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Research Article

Assessment of Genetic Diversity using Morphological and Molecular Markers in Some Cotton Genotypes

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Abstract
Evidence exists that cotton genetic diversity is declining in most breeding programs. Diversity is required for selection and recombination. Assessment of genetic similarity and diversity is prerequisite for objective based population improvement. Twenty one cultivated allotetraploids (15 *G. hirsutium* and 6 *G. babadense*) obtained from various sources, were assessed for genetic diversity using both morphological and molecular markers. Genotypes were significantly different only for number of bolls per plant, seed index and seed cotton yield per plant among the characters studied. At similarity percentage of 60.78, only two major groups with random cluster distribution were identified. The group I contained a unit member and the group II had 20 members. Multiple correspondence analyses explained 82.07% variability in dimension 1 and 80.57% in dimension 2. The expression for majority of the traits studied in all the genotypes has about 80% similarities. PIC varied from 0.00 to 0.80 with an average of 0.57. Genetic similarity co-efficients for each pair of the 21 *Gossypium* genotypes ranged from 0.13 to 1.00. Narrow genetic diversity was also revealed in the dendrogram constructed from the molecular data. Irrespective of sources and species, two major random clusters were identified at similarity coefficient of 0.49. The findings from this study will be helpful in future cotton breeding program.

Keywords: Cotton, Molecular, Diversity, SSR, Markers, Cluster

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Introduction

Cotton provides numerous useful products, millions of jobs and has been a major economic component and driver of economic growth in several countries over the past few decades as it moves from field to fabric (FAO, 2018). Global textile industries depend largely on natural fibre of which cotton otherwise known as 'white Gold" is the crop of interest. Magadum *et al*., (2012) reported that cotton is commercialized in about 111 countries, on a global area of 30.29 million hectares accounting for 105.06 million bales of production with average productivity of 755 kg/ha. They also assayed that the demand for cotton and cotton products is ever on the increase and the world average productivity is never going to meet the demand at the current rate. Deshpande *et al*., (2008) predicted the world cotton demand of 60 million bales by 2025. The task before scientist is enormous, for cotton to effectively compete with synthetic fibre, superior traits like fibre length, fibre strength and yield per hectare has to be improved in all available breeding populations. Cotton hybrids are the main precursor to achieve higher yield and superior quality (Thiyagu *et al.,* 2010). Dhamayanthi (2011) reported the existence and expression of hybrid vigour in both inter-specific and intra-specific hybridization of diploid and tetraploid cotton. Baloch *et al.,* (2015), Shake el*et al*.

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(2014), Rajamani *et al*., (2009), Pathak and Parkash Kumar (2011) and Isong *et al*. (2017) all corroborated the existence of several levels of heterobeltiosis and standard heterosis in cotton. However, Baloch *et al*., (2015) reiterated that production of hybrids is largely dependent on the genetic diversity of the parental lines. Genetic diversity and the knowledge on relationship between genotypes are of great importance to cotton breeding, since it provides information on the allelic variation that can be used to create new favorable gene combinations. Hence, precise estimation of the genetic divergence in the available germplasm will provide a guide for choosing parents, predicting the degree of inheritance, variation and level of heterosis. Generally, diversity is measured by genetic distance or similarity, both of which indicate that there are either genetic differences or similarities, respectively (Weir, 1990). Conventionally, this has been done with morphological markers and recently the efficiency of genetic diversity analysis is accelerated by use of molecular markers such as SSRs (Tyagi *et al.*, 2015; Dahab, 2013; Kusuma, 2018). Genetic variation is a prerequisite for any crop improvement programme. Assessment of the extent and distribution of genetic variation in crop species and their relatives is essential in understanding pattern of diversity and evolutionary relationships among accessions that help to sample genetic resources in a more systematic fashion for conservation and plant improvement (Wu *et al*., 2007). Genetic similarity or dissimilarity (genetic distance) estimates among genotypes are helpful in selecting parental combinations for creating diverse segregating populations and classification of germplasm into heterotic groups in hybrid breeding programme (Ali *et al*., 2008). Traditionally, genetic diversity is assess based on morphological features such as plant height, reproductive features, day length sensitivity, local adaptation and biochemical markers such as isozymes. With these characters, only a part of the total genetic variation may be revealed and this may not provide an accurate indication of the genetic divergence among cultivars/species due to environmental influence and stage or tissue specific gene expression (Winter and Kahl 1995).

The identification of polymorphic markers for varietal and genotype discrimination is essential, in view of the narrow cotton genetic base and insufficiency of morphological descriptors (Zhu *et al.*, 2014). In recent years, the limitations of morphological and biochemical markers has been overcome by molecular markers to analyze genetic relationship and genetic diversity (Winter and Kahl, 1995). High resolution of molecular markers makes them a valuable tool for varietal and parental identification for protection of breeder's rights. Generation of high polymorphism which is independent of the effects related to environmental conditions and physiological stage of plant makes molecular markers a reliable tool for diversity studies. Molecular marker analysis discloses genetic differences at the DNA level in plants. Considering the different types of molecular marker system to study the extent of diversity in cultivated cotton, Simple Sequence Repeats (SSRs) marker is preferred to predict the genetic variation within cultivated diploid and tetraploid cotton (Boopathi *et al*., 2008). SSRs have been employed by several authors to study the extent of genetic diversity among cotton germplasm (Bertini *et al*., 2006; Guo *et al*., 2007 Lacape *et al*., 2007; Wu *et al*., 2007; Boopathi*et al*., 2008). In this study, combination of both morphological and molecular markers was employed to capture the best features of both strategies in genetic diversity analysis. The objective of the study was to assess the genetic diversity among fifteen *G. hirsutum* and six *G. barbadense* genotypes, using morphological and Simple Sequence Repeat (SSR) markers.

Materials and Methods

Research field and molecular laboratory of the Department of Cotton, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, India was where the experiments were performed during the summer season of 2016 and 2017. The genetic materials for the experiments were 21 cotton genotypes (15 *G. hirsutium* and 6 *G. babadense*) collected from the gene bank of the department. These materials are being widely cultivated across the four agroclimatic Zones of Tamil Nadu in India. Table 1 illustrates the origin, pedigree and special features of the chosen experimental materials.

Morphological markers analysis

Diversity analysis based on morphological markers was performed with the 21 genetic materials being exposed to the field condition. The experimental design was Randomized Completely Block Design, having three replications. Four rows each were planted at a spacing of 90 cm between rows and 60 cm between plants. Standard procedure for field maintenance was adhered to, basic agronomic practices like irrigation, fertilizer application, weed and pest control were observed. Data were recorded from five selected plants in each entry for eighteen characters *viz*., days to flowering (DF), days to 50 percent flowering (DFF), days to first bursting (DFB), seed cotton yield per plant (g) (SCYPP), plant height (cm) (PH), number of bolls per plant (NBPP), number of sympodia branches per plant (NSPP), number of monopodia branches per plant (NMPP), boll weight (g) (BW), number of seeds per boll (NSPB), ginning percentage (GP), lint index (LI), seed index (SI), 2.5% span length (mm) (2.5%SL), bundle strength (BS), fibre fineness (FF), elongation percentage (E%) and Uniformity ratio (UR). Seed cotton was pooled from the sampled plants, ginned and the lint obtained was evaluated for fibre quality characters estimation using High Volume Instrument 900 classic. The means for all the observed parameters were worked out and were further subjected to analysis of variance (ANOVA) following the method of Kempthorne (1957). Genetic divergence was determined following the procedure of MINITAB (Package vs14.13) for Cluster Analysis with Euclidean Distance and Single Linkage Method. Multiple correspondence analyses was employed to determine the discrimination measures and detect the strength of the structure for each trait using IBM SPSS statistics version 20.

SSR markers analysis

Diversity analysis based on molecular (SSR) markers was assessed among the selected cotton germplasm using a total of 72 SSRs that span all the chromosomes of *Gossypium* spp. The SSR primer sequences were obtained from cotton Data Base (cottonDB at http://algodon.tamu.edu/cgi-bin/searches/browser) and they were commercially synthesized and procured from 1stBASE, Singapore. The dNTPs, assay Buffer and *Taq*DNA Polymerase were purchased from BangaluruGeNei Ltd., India.Genomic DNA was extracted from fresh and young leaves of cotton plants, as described by Zhang and Stewart (2000). The isolated genomic DNA was quantified using NanoDrop^{TM} 1000 Spectrophotometer. Inactness, homogeneity and purity were checked by electrophoresis in 0.8 per cent agarose gel. After quantification, the final concentration of DNA was adjusted to 20 ng/µL, using low salt Tris-EDTA buffer. The cocktail for PCR amplification was prepared with the 20 ng/µL of DNA, Master mix, Forward primer (100 µM), Reverse primer (100 µM) and sterile distilled water having a total of 15 aliquot (µL**).** The amplification reaction was carried out in thermo cycler (MyCycler®, Bio-Rad Laboratories, California. The program of reaction was set according to Zhang and Stewart (2000).After adding 5 µL of loading dye (40% sucrose, 0.25% bromophenol blue prepared in sterile distilled water), PCR products were resolved using 3% Metaphor agarose gel electrophorosis. Fifteen microlitres each of the product were loaded and subjected to electrophoresis in 1x TBE buffer (0.9 M Tris-HCl, 0.025 M EDTA Na^{2+}, 0.9 M Boric acid) at 100 V. A 100 bp DNA ladder was used to calculate PCR product size. The bands were visualized under UV trans illumination after staining with ethidium bromide and gel images were documented using Alpha Imager TM 1200 (Alpha Innotech Corporation, USA). Fragment length was determined visually by comparing with DNA ladder. After visualizing the gel, amplified fragments of each SSR primer bands were scored in binaries as presence (1) or absence (0). Polymorphism information content (PIC) of SSR markers was calculated using the formula developed by Anderson *et al*. (1993). Genetic diversity estimates were computed using NTSYSpc ver. 2.02i (Rohlf, 2000). Genetic similarity (GS) among the 21 cotton genotypes were calculated using SIMQUAL module and DICE similarity co efficient based on the proportion of shared alleles. Genetic distances (GD) between pairs of lines were estimated as GD = 1 – GS. A dendrogram for clustering of genotypes was done based on a

similarity matrix using the unweighted pair group method of arithmetic average (UPGMA) algorithm following SAHN module.

Results and Discussion

Mean squares, grand means, standard errors and critical differences are presented in Table 2, discrimination measures are in Tables 3. While Allelic distribution and polymorphic information content (PIC) are in Table 4, Genetic similarity coefficient values are presented in Table 5. Figure 1, 2 and 3 present dendrograms constructed based on phenotypic parameters, multiple correspondence analysis and molecular analysis of the genotypes evaluated respectively.

Morphological markers based genetic diversity analysis

The analysis of variance indicated that the genotypes were significantly different in number of branches per plant, seed index and seed cotton yield per plant. This therefore showed that there exists low genetic variability for most of the traits amongst the 21 cotton genotypes. Highest critical differences among the traits were observed in DFB while the least were recorded for NMPP. This result is in agreement with Isong *et al.*, (2017). The cluster analysis grouped the genotypes based on their similarity index for each of the characters. At similarity percentage of 60.78, only two major groups, with random cluster distribution, were identified. The group I contained a unit member and the group II had 20 genotypes. This is an indication of low genetic diversity among the evaluated genotypes. Analysis to quantify nominal data by assigning numerical values to the traits and genotypes and measure discriminations of either close together or far apart, multiple correspondence analysis explained 82.07% variability in dimension 1 and 80.57% in dimension 2. The active total for the traits were 14.50, and 14.78 for dimension1 and 2 respectively. The implication is that the expression for majority of the traits studied in all the genotypes has about 80% similarity. This also confirmed the narrow genetic diversity earlier indicated by cluster analysis.

Molecular marker based genetic diversity analysis

The 72 primer pairs, used for the molecular study, revealed 62 polymorphic primer pairs (86.11%) for both *G. hirsutum* and *G. barbadense* genotypes. The 62 primer pairs amplified a total of 155 alleles with an average of 2.51 alleles per microsatellite locus, similar to that reported by Bertini *et al*., (2006) who used 31 pairs of polymorphic primers that amplified a total of 66 alleles and an average of 2.13 alleles per locus. In the same vein, Liu *et al*.,(2000) used 56 polymorphic primer pairs which produced a total of 325 alleles with average of 5 alleles per locus. The number of bands amplified by the SSR primer pairs ranged from 1 to 5. The PIC value varied from 0.00 to 0.80 with an average of 0.57. The high average PIC values (>0.5) observed is an indication of the effectiveness of the markers. A marker is informative (effective) if its PIC values is higher than 0.5 (Ahmad *et al*., 2015). Similar PIC range (0.05 to 0.82), although with lower average (0.31) in cotton were observed by Liu *et al*.,(2000). Bertini *et al*., (2006) reported PIC range between 0.18 and 0.62, and average value of 0.40 in their work. Genetic similarity co-efficients for each pair of the 21 *Gossypium* genotypes ranged from 0.13 to 1.00. The least similarity co-efficient (highest diversity) was recorded between H7 and H11. Among all genotypes, the least similarity coefficients were obtained between H11 and all other genotypes except H1 where similarity coefficient of 0.51 was observed. Similar narrow genetic diversity was also revealed in the dendrogram constructed from the molecular data. Irrespective of sources and species, two major random clusters were identified at similarity coefficient of 0.49. The cluster I had a unit member (11). The cluster II contained three subgroups, with genotypes (1) and (16) constituting unit member in two subgroups out of the three subgroups.

Conclusion

The results of the present study revealed narrow genetic diversity among the cotton genotypes evaluated at both morphological and molecular levels. The primers used in the study documented high average PIC for future uses. Among all the genotypes, genotype H11 showed high genetic distance from other genotypes in the study. This result will be helpful in future cotton breeding

program.

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Table 1. Details of genotypes used in this study

S/No	Genotype	Parentage	Source	Special features
1	MCU13	Multiple cross derivative	TNAU,Coimbatore	Short duration, winter irrigation
2	SVPR4	Derivative from MCU5 x S4727	CRS,Srivilliputhur	Summer irrigated and rice fallow
3	KC3	TKH 497 × KC 1	RRS, Kovilpatty	Suitable for rainfed cultivation
4	TCH1777	MCU 12 x LRA 5166) x TCH 1569	TNAU,Coimbatore	
5	MCU9	MCU 8 × MCU 5	TNAU,Coimbatore	Extra-long staple, winter irrigation
6	TCH1716	(MCU 5 / TCH 92-7) / MCU 5 -1	TNAU,Coimbatore	Good ginning out turn, ELS cotton
7	MCU7	X – ray mutant of L 1143EE	TNAU,Coimbatore	Suitable for rice fallow and earliness
8	TCH1819		TNAU,Coimbatore	
9	COD-5-1-2			
10	African1-2			
11	VS-9-S11-1			
12	TCH1705-101	Selection from AKH 2053	TNAU,Coimbatore	Drought tolerant
13	KC2	MCU 10 × KC 1	Kovilpatty	Jassid resistant, winter irrigation
14	TSH 0250		CRS,Srivilliputhur	
15	Surabhi	MCU 5 VT (MCU 5 x *G. mexcicanum*)	CICR, Coimbatore	Normal type
16	TCB 26		TNAU, Coimbatore	
17	TCB 37		TNAU,Coimbatore	
18	TCB 209		TNAU,Coimbatore	
19	CCB36		CICR, Coimbatore	
20	DB3		UAS, Dharward	
21	Suvin	Sujatha × SIV 135	CICR, Coimbatore	Extra long staple

Table 2. Mean squares, grand means, standard errors and critical differences for traits in 21 cotton genotypes

Sources of variation	df	DF	DFF	DFB	PH	NBPP	NSPP	NMPP	BW	GP	LI	SI	2.5%SL	UR	BS	E%	FF	NSPB	SCYPP
Replication	2	8.1	0.1	13.9	26.0	0.2	1.0	0.03	0.1	64.6	0.0	6.9	6.8	42.7	23.4	2.8	0.2	40.92	20.2
Treatment	20	11.8	40.1	32.4	294.2	36.7**	11.7	0.3	0.4	25.8	0.5	1.6*	24.7	17.2	9.1	1.7	0.9	15.0	792.7**
Error	40	49.4	48.7	131.9	295.2	14.7	19.7	0.3	0.9	41.7	0.8	0.8	25.6	47.2	99.1	3.5	0.9	37.1	70.5
Means		50.8	59.0	90.2	99.8	24.7	16.6	1.5	2.9	29.8	3.1	7.2	29.8	48.9	23.3	5.7	4.3	19.8	89.2
SEd		9.5	9.4	15.5	13.9	1.6	1.9	0.2	0.4	2.7	0.4	0.8	6.1	8.6	4.2	0.8	0.4	2.6	3.6
CD (5%)		18.7	18.3	30.3	27.1	3.2	3.7	0.4	0.8	5.4	0.8	1.6	12.0	16.9	8.3	1.6	0.8	5.1	7.0
CD (1%)		24.6	24.1	39.9	35.7	4.2	4.9	0.6	1.1	7.1	1.0	2.1	15.8	22.2	10.9	2.1	1.1	6.7	9.2

*** Significant at 5% level** ** **Significant at 5% level**

Table 3. Discrimination measures for all the parameters studied

		DF	DFF	DFB	PH	NBPP	NSPP	NMPP	BW	NSPB	SCYPP	GP	LI	SI	SL	UR	BS	EP	FF	Active Total	% Variance
Dimension	1	0.91	0.99	0.93	0.92	0.36	0.67	0.87	0.99	0.92	0.98	0.77	0.62	0.54	0.98	0.93	0.85	0.96	0.58	14.78	82.07
	2	0.86	0.83	0.72	0.73	0.72	0.62	0.59	0.89	0.79	0.95	0.86	0.75	0.73	0.96	0.95	0.73	0.89	0.88	14.50	80.57
Mean		0.89	0.91	0.83	0.82	0.54	0.64	0.73	0.95	0.86	0.97	0.81	0.68	0.64	0.97	0.94	0.79	0.93	0.74	14.64	81.32

Table 4. Allelic distribution and polymorphic information content (PIC) values of SSR markers across 21 genotypes of cotton

S/No.	Name of Primers	Number of alleles						PIC values
		1	2	3	4	5	Total	
1	BNL358	6	15				2	0.50
2	BNL387	11	1	6	1	1	5	0.80
3	BNL390	4	10	7			3	0.67
4	BNL391	11	10				2	0.50
5	BNL409	11	2	9			3	0.64
6	BNL448	16	5				2	0.50
7	BNL530							
8	BNL542	12	9				2	0.50
9	BNL569							
10	BNL673	15	6				2	0.50
11	BNL3482	14	7				2	0.50
12	BNL3955	21					1	0.00
13	BNL3994	16	5				2	0.50
14	CIR019	13	5	3			3	0.63
15	CIR020	15	5	1			3	0.67
16	CIR021	21					1	0.00
17	CIR022	15	6				2	0.44
18	CIR023	18	3				2	0.50
19	CIR024	21					1	0.00
20	CIR025	9	12				2	0.50
21	CIR407	11	10				2	0.50
22	CIR413	10	8	3			3	0.67
23	JESPR17	10	11				2	0.50
24	Gh473							
25	Gh474	8	9	4			3	0.67
26	Gh475	12	9				2	0.50
27	Gh476	11	10				2	0.44
28	Gh477	15	5	1			3	0.67
29	Gh478	16	5				2	0.44
30	Gh479							
31	HAU0298	9	12				2	0.50
32	HAU0299	13	8				2	0.44
33	HAU0300							
34	HAU0301	6	9	6			3	0.67
35	HAU0302	7	9	4	1		4	0.74
36	HAU0303	12	8				2	0.50

S/No.	Name of Primers	Number of alleles						PIC values
		1	2	3	4	5	Total	
37	HAU0304							
38	HAU0305	10	5	3	3		4	0.67
39	HAU0306	6	9	6			3	0.74
40	HAU0307	11	7	4			3	0.50
41	HAU0308	21					1	0.67
42	HAU0309	15	5	1			3	0.74
43	HAU0310	12	9				2	0.50
44	HAU0311	9	8	4			3	0.67
45	HAU0312	14	7				2	0.74
46	HAU0313							
47	HAU0314	8	8	5			3	0.67
48	HAU0315	16	5				2	0.44
49	HAU0316	12	7	1	1		4	0.75
50	HAU0317	10	8	2	1		4	0.75
51	HAU1219							
52	HAU1220	13	4	4			3	0.67
53	NAU1043_238	12	9				2	0.50
54	JESPR271	14	7				2	0.50
55	JESPR272							
56	JESPR273	10	11				2	0.50
57	JESPR274	9	12				2	0.50
58	JESPR275	6	13	2			3	0.67
59	JESPR276	11	10				2	0.50
60	JESPR277	21					1	0.00
61	NAU1193	13	8				2	0.50
62	NAU1194	9	8	4			3	0.63
63	NAU1195	5	15	1			3	0.67
64	NAU1196	3	15	2	1		4	0.72
65	NAU1197	11	6	4			3	0.63
66	NAU1198	21					1	0.00
67	NAU1199	13	5	3			3	0.67
68	NAU1200	16	5				2	0.50
69	NAU1201	9	5	2	4	1	5	0.76
70	NAU1202	14	7				2	0.50
71	NAU1211	10	11				2	0.50
72	NAU1212	8	5	8			3	0.67

Table 5. Genetic similarity co-efficient values between genotypes of *G. hirsutum* and *G. barbadense*

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	B1	B2	B3	B4	B5	B6
H1	1.00	0.75	0.75	0.75	0.30	0.62	0.75	0.73	0.75	0.75	0.51	0.56	0.56	0.75	0.68	0.59	0.65	0.67	0.70	0.75	0.60
H2		1.00	1.00	1.00	0.56	0.84	1.00	0.98	1.00	1.00	0.25	0.81	0.81	1.00	0.81	0.71	0.87	0.89	0.92	0.97	0.86
H3			1.00	1.00	0.56	0.84	1.00	0.98	1.00	1.00	0.25	0.81	0.81	1.00	0.81	0.71	0.87	0.89	0.92	0.97	0.86
H4				1.00	0.56	0.84	1.00	0.98	1.00	1.00	0.25	0.81	0.81	1.00	0.81	0.71	0.87	0.89	0.92	0.97	0.86
H5					1.00	0.68	0.56	0.54	0.56	0.56	0.41	0.37	0.37	0.56	0.52	0.52	0.56	0.54	0.51	0.52	0.54
H6						1.00	0.84	0.83	0.84	0.84	0.13	0.65	0.65	0.84	0.68	0.62	0.71	0.73	0.76	0.81	0.73
H7							1.00	0.98	1.00	1.00	0.25	0.81	0.81	1.00	0.81	0.71	0.87	0.89	0.92	0.97	0.86
H8								1.00	0.98	0.98	0.24	0.79	0.79	0.98	0.79	0.70	0.86	0.87	0.90	0.95	0.84
H9									1.00	1.00	0.25	0.81	0.81	1.00	0.81	0.71	0.87	0.89	0.92	0.97	0.86
H10										1.00	0.25	0.81	0.81	1.00	0.81	0.71	0.87	0.89	0.92	0.97	0.86
H11											1.00	0.44	0.44	0.25	0.38	0.41	0.35	0.37	0.30	0.29	0.30
H12												1.00	1.00	0.81	0.68	0.65	0.75	0.79	0.79	0.81	0.76
H13													1.00	0.81	0.68	0.65	0.75	0.79	0.79	0.81	0.76
H14														1.00	0.81	0.71	0.87	0.89	0.92	0.97	0.86
H15															1.00	0.65	0.71	0.73	0.79	0.81	0.73
B1																1.00	0.81	0.67	0.67	0.68	0.57
B2																	1.00	0.79	0.79	0.84	0.73
B3																		1.00	0.87	0.86	0.78
B4																			1.00	0.92	0.78
B5																				1.00	0.86
B6																					1.00

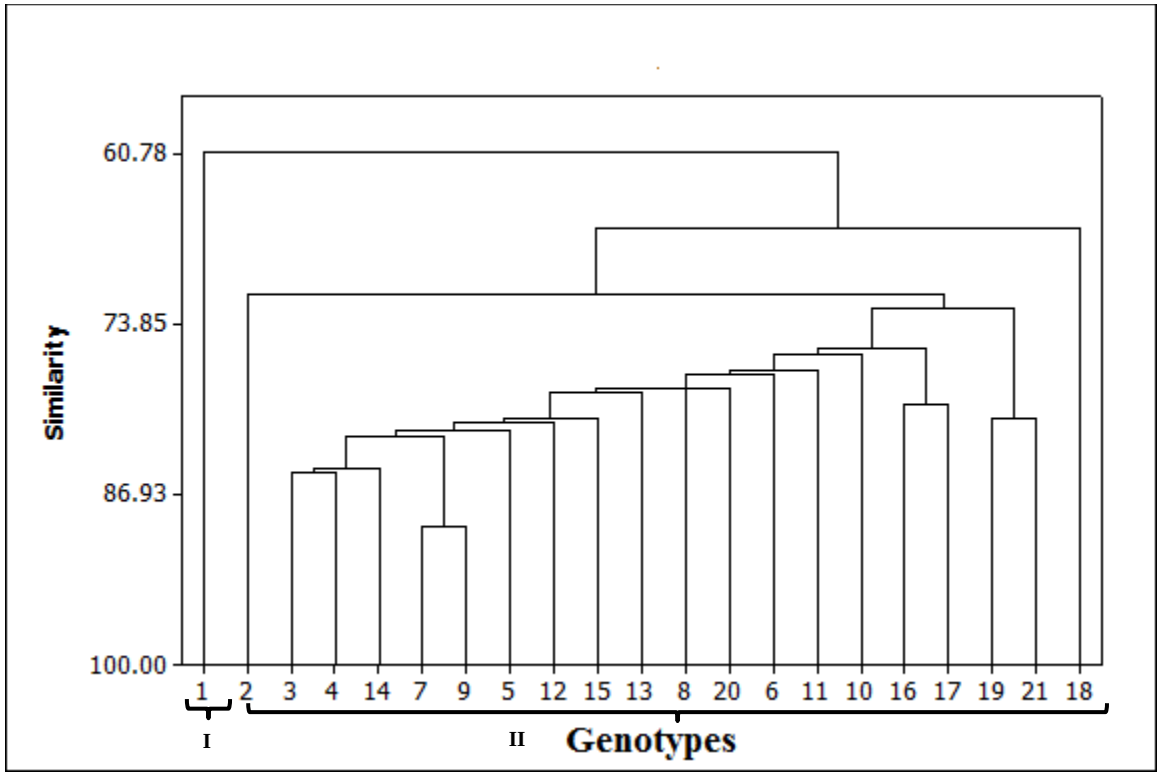

Figure 1.Dendrogram for Agglomerative Cluster Analysis of 21 Cotton Genotypes

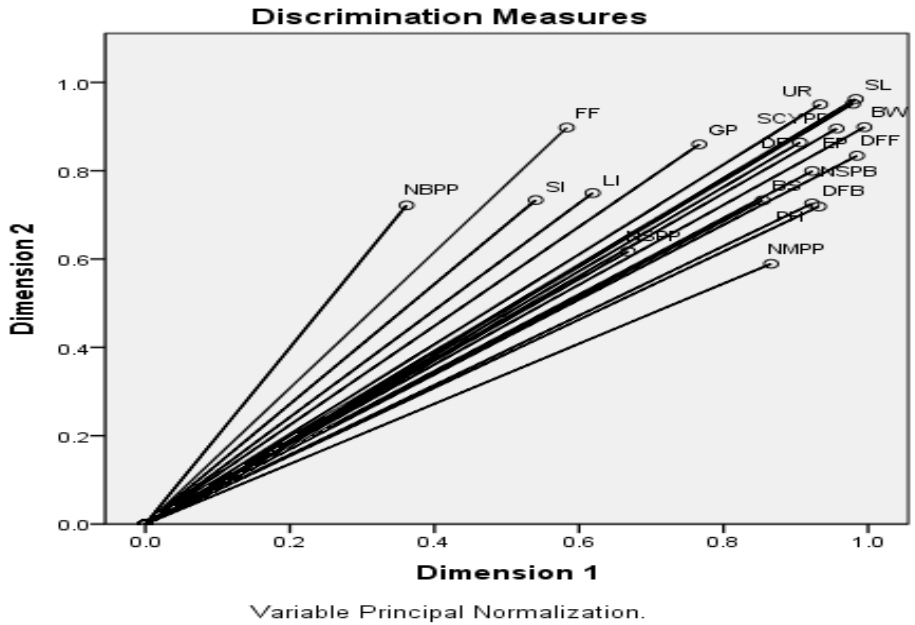

Figure 2. Plot of multiple correspondence analysis showing Discrimination Measures

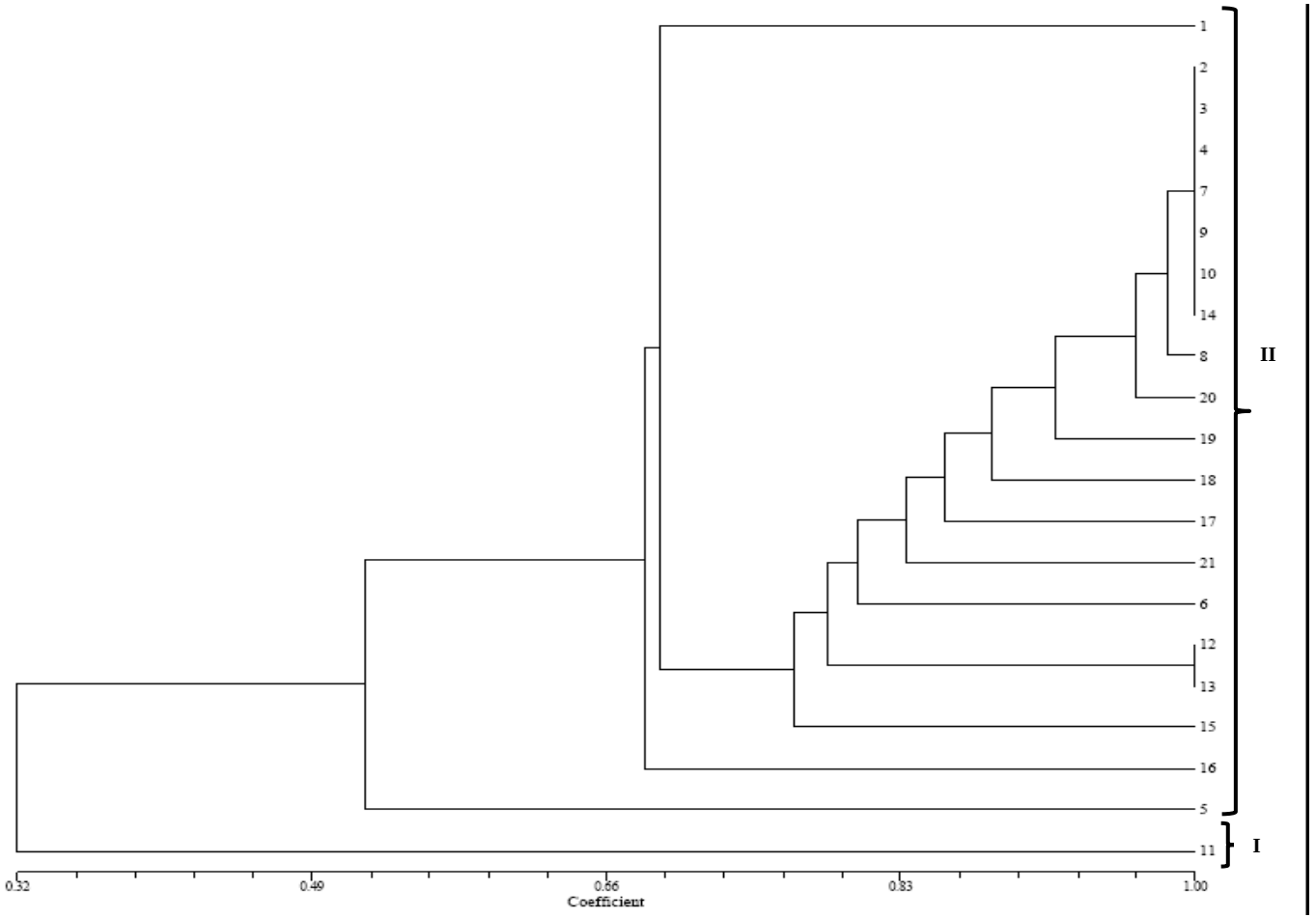

Figure 3. Dendrogram for molecular diversity analysis of 21 genotypes