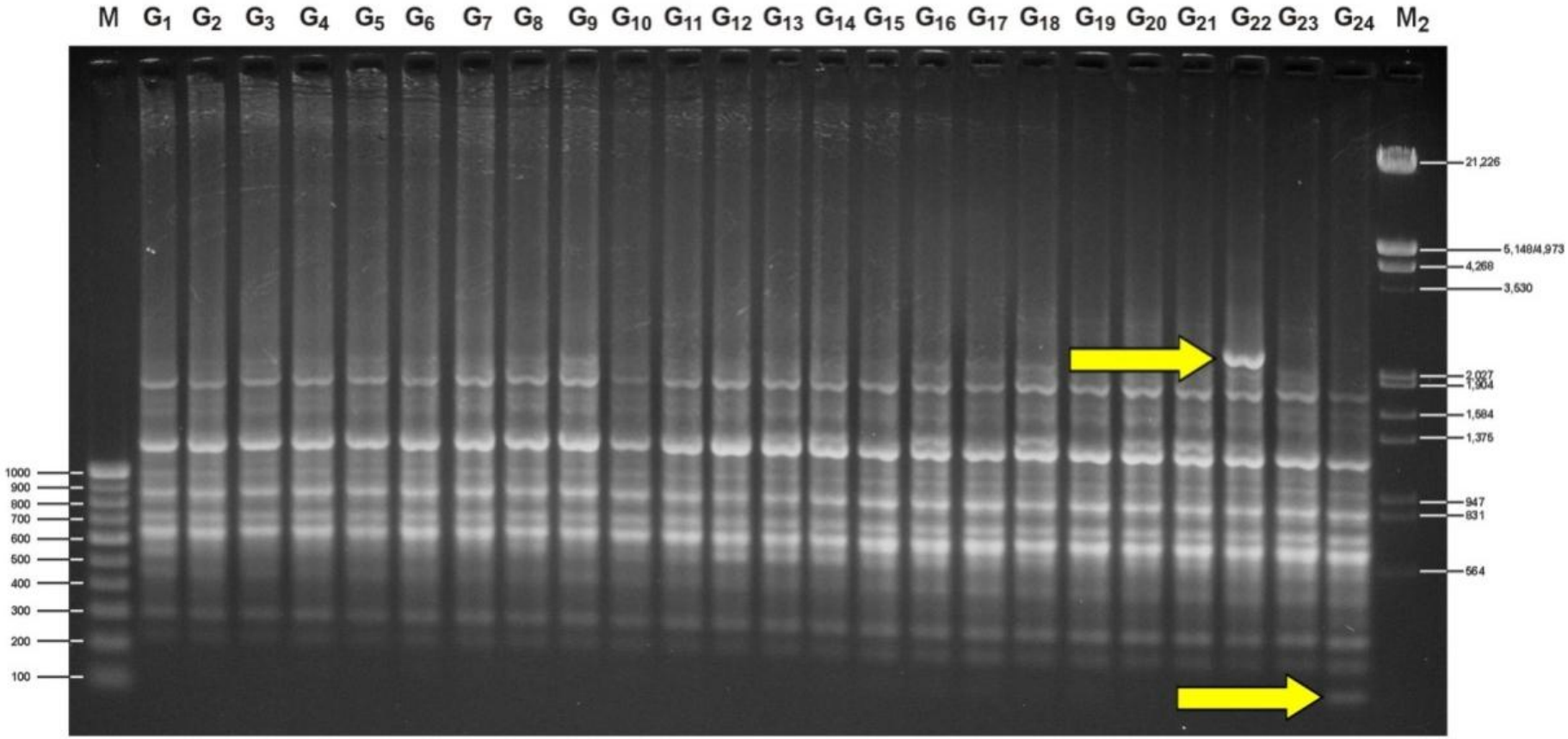

Figure 1. RAPD banding pattern generated through primer OPA-02 (M_1=100 bp DNA ladder; M_2= Lambda DNA/*Eco*R I/ *Hind* III double digest, G_1-G_{24} are code of different genotypes as listed in Table 1). Arrows indicate putative genotype specific bands.

and ISSR were very low (r=0.28), which indicates poor fit among RAPD and ISSR marker systems.

DISCUSSION

The genotypes of Isabgol are not well demarcated due to similarities in their morphology. Thus, there is an urgent need to characterize the cultivated genotypes for their genetic diversity (Rohilla et al., 2012). The present work evaluates the genetic diversity and relationships among 24 Isabgol genotypes using RAPD and ISSR markers.

The results indicate that the average percentage polymorphism of RAPD (47.12%) was higher than that of ISSR (19.35 %). Moreover, the number of polymorphic bands per primer of RAPD (4.45) was more than that of ISSR (1.5) which suggested that the RAPD markers were superior to ISSR markers in the capacity of revealing more informative bands in a single amplification. Similar

Table 5. Similarity matrix (Jaccard's coefficient) of 24 isabgol genotypes revealed by RAPD markers.

Genotype	PB-62	Gumary	GI-4	Palampur-2	GI-2	MIB-125	PB-7	RI-145	RI-150	RI-153	RI-148	RI-147	RI-89	RI-155	RI-166	RI-165	RI-154	RI-139	RI-142	RI-138	RI-136	RI-137	RI-167	RI-168
PB-62	1.00																							
Gumary	0.82	1.00																						
GI-4	0.86	0.88	1.00																					
Palampur-2	0.90	0.87	0.93	1.00																				
GI-2	0.89	0.86	0.90	0.95	1.00																			
MIB-125	0.85	0.84	0.89	0.90	0.87	1.00																		
PB-7	0.86	0.90	0.93	0.94	0.91	0.90	1.00																	
RI-145	0.86	0.88	0.91	0.94	0.92	0.90	0.96	1.00																
RI-150	0.84	0.86	0.90	0.91	0.90	0.89	0.92	0.93	1.00															
RI-153	0.81	0.80	0.81	0.82	0.83	0.81	0.82	0.82	0.84	1.00														
RI-148	0.89	0.81	0.84	0.88	0.87	0.86	0.85	0.85	0.88	0.87	1.00													
RI-147	0.85	0.83	0.82	0.87	0.86	0.83	0.85	0.87	0.84	0.85	0.88	1.00												
RI-89	0.86	0.84	0.83	0.87	0.86	0.84	0.86	0.87	0.86	0.83	0.89	0.95	1.00											
RI-155	0.85	0.82	0.84	0.88	0.87	0.84	0.86	0.87	0.86	0.81	0.88	0.94	0.95	1.00										
RI-166	0.87	0.82	0.88	0.90	0.86	0.88	0.89	0.87	0.84	0.81	0.88	0.87	0.87	0.89	1.00									
RI-165	0.83	0.88	0.91	0.89	0.88	0.87	0.94	0.91	0.90	0.81	0.84	0.83	0.85	0.85	0.88	1.00								
RI-154	0.85	0.87	0.89	0.92	0.88	0.90	0.93	0.94	0.91	0.80	0.83	0.84	0.85	0.84	0.88	0.91	1.00							
RI-139	0.83	0.84	0.88	0.90	0.87	0.86	0.93	0.95	0.91	0.80	0.83	0.84	0.86	0.85	0.85	0.92	0.93	1.00						
RI-142	0.85	0.85	0.90	0.93	0.88	0.88	0.93	0.92	0.91	0.81	0.84	0.85	0.84	0.87	0.87	0.90	0.94	0.95	1.00					
RI-138	0.86	0.86	0.92	0.93	0.89	0.88	0.94	0.92	0.91	0.81	0.83	0.85	0.87	0.88	0.88	0.93	0.94	0.95	0.95	1.00				
RI-136	0.85	0.87	0.91	0.91	0.86	0.87	0.93	0.93	0.89	0.80	0.82	0.84	0.86	0.87	0.87	0.93	0.93	0.95	0.95	0.97	1.00			
RI-137	0.84	0.85	0.91	0.91	0.86	0.86	0.92	0.91	0.91	0.80	0.82	0.85	0.86	0.88	0.86	0.90	0.91	0.92	0.93	0.95	0.93	1.00		
RI-167	0.84	0.79	0.86	0.86	0.85	0.84	0.84	0.81	0.83	0.80	0.83	0.79	0.78	0.79	0.83	0.84	0.85	0.82	0.86	0.85	0.83	0.83	1.00	
RI-168	0.82	0.80	0.84	0.82	0.82	0.78	0.82	0.80	0.78	0.79	0.78	0.77	0.77	0.77	0.81	0.84	0.81	0.80	0.81	0.81	0.82	0.79	0.82	1.00

results were observed by the number of plant species (Fernandez et al., 2002; Bhattacharya et al., 2010). Eleven genotype specific bands detected in this study will be useful in genetic analysis of Isabgol germplasm. The putative genotype specific bands can be used as probes to ascertain whether they are in low or high copy numbers in the Isabgol genome, and such specific bands may be used for genotype characterization and grouping germplasm accessions. Further, putative genotype specific bands could be converted to sequence characterized amplification regions (SCARs) after sequencing and designing primer pairs to develop robust genotype specific markers (Samantaray et al., 2010; Noormohammadi et al., 2012).

The narrow variation in genetic distance among the different Isabgol genotypes revealed by both RAPD and ISSR markers reflects narrow genetic base and self-crossing nature of the crop. Earlier study by Singh et al. (2009) using the RAPD technique also showed a very low (2-17%) genetic variation among 80 germplasm accessions of Isabgol. RAPD and ISSR analysis showed two distinct genetic grouping of genotypes. Such variation between RAPD and ISSR may be due to the fact that the PCR amplified profiles in the two marker assays originated from different repetitive and non-repetitive regions of the genomes, and the possibility that many co- migrating bands may

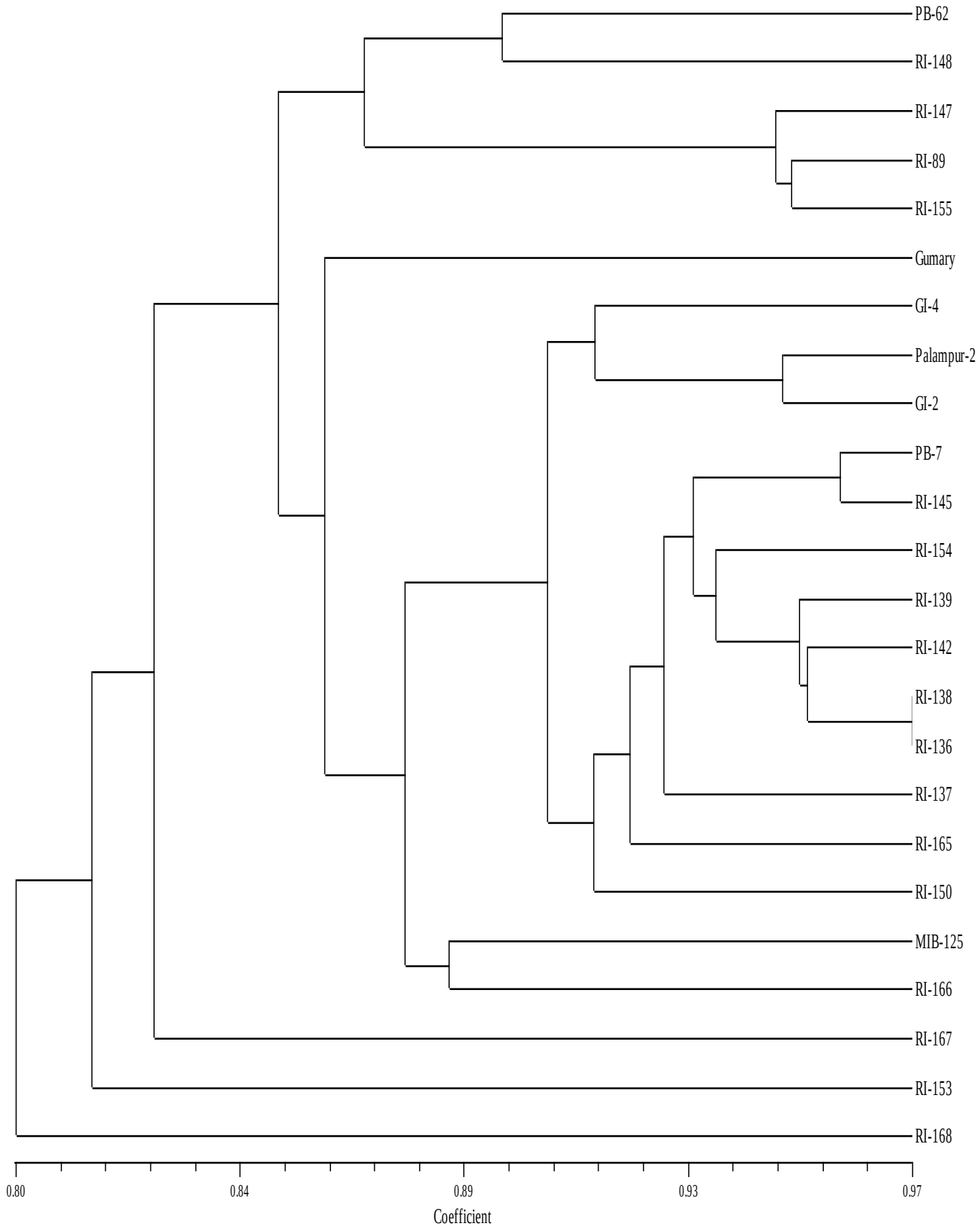

Figure 2. Dendrogram constructed with UPGMA clustering method among 24 genotypes of Isabgol using RAPD primers.

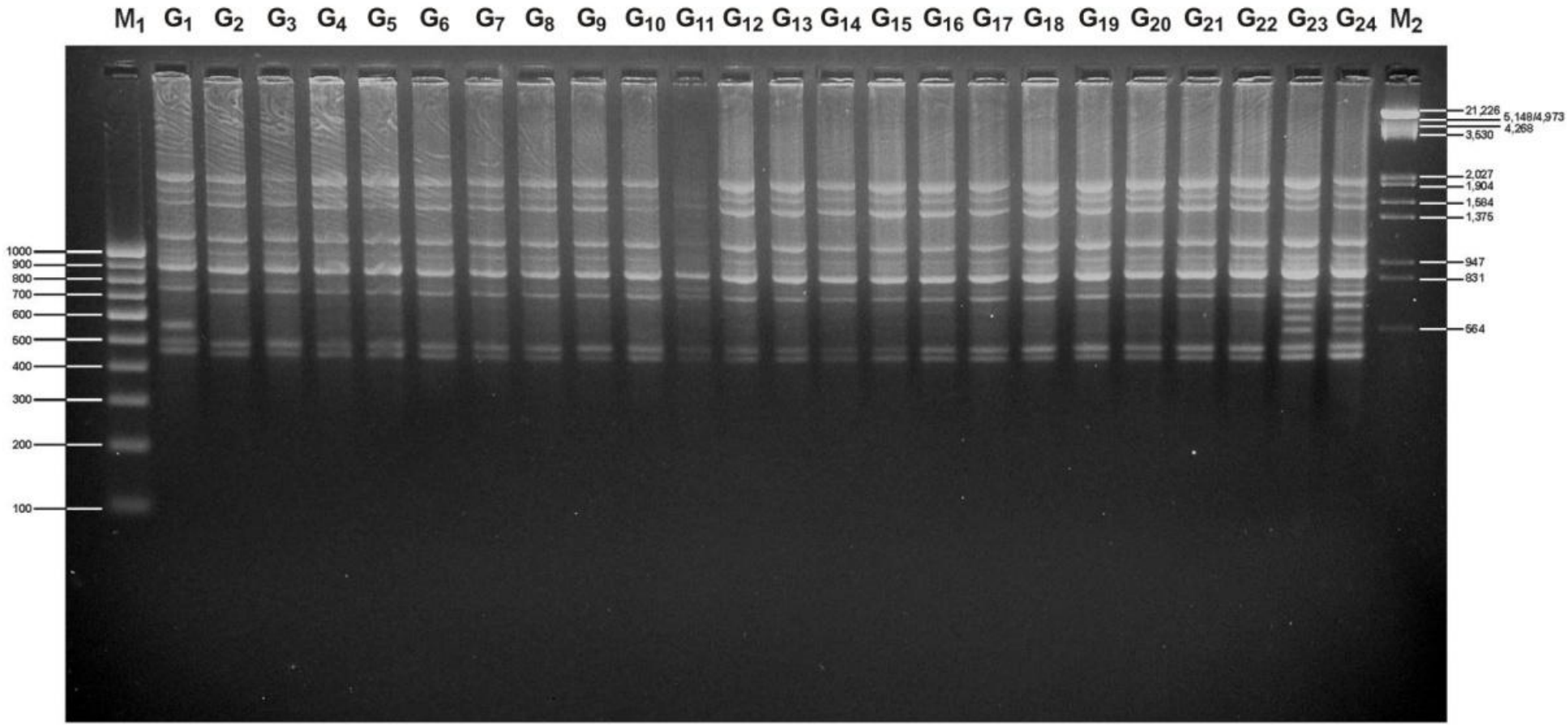

Figure 3. ISSR banding pattern generated through Primer -14 (M_1=100 bp DNA ladder ; M_2= Lambda DNA/*Eco*R I/ *Hind* III double digest, G_1-G_{24} are code of different genotypes as listed in Table 1).

be non-homologous, producing a background noise that could influence the results (Thormann et al., 1994; Khurana-Kaul et al., 2012). Critical examination of phylogenetic trees (based on combined and individual markers) revealed that there is no correlation between grouping pattern of genotypes and their geographical regions. Similar results were reported in Shishm (Arif et al., 2009). Most of the studied genotypes showed high level of genetic similarity. Genotypes RI-168, RI-167, RI-137, PB-62, RI-153, RI-148 and Gumary were spotted as genetically diverse in both sets of markers and could be efficiently utilized in crop improvement programmes. The information obtained from the present study could be of practical use for mapping the Isabgol genome as well as for classical breeding.

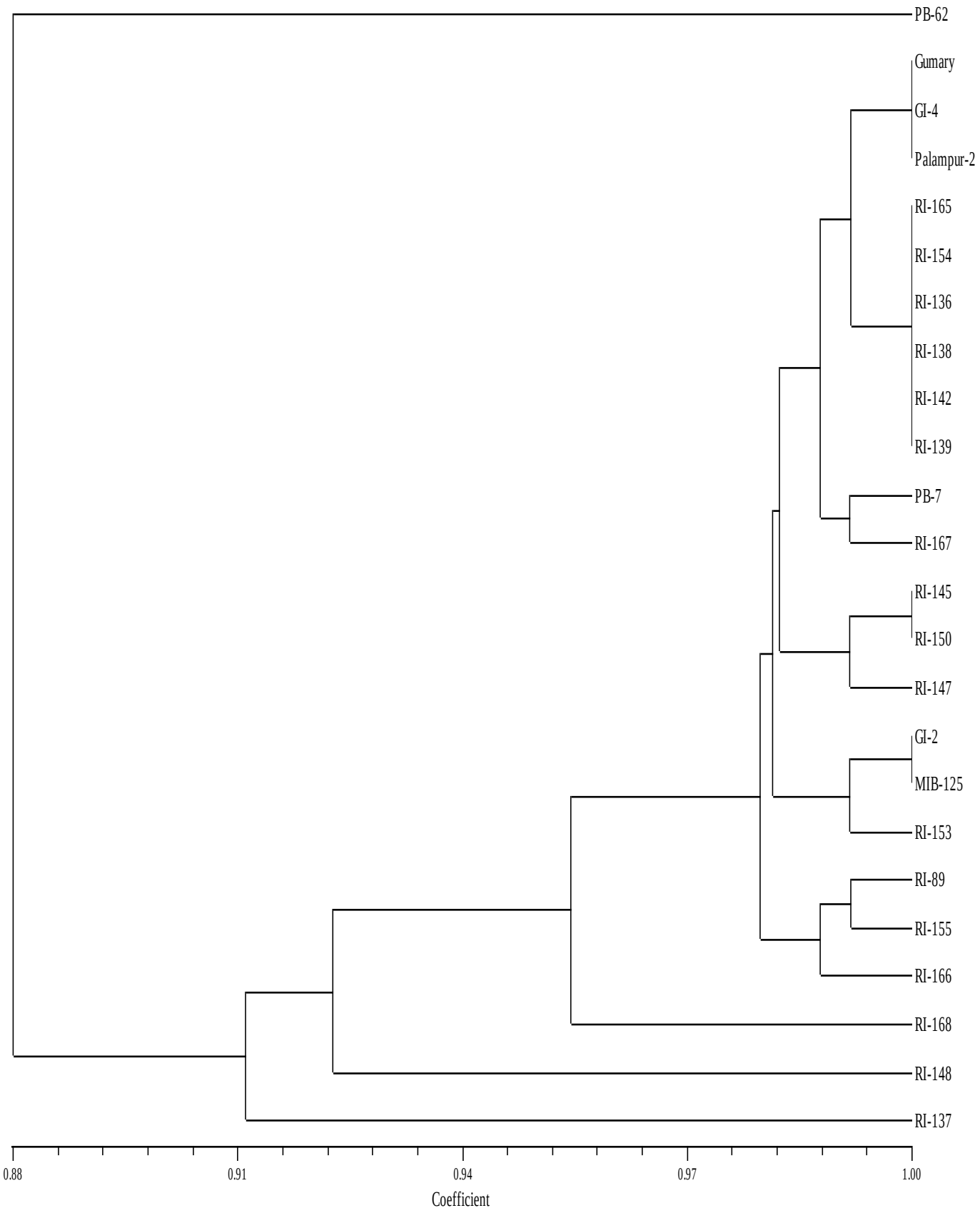

Figure 4. Dendrogram constructed with UPGMA clustering method among 24 genotypes of Isagol using ISSR primers.

Table 6. Similarity matrix (Jaccard's coefficient) of 24 isabgol genotypes revealed by ISSR markers.

Genotype	PB-62	Gumary	GI-4	Palampur-2	GI-2	MIB-125	PB-7	RI-145	RI-150	RI-153	RI-148	RI-147	RI-89	RI-155	RI-166	RI-165	RI-154	RI-139	RI-142	RI-138	RI-136	RI-137	RI-167	RI-168
PB-62	1.00																							
Gumary	0.89	1.00																						
GI-4	0.89	1.00	1.00																					
Palampur-2	0.89	1.00	1.00	1.00																				
GI-2	0.88	0.99	0.99	0.99	1.00																			
MIB-125	0.88	0.99	0.99	0.99	1.00	1.00																		
PB-7	0.88	0.99	0.99	0.99	0.98	0.98	1.00																	
RI-145	0.88	0.99	0.99	0.99	0.98	0.98	0.98	1.00																
RI-150	0.88	0.99	0.99	0.99	0.98	0.98	0.98	1.00	1.00															
RI-153	0.89	0.98	0.98	0.98	0.99	0.99	0.98	0.98	0.98	1.00														
RI-148	0.83	0.93	0.93	0.93	0.93	0.93	0.92	0.92	0.92	0.94	1.00													
RI-147	0.87	0.98	0.98	0.98	0.98	0.98	0.98	0.99	0.99	0.97	0.93	1.00												
RI-89	0.88	0.98	0.98	0.98	0.97	0.97	0.97	0.98	0.98	0.96	0.92	0.99	1.00											
RI-155	0.89	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.93	0.98	0.99	1.00										
RI-166	0.88	0.98	0.98	0.98	0.98	0.98	0.97	0.97	0.97	0.98	0.93	0.98	0.98	0.99	1.00									
RI-165	0.89	0.99	0.99	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.92	0.98	0.98	0.99	0.98	1.00								
RI-154	0.89	0.99	0.99	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.92	0.98	0.98	0.99	0.98	1.00	1.00							
RI-139	0.89	0.99	0.99	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.92	0.98	0.98	0.99	0.98	1.00	1.00	1.00						
RI-142	0.89	0.99	0.99	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.92	0.98	0.98	0.99	0.98	1.00	1.00	1.00	1.00					
RI-138	0.89	0.99	0.99	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.92	0.98	0.98	0.99	0.98	1.00	1.00	1.00	1.00	1.00				
RI-136	0.89	0.99	0.99	0.99	0.98	0.98	0.98	0.98	0.98	0.98	0.92	0.98	0.98	0.99	0.98	1.00	1.00	1.00	1.00	1.00	1.00			
RI-137	0.81	0.93	0.93	0.93	0.92	0.92	0.92	0.92	0.92	0.91	0.85	0.91	0.90	0.91	0.90	0.92	0.92	0.92	0.92	0.92	0.92	1.00		
RI-167	0.89	0.98	0.98	0.98	0.98	0.98	0.99	0.98	0.98	0.97	0.91	0.97	0.98	0.98	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.91	1.00	
RI-168	0.90	0.95	0.95	0.95	0.96	0.96	0.96	0.94	0.94	0.97	0.91	0.94	0.94	0.95	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.88	0.97	1.00

Table 7. Similarity matrix (Jaccard's coefficient) of 24 isabgol genotypes revealed by combined RAPD+ISSR markers.

Genotype	PB-62	Gumary	GI-4	Palampur-2	GI-2	MIB-125	PB-7	RI-145	RI-150	RI-153	RI-148	RI-147	RI-89	RI-155	RI-166	RI-165	RI-154	RI-139	RI-142	RI-138	RI-136	RI-137	RI-167	RI-168
PB-62	1.00																							
Gumary	0.86	1.00																						
GI-4	0.88	0.94	1.00																					
Palampur-2	0.90	0.93	0.96	1.00																				
GI-2	0.89	0.92	0.94	0.97	1.00																			
MIB-125	0.87	0.91	0.94	0.94	0.93	1.00																		

Table 7. Contd.

PB-7	0.88	0.94	0.96	0.97	0.95	0.94	1.00																		
RI-145	0.88	0.93	0.95	0.96	0.95	0.94	0.97	1.00																	
RI-150	0.86	0.92	0.94	0.95	0.94	0.94	0.95	0.96	1.00																
RI-153	0.86	0.89	0.89	0.90	0.91	0.90	0.90	0.89	0.91	1.00															
RI-148	0.88	0.87	0.89	0.91	0.91	0.90	0.89	0.89	0.91	0.91	1.00														
RI-147	0.87	0.90	0.89	0.92	0.91	0.90	0.91	0.92	0.91	0.91	0.91	1.00													
RI-89	0.87	0.90	0.90	0.92	0.91	0.90	0.91	0.92	0.92	0.89	0.91	0.97	1.00												
RI-155	0.87	0.89	0.90	0.93	0.92	0.90	0.92	0.92	0.91	0.89	0.91	0.96	0.97	1.00											
RI-166	0.89	0.89	0.93	0.94	0.92	0.93	0.93	0.92	0.90	0.89	0.91	0.92	0.92	0.94	1.00										
RI-165	0.87	0.93	0.95	0.94	0.93	0.93	0.96	0.95	0.94	0.89	0.88	0.90	0.91	0.92	0.93	1.00									
RI-154	0.88	0.92	0.94	0.95	0.93	0.94	0.96	0.96	0.95	0.89	0.87	0.90	0.91	0.91	0.93	0.95	1.00								
RI-139	0.87	0.91	0.93	0.94	0.92	0.92	0.96	0.97	0.95	0.89	0.87	0.90	0.92	0.92	0.91	0.96	0.96	1.00							
RI-142	0.88	0.92	0.94	0.96	0.93	0.93	0.95	0.95	0.95	0.89	0.88	0.91	0.91	0.92	0.92	0.95	0.97	0.97	1.00						
RI-138	0.88	0.92	0.95	0.96	0.93	0.93	0.96	0.95	0.95	0.89	0.88	0.91	0.92	0.93	0.93	0.96	0.97	0.97	0.98	1.00					
RI-136	0.87	0.92	0.95	0.95	0.92	0.92	0.96	0.95	0.94	0.88	0.87	0.90	0.92	0.92	0.92	0.96	0.96	0.98	0.97	0.98	1.00				
RI-137	0.84	0.89	0.92	0.92	0.89	0.89	0.93	0.92	0.92	0.86	0.84	0.88	0.89	0.90	0.89	0.92	0.92	0.93	0.93	0.94	0.93	1.00			
RI-167	0.87	0.88	0.92	0.92	0.91	0.90	0.91	0.89	0.90	0.88	0.87	0.87	0.87	0.87	0.90	0.91	0.92	0.90	0.92	0.91	0.90	0.87	1.00		
RI-168	0.86	0.87	0.90	0.88	0.89	0.87	0.89	0.87	0.86	0.87	0.84	0.84	0.85	0.85	0.88	0.90	0.88	0.87	0.88	0.88	0.89	0.84	0.89	1.00	

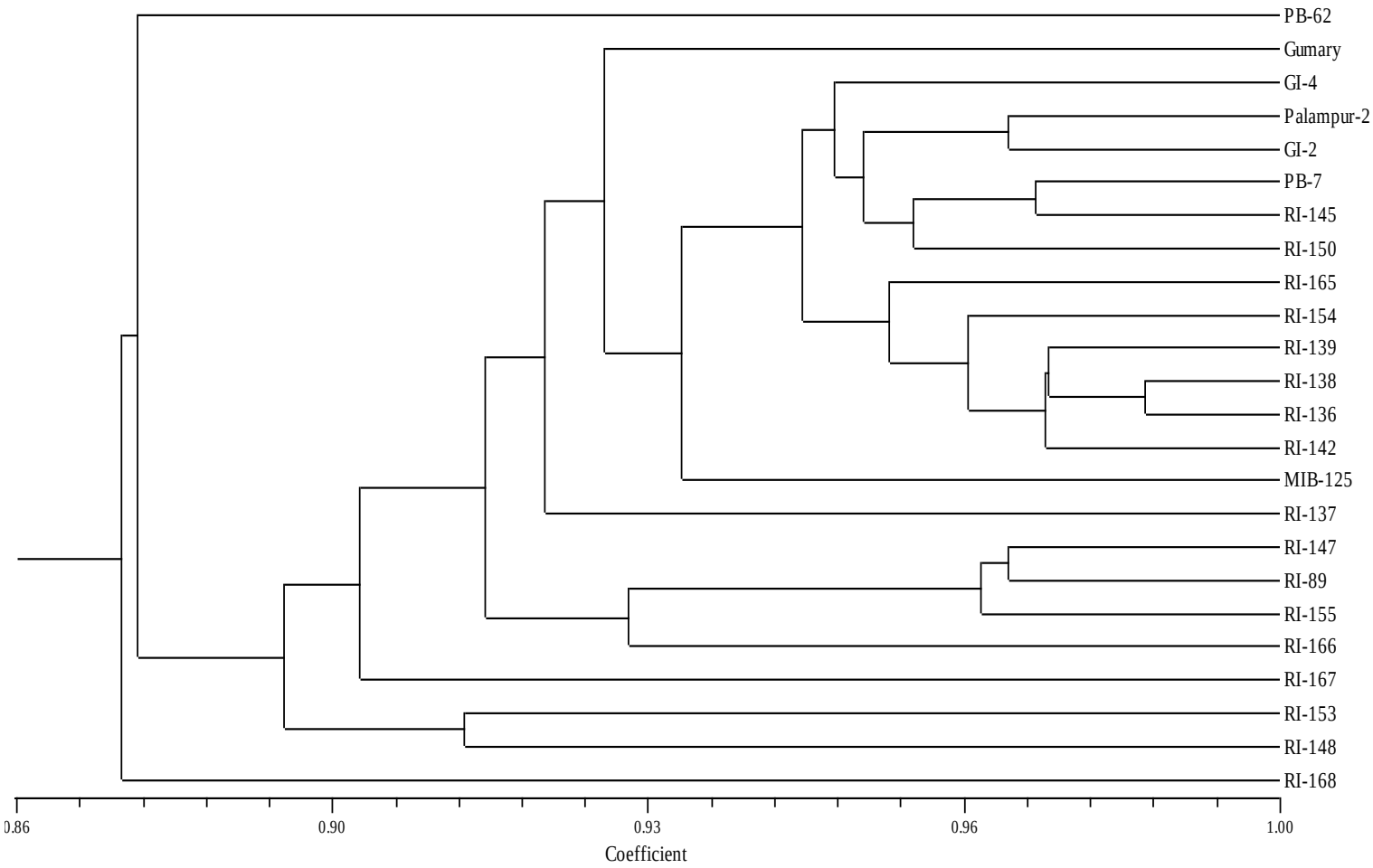

Figure 5. Dendrogram constructed with UPGMA clustering method among 24 genotypes of Isabgol using combined RAPD+ISSR primers.

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