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Comparative assessment of microsatellite and RAPD markers and their efficiency in DNA fingerprinting of upland cotton (*Gossypium hirsutum*)

VIJAY N WAGHMARE[1], S R SUTAR[2], P B THAKRE[3], D R SAPKAL[4], R V SALAME[5], A M BADOLE[6] and B R PATIL[7]

Central Institute for Cotton Research, Nagpur, Maharashtra 440 010

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ABSTRACT

A statistical approach was employed to compare the potential of microsatellite (SSR) and RAPD markers for identification, diversity analysis and genotype discrimination. Twentyfour SSR and seventeen RAPD primers were screened across 91 upland cotton (50 maintainer and 41 restorers) accessions. Major parameters, i e polymorphism information content (PIC), resolving power (Rp) and marker index (MI), were computed and compared for their ability to discriminate among the accessions. PIC, Rp and MI values displayed wide variation among SSR and RAPD markers. MI values showed excellent linear relationship with number of genotypes identified (r^2 = 0.70 and 0.69) by SSR and RAPD markers than Rp (r^2= 0.63, 0.56). SSR and RAPD markers selected based on informative PIC, high Rp and MI values independently discriminate majority of accessions. However, combined use of SSR and RAPD markers, increases the efficiency and discrimination power. Minimum of 8 markers (4 SSR and 4 RAPD) possessing high MI and Rp values were found to discriminate as many as 90 out of 91 accessions. This study reveals that selection of markers based on Rp and MI values for DNA fingerprinting and diversity studies was highly reliable and such selective markers could be effectively used for characterization of large germplasm accessions.

Key words: DNA fingerprinting, *Gossypium*, Marker index, Polymorphism information content, Resolving power, Upland cotton

Cotton (*Gossypium hirsutum* L.) is the world leading natural fibre crop. Cotton belongs to genus *Gossypium* and it comprises 45 diploid and five allotetraploid species that occur naturally through the semi-arid and arid region of Africa, Australia, Central and South Africa, the Indian sub-continent, Arabia, Galapagoes, and Hawaii (Fryxell 1979). During the course of evolution, four species whose seed fibres were long and spinnable were domesticated. India maintains second largest germplasm bank of all four cultivated cotton species with total of about 9954 accessions at Central Institute for Cotton Research, Nagpur. Conservation and management of such huge accessions is a challengeable task and requires reliable means of genotype/cultivar identification that can be applied routinely to large number of accessions.

[1]Senior Scientist (e mail: vijayvnw@yahoo.com), [2]Senior Research Fellow (e mail: satyam111222@yahoo.co.in), [3]Senior Research Fellow (e mail: prajwal_002@rediffmail.com), [5]Technical Officer (e mail: rvsalame@gmail.com), [6]Technitian (e mail: badoleam@rediffmail.com), Division of Crop Improvement

[4]PhD Student (e mail: deepaksapkal7878@rediffmail.com), [7]Senior Cotton Breeder (e mail; brpcotton@ Dr Punjabrao Deshmukh Krishi Vidyapeeth, Akola

DNA fingerprinting is an important tool for characterization of germplasm and establishment of identity of varieties/ hybrids/ parental genotypes in plant breeding and germplasm management. For protecting proprietary germplasm, molecular markers have played an important role in securing plant variety rights by virtue of their unique efficiency in distinguishing even the closely related germplasm accessions. Several PCR-based marker have been developed in last two decades. The random amplified polymorphic DNA (RAPD) has been used for genotype identification in several crops plants (Dongre and Kharbikar 2004, McGregor *et al*. 2000, Mondal *et al*. 2008, Rana and Bhat 2004). Simple sequence repeat (SSR) markers are potentially powerful for DNA fingerprinting and have widespread application in plant genome analysis (Morgante and Olivieri 1993). The availability and abundance of microsatellite (or SSR) markers throughout the cotton genome and the fact that they are polymorphic, codominant and are polymerase chain reaction (PCR)-based, make them particularly useful in genetic diversity studies in cotton (Reddy *et al*. 2001). Microsatellite markers have been used for DNA fingerprinting by several researchers (Belaj *et al*.

Table 1 *Gossypium hirsutum* cultivars/accessions used in genetic diversity studies

Species	Genome	Cultivars/ accessions				
Gossypium hirsutum	AD1 (B lines)	1. AK 32	11. AKH 45	21. AKH 71HB	31. AKH 97	41. AKH 2173
		2. AKH 07	12. AKH 45LS	22. AKH 75	32. AKH 98	42. AKH 3450
		3. AKH 08	13. AKH 48	23. AKH 75 LS	33. AKH 108	43. AKH 8660
		4. AKH 23	14. AKH 49	24. AKH 83	34. AKH 109	44. DS 28
		5. AKH 24	15. AKH 52	25. AKH 84	35. AKH 118	45. JLH 168
		6. AKH 37	16. AKH 53	26. AKH 85	36. AKH 143	46. LCMS 2
		7. AKH 38	17. AKH 58	27. AKH 87	37. AKH 431	47. LRK 516
		8. AKH 40	18. AKH 59	28. AKH 88	38. AKH 1234	48. NH 258
		9. AKH 42	19. AKH 65	29. AKH 93	39. AKH 1547	49. NS 15
		10. AKH 43	20. AKH 68	30. AKH 95	40. AKH 2160	50. PKV Rajat
	AD1 (R lines)	51. AKH 01	60. AKH 31/7	69. AKH 785	78. AKH 1130	87. AKH 3617
		52. AKH 02	61. AKH 73	70. AKH 814	79. AKH 1162	88. AKH 4943
		53. AKH 02 LS	62. AKH 77	71. AKH 859	80. AKH 1163	89. AKH 4952
		54. AKH 04	63. AKH 76	72. AKH 860	81. AKH 1167	90. AKH 5120
		55. AKH 05	64. AKH 545	73. AKH 912	82. AKH 1172	91. AKH 8830
		56. AKH 16	65. AKH 773	74. AKH 969	83. AKH 1174	
		57. AKH 27	66. AKH 780	75. AKH 970	84. AKH 1178	
		58. AKH 28	67. AKH 781	76. AKH 976	85. AKH 3614	
		59. AKH 31	68. AKH 784	77. AKH 1124	86. AKH 3614 10	

2003, McGregor *et al*. 2000, Milbourne *et al*. 1997) including cultivar discrimination studies in cotton (Bertini *et al*. 2006).

The aim of the present study was to employ statistical approach for systematic evaluation of different parameters such as polymorphism information content (PIC), resolving power (Rp) and marker index (MI) to detect their efficiency in genotype discrimination (Milbourne *et al*. 1997, Mondal *et al*. 2008, Pejic *et al*. 1998, Prevost and Wilkinson 1999) that in turn could be used for the purpose of identification of cultivars/ germplasm accessions.

MATERIALS AND METHODS

A total of ninetyone genotypes of *Gossypium hirsutum* L., 50 maintainer (B lines) and 41 restorers (R lines) were used in this study (Table 1). The B lines were elite varieties/ advanced breeding lines of upland cotton while R lines were developed and maintained at Cotton Research Station, Dr Punjabrao Deshmukh Krishi Vidyapeeth, Akola, India. B and R lines were grown in the field in one row plots (10 plants spaced at 60 cm apart) during the crop season 2008 at Dr PDKV, Akola. Genomic DNA was extracted from 2 g of young leaves collected from 2-3 random plants of each genotype as per Sharma *et al*. (2002).

Three hundred thirty microsatellite (SSR) were screened against diverse upland genotypes of which 30 primers were sampled based on scorable polymorphism across 91 cotton accessions. Twentyfour SSRs were found informative which were subsequently used for analysis (Table 2). Sixty RAPD primers were initially screened against diverse cotton genotypes of which 23 primers based on scorable polymorphism were sampled. Seventeen of the 23 RAPDs

Table 2 SSR markers indicating polymorphism, resolving power, marker index and discrimination of cotton accessions.

SSR markers	n_p	PIC	RP	MI	Number of genotypes/ clusters identified
A 1222	1	0.06	0.06	0.06	2
BNL 1053	2	0.06	0.13	0.12	3
BNL 1672	2	0.18	0.41	0.37	2
BNL 2590	2	0.08	0.7	0.17	2
BNL 3255	5	0.22	1.84	1.12	8
CIR 09	1	0.35	0.46	0.35	2
CIR 96	2	0.23	0.72	0.48	3
CIR 105	3	0.18	0.68	0.55	5
CIR 175	2	0.21	0.57	0.42	3
CIR 179	3	0.32	1.41	0.98	2
CIR 203	4	0.12	0.5	0.47	7
CIR 222	2	0.3	0.86	0.61	3
CIR 242	1	0.12	0.13	0.12	2
CIR 246	4	0.36	1.91	1.42	11
CIR 251	1	0.02	0.02	0.02	2
CIR 253	3	0.04	0.13	0.12	5
CIR 255	2	0.09	0.2	0.19	3
CIR 261	1	0.43	0.61	0.43	2
CIR 295	2	0.24	0.79	0.49	3
CIR 320	1	0.46	0.72	0.46	2
CIR 343	2	0.21	0.57	0.43	2
CIR 370	4	0.22	1.21	0.91	6
CIR 401	4	0.04	0.2	0.19	2
CIR 411	2	0.42	1.45	0.85	4

screened against 91 accessions were found informative (Table 3) which were used for diversity analysis.

Polymerase chain reaction (PCR) for SSR was carried out in 25 µl volume consisting of 20 ng template DNA, 1X assay buffer, 1 mM $MgCl_2$, 0.4 mM dNTPs, 0.2 µM primer and 0.5 unit Taq DNA polymerase. Amplification was performed by using Eppendorf Mastercycler gradient PCR programmed for initial denaturation at 94^0C for 6 min., followed by 35 cycles consisting of denaturation at 94^0C for 1 min., annealing at 50^0 - 60^0C for 1 min, extension at 72^0C for 1 min and final extension at 72^0C for 10 min. PCR reaction was set for RAPD primer consisting of same ingredients and cyclic parameters indicated for microsatellites except 0.75 unit of Taq DNA polymerase, 40 denaturation cycles at 94^0C for 45 sec. and primer annealing at 36^0C for 1 min. Amplified DNA fragments were resolved in horizontal electrophoresis system using 3.5% metaphore agarose gel for SSR and 2% agarose gel for RAPD in 1X TAE buffer. The size of amplification products were determined by comparing with 100 bp and 1 kb DNA ladder. The gels containing ethidium bromide (1µg/ml) were visualized and images were captured using Alpha Imager gel documentation system.

The microsatellite and RAPD amplified fragments were scored as present or absent of a binary character where 1 = present and 0 = absent for all the 91 accessions. The SIMQUAL programme of the numeric taxonomy multivariate analysis system (NTSYS-pc) version 2.02 (Rohlf 1990) was used to calculate the Jaccard's coefficient (Jaccard 1908). SAHN programme of NTSYS-pc software was used for cluster analysis based on similarity matrix data. Dendrogram was generated based on individual polymorphic marker data using unweighed pair group method of arithmetic average (UPGMA) to access the clustering pattern of the accessions and also discrimination power of each marker. Polymorphic markers, individually and in combination (SSR, RAPD and SSR+RAPD) were considered for genotype discrimination analysis. To estimate the congruence among similarity matrices, cophenetic matrices of each marker system were compared for matrix correspondence as per Mantel (1967).

Table 3 RAPD markers indicating polymorphism, resolving power, marker index and discrimination of cotton accessions.

Primer	n_p	PIC	RP	MI	Number of genotypes/ clusters identified
OPB01	5	0.32	2.53	1.60	12
OPB02	2	0.22	0.53	0.46	2
OPB03	7	0.14	1.34	0.99	13
OPB07	2	0.49	1.91	0.99	2
OPB08	5	0.18	1.06	0.91	9
OPB10	4	0.11	0.53	0.43	5
OPB11	5	0.12	0.66	0.59	8
OPB18	1	0.04	0.04	0.04	2
OPB20	4	0.22	1.16	0.88	7
OPC04	3	0.32	1.54	0.96	5
OPC07	3	0.13	0.42	0.38	2
OPC08	2	0.24	0.66	0.47	4
OPC09	5	0.26	1.61	1.31	9
OPC11	3	0.12	0.37	0.35	6
OPC13	5	0.05	0.24	0.24	4
OPC14	2	0.19	0.48	0.38	3
OPC16	4	0.09	0.40	0.35	6

To compare the efficiency of the markers (SSR and RAPD) for distinguishing cotton accessions, following parameters were estimated for each assay unit (U).

Number of polymorphic bands (n_p),

Number of non-polymorphic bands (n_{np}),

Average number of polymorphic bands per assay unit (n_p/U),

Number of loci (L), in case of RAPD, L= (n_p+ n_{np}),

Number of loci per assay unit ($_{nu}$) =L/U,

Average number of alleles per locus (n_{av}). For SSRs the average number of alleles per locus, $n_{av} = n_p/L$. For RAPD two alleles per assay are considered, $n_{av} = 2$,

Effective number of alleles per locus as per Morgante and Olivieri (1993), $ne = 1/\Sigma pi^2$, where p is the frequency of the i^{th} allele,

Total number of effective alleles (Ne) as defined by Pejic *et al*. (1998), $Ne = \Sigma n_e$;

Assay efficiency index (Ai) as per Pejic *et al*. (1998), $A_i = N_e/U$

Polymorphic information content (PIC) for each marker, $PIC = 1-p^2-q^2$ where p is the frequency of presence and q is frequency of absence of band (Ghislain *et al*. 1999),

Average PIC for each assay unit = $\Sigma 1-p^2-q^2$ / n, where n is number of polymorphic bands for that assay unit,

Band informativeness according to Prevost and Wilkinson (1999), $I_b = 1-(2X|0.5-p|)$, where p is the proportion of genotype under study containing the band,

Resolving power, $Rp = \Sigma I_b$ (Prevost and Wilkinson 1999),

Fraction of polymorphic loci for an individual assay, $\beta = n_p/n_p+n_{np}$ (Powell *et al*. 1996),

Diversity index of a primer, DI= Av PIC/ β (Powell *et al*. 1996),

Effective multiplex ratio (EMR), EMR= β x n_p (Milbourne *et al*. 1997),

Marker Index of the assay unit, MI= DI x EMR (Powell *et al*. 1996).

RESULTS AND DISCUSSION

Polymorphism in cotton

A total of 24 polymorphic microsatellite (SSR) markers were screened against 91 upland cotton accessions (Table 2) that produced 71 fragments of which 56 (78.9%) were polymorphic. Seventeen RAPD primers amplified 94

fragments of which 62 (66.0%) were polymorphic (Table 3). The microsatellite and RAPD primers together generated total of 165 fragments, of which 118 (71.5%) were polymorphic. The size of each amplified fragment ranged from 280 bp to 2.3 kb in case of RAPD and 100 to 700 bp for SSR primers.

The polymorphic fragments produced by RAPD markers were comparatively more (62 nos) than in SSR, however, the average polymorphism revealed by SSR was higher (78.9%) than RAPD (66%) and SSR+RAPD (71.5%) markers. In several genetic diversity studies, it has been shown that numbers of polymorphic fragments detected by SSR were relatively less in comparison to RAPD markers but level of polymorphic information revealed by SSR was generally higher. Chen and Du (2006) studied genetic diversity among 43 upland cotton genotypes using 36 SSR primer pairs and reported polymorphism to the extent of 80%. The results of the present investigation are in agreement with previous studies in different crop plants where SSRs were compared to different type of markers (Powell *et al*. 1996, Russell *et al*. 1997; Pejic *et al*. 1998). The hyper variability observed at SSR loci was expected because of the unique mechanism by which this variation is generated; replication slippage is thought to occur more frequently than single nucleotide mutations (insertions/ deletion) events (Powell *et al*. 1996, Milbourne *et al*. 1997). The codominant nature of these markers permit the detection of a high number of alleles per locus and contributes to higher information than would be possible with RAPDs and AFLPs; although extent of diversity also depends on the type of material and evolutionary history of the crop species under study.

Genetic relationship analysis

The similarity matrices for RAPD, SSR and RAPD+SSR markers were compared using Mantel's test for matrix correspondence. The cophenetic matrices of RAPD and SSR marker data showed positive correlation (r = 0.81) while the combined RAPD+SSR matrix showed significant positive correlation with both RAPD (r = 0.93) and SSR (r = 0.92). The mean similarity coefficient among 91 accessions was 0.85 using combined (SSR+RAPD) data that display moderate to high level of similarity among the accessions under study.

The dendrogram was generated separately for an individual assay unit of SSR and RAPD primers and also based on 24 SSR, 17 RAPD and combined 41 (SSR+RAPD) primers. The number of genotypes discriminated by an individual assay of both SSR and RAPD are illustrated (Tables 2, 3). The dendrogram based on 24 SSR marker data discriminated 89 of the 91 accessions; two pair of accessions namely, AKH 65, AKH 84 and AKH 1163, AKH 1162 remained non-distinguishable. Seventeen RAPD and combined 41 (SSR+RAPD) markers independently distinguished 90 accessions clearly into distinct group/ subgroups while only one pair of accessions JLH 168, AKH 2160 and AKH 2160, AKH-24 remained non-distinguishable by 17 RAPD and 41 combined markers, respectively.

Informativeness of markers

Of the 24 SSR used for DNA fingerprinting, 11 primers (i.e. 45.8%) amplified more than one locus per primer. A total 40 loci were amplified by 24 SSR primers and number of alleles ranged from 1 to 4 with an average of 1.4 alleles per locus. Seventeen RAPD's amplified total 94 loci (each amplicon is considered as one locus) with an average of two alleles per locus. An amplification of two or more loci by SSR has also been reported by Liu *et al.* (2000) and Bertini *et al*. (2006). Buteler *et al*. (1999) claimed that the multilocus amplification of the SSR is common in species with allopolyploid origin due to genome fusion and chromosome duplication event during evolution.

The total number of effective alleles for SSR and RAPD were 56.4 and 120, respectively; indicating low to moderate level of allelic variation. The high value of assay efficiency index by RAPDs than SSRs has been observed on account of high range of multiple polymorphic fragments (3.64) per single reaction as compared to SSR (2.33). Although number of polymorphic loci obtained with SSR markers were less, they exhibited higher polymorphism (78.9 %) than RAPD markers (66%). Assay efficiency index (Ai) is related to the number of effective alleles identified per assay. High Ai reflects the ability of RAPDs to produce large number of amplified fragments than the actual polymorphism. The high assay efficiency index by RAPDs than SSRs were earlier reported in maize (Pejic *et al*. 1998) and olive (Belaj *et al*. 2003).

PIC values ranged from 0.02 to 0.46 for microsatellite primers while 0.04 to 0.49 for RAPDs. The PIC, that estimate the informativeness of the markers, showed relatively narrow range and significantly less average PIC than the value reported by Liu *et al*. (2000) and Bertini *et al*. (2006) in upland cotton. Liu *et al*. (2000) demonstrated range of PIC values from 0.05 to 0.82 with an average value of 0.31. Similarly, Bertini *et al*. (2006) estimated the PIC values ranged from 0.18 to 0.62 with an average of 0.40. Significantly lower PIC values found in the present investigation might be due to use of plant material developed from closely related breeding lines/ cultivars with a narrow genetic base. In contrast, Liu *et al*. (2000) used wild *hirsutum* accessions that might explain greater diversity and polymorphism (up to 5 alleles/locus).

In genetic diversity and fingerprinting studies, the distinction of genotypes is based on the presence or absence of a band at a particular position. In ideal situation, a marker is considered to be the most informative if each band at certain position is present in half of the genotypes and absent from the other half. The estimates of resolving power is based on distribution of alleles among the sampled genotypes. Rp values ranged from 0.06 to 1.91 and 0.04 to 2.52 for SSR

and RAPDs, respectively; significantly higher with RAPDs (mean Rp: 0.91) than SSR (mean Rp: 0.68) markers. Similar results have also been reported in tetraploid potato using ISSR marker (Prevost and Wilkinson 1999) and in groundnut using ISSR and RAPD markers (Mondal *et al*. 2008).

The estimates of marker index (MI) largely depend on number of polymorphic fragments obtained in each assay than allelic heterozygosity among the cultivars. MI values ranged from 0.02 to 1.42 and 0.04 to 1.59 for SSRs and RAPDs, respectively. RAPDs produced more polymorphic fragments translating in higher effective multiplex ratio and also high MI (mean MI: 0.67) than SSRs (mean MI: 0.47). Belaj *et al*. (2003) also reported high MI value with AFLP and RAPD than with SSR markers in olive. On the contrary, Powell *et al*. (1996) reported higher MI with SSR than with RAPD markers in soybean. The extent of diversity (DI) obtained using SSR (0.36) was slightly higher than RAPD markers (0.34). All indices except mean PIC and mean DI were higher for RAPDs than for SSR markers (Table 4).

Discrimination power based on PIC, Rp and MI

Polymorphism information content (PIC), resolving power (Rp) and marker index (MI) were computed and compared to determine the ability of individual SSR and RAPD primers to discriminate among the accessions (Tables 2, 3). Only polymorphic amplicons of microsatellite and RAPD primers were considered to detect efficiency of markers. No significant linear relationship was observed between PIC and the number of genotypes identified by SSR and RAPD markers (r = 0.10, 0.01). It has been observed that SSR markers with high PIC values, such as CIR 320 (0.46), CIR 261 (0.43) and RAPD marker OPB07 (0.50) could discriminate 91 genotypes only into two groups while the markers with less PIC values, such as CIR 246 (0.36), BNL 3255 (0.22), OPB03 (0.14), OPB08 (0.18), OPB11 (0.12) etc., were able to discriminate the accessions/ genotypes into several groups. The PIC values were found to have negative correlation with number of polymorphic fragments (r = –0.12 for SSR and r = –0.17 for RAPD markers) and also very negligible or no correlation with genotype identification/ discrimination. Selection of markers based on informative PIC values containing more polymorphic fragments than higher PIC values helps to discriminate more number of genotypes.

Table 4 Comparison of marker information indices between SSR and RAPD markers.

Index with their abbreviation	Symbols	Markers	
		RAPD	SSR
No of assay unit	U	17	24
No of polymorphic bands	n_p	62	56
No of nonpolymorphic bands	n_{np}	32	15
Average no of polymorphic bands/ assay unit	n_p/U	3.64	2.33
No of loci	L	94	40
No of loci/assay unit	n_u	5.52	1.66
Average no of alleles per locus	n_{av}	2	1.4
Effective no of alleles per locus	n_e	1.93	1.65
Total no of effective alleles	N_e	120	56.4
Assay efficiency index	A_i	7.05	2.35
Average Polymorphism information content per assay unit	PIC	0.19	0.2
Average Resolving power per assay unit	Rp	0.91	0.68
Average Effective multiplex ratio per assay unit	EMR	2.75	2
Average Diversity index per assay unit	DI	0.34	0.36
Average Marker index per assay unit	MI	0.67	0.47
Average number of genotypes identified		5.82	3.58

Marker index (MI) showed excellent linear relationship with number of genotypes identified (r = 0.70 and 0.69) by SSR and RAPD primers (Figs 1a, 1b), respectively than with Rp (r = 0.63, 0.56)**.** A linear relationship between MI and number of genotypes discriminated was given by the equation $MI_{(SSR)} = 0.15 + 0.07\times$ and $MI_{(RAPD)} = 0.12 + 0.03\times$ for SSR and RAPD, respectively while the relationship between Rp and number of genotypes discriminated was correspondingly described by the equation $Rp_{(SSR)} = 0.23 + 0.15 \times$ and $Rp_{(RAPD)} = 0.19 + 0.23 \times$ (Fig. 1c, 1d). Milbourne *et al*. (1997) used MI as a basis for comparing different marker and found that it was very useful to evaluate utility of RAPD, SSR and AFLP in analysis of genetic relationship in cultivated potato.

Most informative markers were sampled based on estimates of Rp and MI. Employing Rp and MI parameters independently, same set of SSR and RAPD markers were identified. Five selected microsatellite markers (BNL 3255, CIR 179, CIR 246, CIR 370 and CIR 411) based on informative PIC, high Rp and MI values produced 18 amplicons and discriminated 75 accessions while five RAPD markers (OPB 01, OPC 09, OPB 07, OPB 03, and OPC 04) produced 22 amplicons and discriminated 83 accessions. The combined use of five SSR+RAPD markers (BNL 3255, CIR 246 OPB 01, OPC 09 and OPB 03) produced 26 amplicons and discriminated as many as 85 of 91 accessions. However, all 24 SSR or 17 RAPD or 41 (SSR+ RAPD) markers polymorphic data could discriminate 89, 90 and 90 accessions, respectively. Attempts were made to find out minimum number of markers required to discriminate all 91 accessions. A total of eight markers (4 SSR: BNL 3255, CIR 179, CIR 246, CIR 370 and 4 RAPDs: OPB 01, OPC 09, OPB 03, OPC 04) selected strictly based on high MI and Rp values discriminated maximum of 90 accessions (Fig 2). Dendrogram generated using eight markers data displayed a total of five main clusters (A-E), seven sub-clusters (A1-A3 and B1-B4) and two accessions as outgroup members with

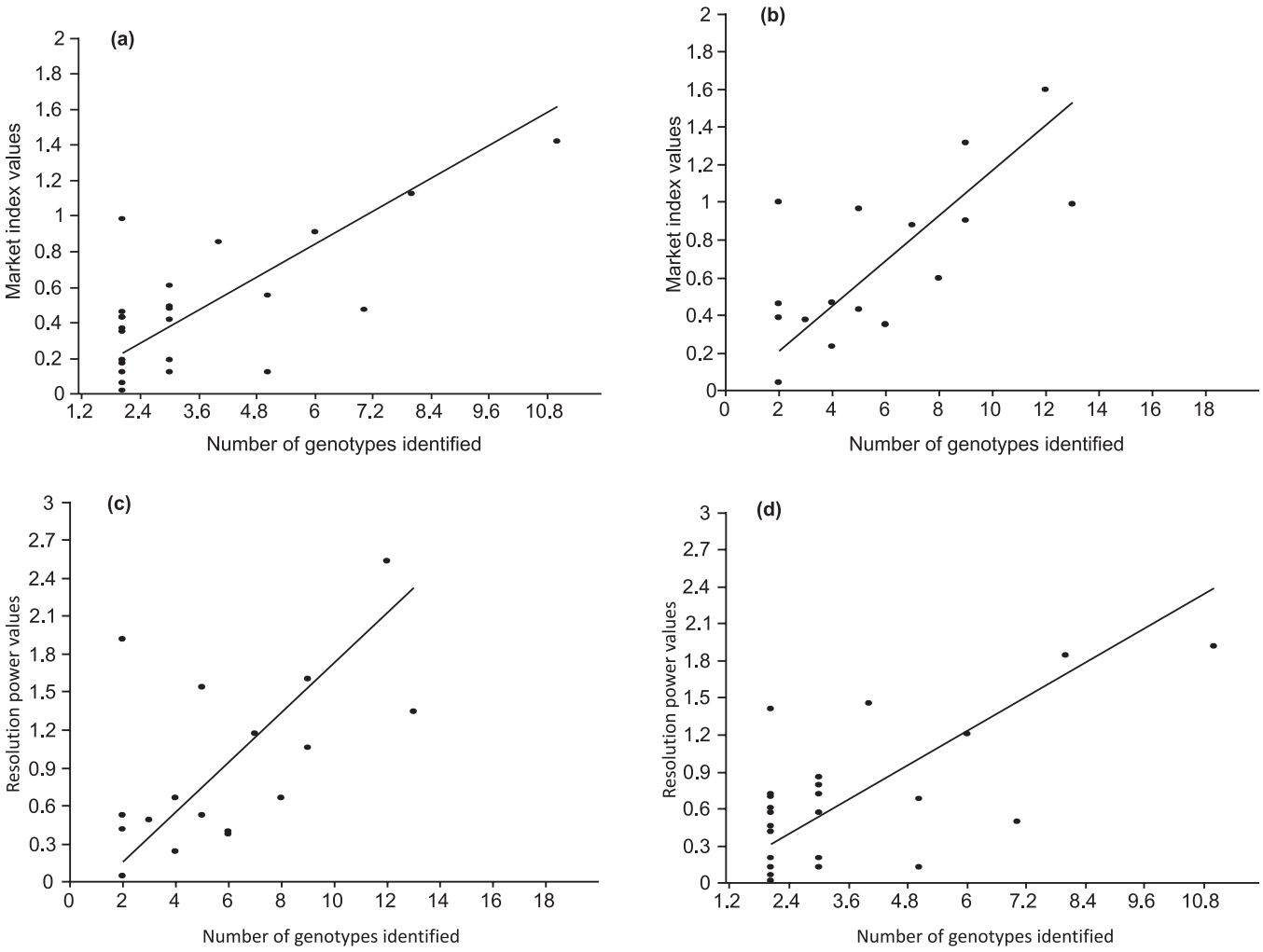

Fig 1 Regression analysis indicating distribution of accessions
(*a*) Relationship between MI and number of genotypes distinguished using 24 microsatellite primers (r = 0.70),(*b*) Relationship between MI and number of genotypes distinguished using 17 RAPD primers (r = 0.69), (*c*) Relationship between Rp and number of genotypes distinguished using 24 microsatellite primers (r = 0.63), (*d*) Relationship between Rp and number of genotypes distinguished using 17 RAPD primers (r = 0.56).

similarity coefficients ranged between 0.45 and 0.96. Interestingly, use of 17 RAPD or 41 (SSR+ RAPD) markers could also distinguish 90 accessions in the present study.

Marker index and resolving power were found to be strongly correlated with genotype identification in this study. Based on correlation estimates, efficiency of parameters in identification of markers that might discriminate higher possible number of genotypes/ accessions, may be arranged in the order, MI > Rp. The correlation between MI and Rp was found to be similar and strong in SSR (r = 0.967) and RAPD (r = 0.966) markers, that might supplement each other in marker identification. Relatively high MI and Rp values used for sampling the markers (SSR and RAPD) have been shown to be directly related with the discrimination capacity when handling large number of accessions. From the germplasm bank management perspectives, it is very important that numerous cultivars/ accessions need to be accurately characterized and identified with the use of minimum number of markers. In this study, we could identify minimum of 8 markers (4 SSRs and 4 RAPDs) based on high MI and Rp values that distinguished maximum 90 of 91 accessions. Discrimination power of different markers based on valued parameters such as MI and Rp may further be confirmed with more studies in cotton. Based on the reliable and convincing results obtained from this study, we suggest use of MI and Rp for selection of markers to be applied for varietal identification and germplasm characterization studies. In general, it is accepted that the utility of given marker is a balance between the level of polymorphism it can detect and its capacity to identify multiple polymorphisms (Powell *et al*. 1996).

Estimates of different parameters based on polymorphic

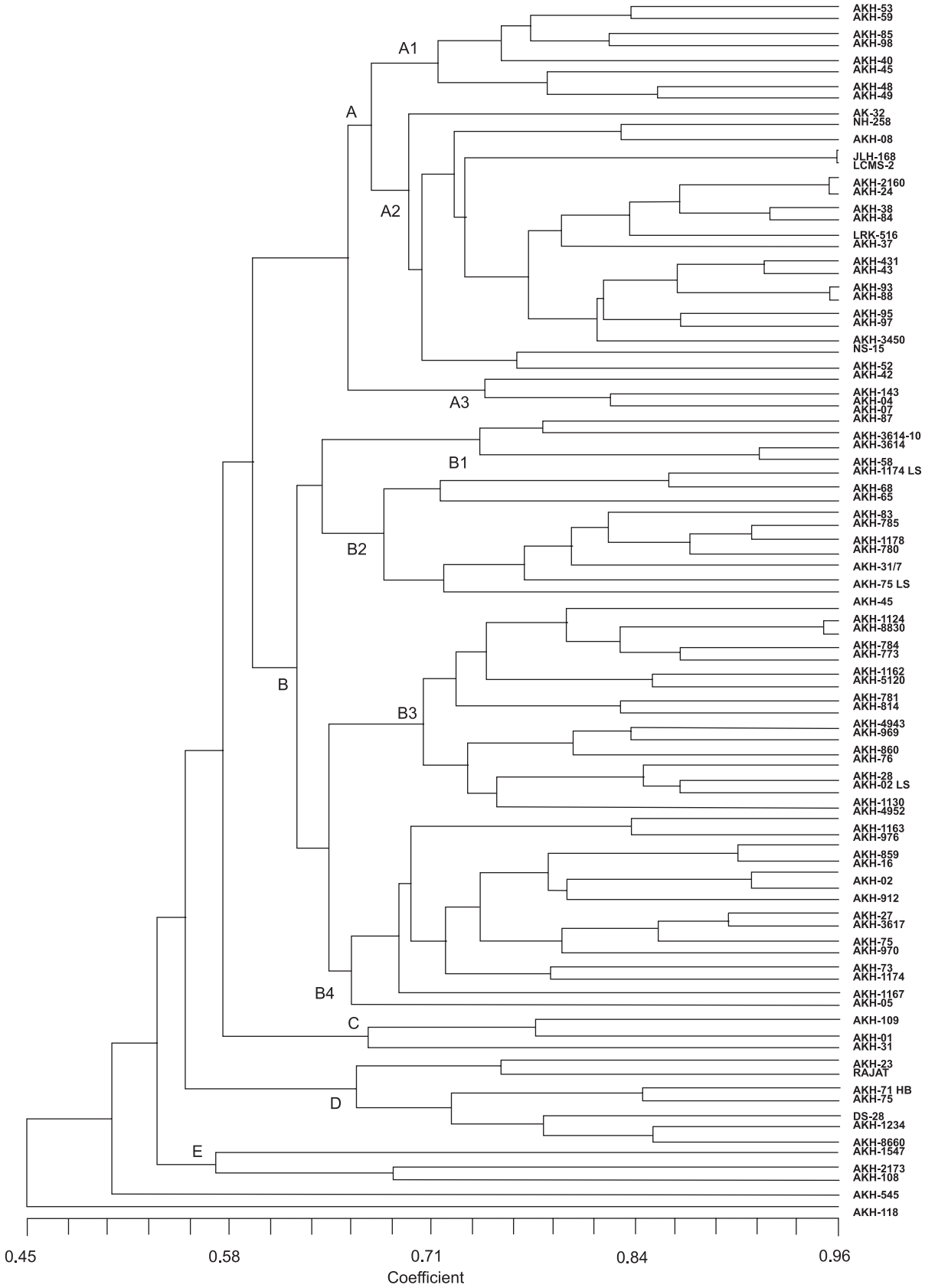

Fig 2 Dendrogram of upland cotton accessions (maintainer and restorer) based on 8 (SSR+RAPD) markers using UPGMA cluster analysis

data revealed that RAPDs provide better estimates, except average diversity index and average polymorphism information content per assay unit than SSR markers. We employed prescreened, informative SSR and RAPD markers for studying genetic diversity of maintainer and restorer accessions and were successful in identification/ distinguishing closely related accessions.

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