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Research Article

Morphological characterization of asiatic cotton (*G. arboreum*) germplasm of India

A. Manivannan[1*], Punit Mohan[2], M. Saravanan[2] and V. N. Waghmare[2]
[1]ICAR-Central Institute for Cotton Research, Regional Station, Coimbatore 641003, India
[2]ICAR-Central Institute for Cotton Research, Nagpur 440010, India
*E-Mail: manivannan461@gmail.com

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Abstract
A set of 816 accessions of *desi* cotton (*G.arboreum*) were evaluated in augmented design 1 during *Kharif* 2015-16. Traits namely stem color, leaf size, leaf lobbing, leaf shape, leaf color, petal color, bract size, boll shape, boll size, leaf nectaries, and plant type were recorded. Principal Component Analysis (PCA) identified five principal components were accounted for a cumulative variation of 86.36%. The first principal component accounted for 40.37%, second for 17.43%, third for 11.32%, fourth for 9.75% and fifth for 7.50% of total variation. First principal component (PC 1) was correlated with boll shape (0.94) and bract size (0.10). Second principal component (PC 2) was associated with leaf shape (0.98) and bract size (0.11). PC 3 was correlated with bract size (0.74) and boll size (0.24). Forth PC (PC 4) was associated with nectarines (0.82) and bract size (0.57). Fifth PC (PC 5) was related with leaf lobbing (0.97). Maximum diversity index was recorded for the trait stem color (6.70). The trait leaf shape showed lowest diversity index (6.472), followed by boll shape (6.492) and nectar (6.570). Lower the diversity index discriminated the accessions in higher order. Principal Component Analysis (PCA) based on qualitative traits revealed that traits namely boll shape, leaf shape and bract size distinguished all genotypes in higher order than other traits. Convex of the hull occupied by the genotypes namely A 32, AC 30, AC 32, AC 543, AC 544 and 78/1A, these genotypes showed the highest point among the factors. All the genotypes were grouped into six distinguished clusters.

Key words
desi cotton, morphological traits, principal component analysis, clustering

Introduction

Cotton is a major source of natural fibre. The genus *Gossypium* has two distinct set of cultivated species namely old world and new world cotton. Old world cotton has the ploidy level of 2n=2x=26 (diploid, AA) and consists of two species namely *Gossypium arboreum* (*karunganni/nadan* cotton) and *Gossypium herbaceum* (*uppam* cotton), by and large known as *desi* cotton (native genotype) or Asiatic cotton. Among all the species, desi cotton is important because of its ability to grow in marginal environment conditions and resistance to variety of pests and diseases (Stewart, 1994), these adoptive traits contributed to the improvement of new world cotton species. The new world cotton comprising of *Gossypium hirsutum* (upland cotton) and *Gossypium barbadense* (egypitan cotton) with 2n=4x=52, an allotetraploid(AADD).

After the American civil war, British government introduced the *Gossypium hirsutum* to India and other colonized countries to cater the need of textile industries of England. At the time of independence, *desi* cotton occupied 98 per cent area (2.79 mha of *arboreum* and 1.39mha of *herbaceum*) and American cotton shared only 2 per cent (0.14mha) (Santhanam, 1997). During 2000 the area of *arboreum* reduced to 1.46mha and *herbaceum* to 0.95mha due to increase in area under hybrids (3.64mha) and *hirsutum* (2.48mha) cotton. Since the introduction of Bt cotton; area under *desi* cotton has been reduced to less than 3 per cent (Kranti, 2015 & Narayanan *et al.*, 2014).

Desi cotton is known for its ability to grow in marginal environments. *Desi* cotton possesses resistance to range of insect pests namely hoppers, white flies, thrips and aphids. *Desi* cotton almost exhibits immune to the vector borne leaf curl virus. Due to short, coarse and weak fiber traits make them unsuitable for modern machine textile industries. However during 17-18th century, *desi* cotton was being spun by handcrafters, the cloth was so fine *e.g.* Dhaka muslin (Dhaka *malmal*). Machine and electrospinning needs high strength fibre, this property lacks in *desi* cotton. In spite of poor fibre traits, these short stable cotton used for denim, upholstery and surgical cloths. Recent past the trend is reversing, the demand for short stable cotton is 2% however the production is 1%; this makes a lot of hope for increasing the area of *desi* cotton.

Variation in germplasm collections has been utilized for identifying desirable genotypes to enhance yield improvement. Distinct qualitative traits are called morphological markers, often considered for germplasm characterization and associated trait for selection; such qualitative traits are stable in expression across the environment. Characterization of germplasm plays a vital role in crop improvement. Characterization, evaluation of germplasm and quantification of genetically diverse genotypes is indispensable for a pragmatic use of plant genetic resources and also for determining evolutionary relationships (Zada *et al.,* 2013). Studies of the variation present in germplasm collections have been carried out employing plant morphological attributes as characterization tool. To identify the desired genotypes, leaf, stem and floral morphological traits are being used in cotton (Sangwan *et al.,* 2008; Iqbal *et al.,*2015; Ranjan *et al.,*2014).

By characterizing genetic diversity between and within groups, breeding efforts can be greatly improved through better parental selection for generating segregating populations. Genetic diversity information is also helpful to identify heterotic groups, understand population structure, and identify a core set of lines for genetic analysis studies. Thus, assessment of genetic diversity and population structure is important in the *desi* cotton.

Material and Methods

A set of 816 racially diverse accessions of *G.arboreum* belonging to race namely *bengalense, indicum,* and *cernum* were selected and were grown in augmented design at ICAR- Central Institute for Cotton Research, Regional Station, Coimbatore, India during *kharif* crop season 2015 and 2016. These study material was procured from Cotton Gene Bank of ICAR- Central Institute for Cotton Research, Nagpur, Maharashtra, India. Experiment was conducted as per augmented design 1; each entry occupied a three-meter row with spacing of 60 × 30 cm. All agronomical practices were followed as prescribed by the agronomists. Qualitative characters were taken under consideration for evaluating the substantial variation and relationship among desi cotton genotypes. Traits namely stem color, leaf size, leaf lobbing, leaf shape, leaf color, petal color, bract size, boll shape, boll size, leaf nectaries, and plant type were used to describe genetic diversity. Scores were assigned for each trait as per the guide lines by DUS (Distinct, Uniformity, and Stability) guide line formulated by PPV& FRA, 2001 (Table 1). Pearson correlation coefficient was worked out for seven qualitative traits and correlation matrix was prepared for comparing different traits. Principal component analyses (PCA) based on seven qualitative traits was performed to find out the relative importance of different traits in capturing the genetic variation in *desi* cotton. The factors of these traits were used to determine the contribution of each factor towards variation. The standardized values were used to perform PCA using PAST 3 (Hammer *et al.,* 2001). A scree plot was drawn from the eigen values associated with a component or factor in descending order versus the number of the component or factor. Scree plot used for visually assess which components or factors explain most of the variability in the data. Dissimilarity matrix based on EUCLIDEAN distance was calculated using these traits by DARwin 5. Most dissimilar and least dissimilar accessions were identified in *desi* cotton genotype based on dissimilarity matrix. A hierarchical cluster analysis for pooled data was performed using scores of dissimilarity matrix (Ward, 1963).

Results and Discussion

Discrete morphological traits of qualitative nature are very much helpful in characterization of the germplasm because they are less influenced by the environment unlike that of quantitative traits. They form distinct phenotypic classes and therefore are useful tools in classifying germplasm and can be predominantly assessed visually and even by naïve. Because of these attributes large number of DUS (distinctiveness, uniformity and stability) characters for plant cultivar registration are defined using qualitative traits. Very few such qualitative traits are available in practical terms (Kruskal, 1978).

Observations on 11 qualitative traits were recorded for all the 816 genotypes and the scores were analyzed (Table 1). Majority of the genotypes were purple green in stem color(98.7%), eight genotypes were purple (AC 622-SP2, Gao 16 CB-9, H 209, H 460, PBN 48, Sanguineum / G 26, 320-1 and 1173 WR) and only two genotypes were expressed green stem color(AC38 and AC 216). Higher frequency of medium leaf size was observed (89.3%) when compared with 5% of small and large leaf size respectively. Eight distinct groups were observed for the leaf lobbing trait, eleven genotypes were having 5-7 lobe and two genotypes were having two lobes, however five lobe categories was most predominant (78.5%). Two category of leaf shape was observed *viz.*, deeply palmated long lobes and deeply palmated lobes with proportion of 75 and 25% respectively. Green leaf color was noticed in 804 genotypes and 12 genotypes were purple color (AC 622-SP2, AC 727-SP1, Gao 16 CB-9, H 52-473, H 209, H 460, PBN 48, Sanguineum / G 26, Vira-6, 320-1 1173 WR and Desi 10). Among the genotypes, three classes of bracts were observed namely medium (418) and large (393) and small bracts (5 genotypes namely A 32, AC 3–SP1, AC 32, AC 63 and DC 46-21). In case of petal color, higher frequency of yellow color petal with red spot was observed (98.9%), seven genotypes were having white color petal with red spot (CJDC-93-32, W 5-65-1258, 30810, AC 3370, Yaganti, AKA5 and AKA7) and distinctly two genotypes were yellow color petal without any spot (Sanguineum / G 26 and Vira-6). In boll shape class, 457 of conical, 354 of ovate and five round shape was observed. The trait leaf necatries was observed in 723 genotypes only and remaining were nectariless (93). Among the genotypes, two categories of plant types were observed, higher proportion of semi spreading genotypes (791) and lesser proportion of spreading type (25). Leaf lobbing, stem color and petal color has been used for grouping of 138 *G.arboreum* accessions (Sangwan *et al.,* 2008)

Principal Component Analysis (PCA) is the most preferred multivariate statistical technique that carries out a data reduction among independent and interdependent variables while retaining those characteristics of the dataset that contribute most to its variance, by keeping lower-order principal components and ignoring higher order ones. As a measure of reducing the multiple variables to a handful of principal components that explain hierarchically lowering levels of variance components, the PCA of the qualitative data derived from 816 genotypes, revealed at least four independent variable groupings accounting to 79 % of total variation (Eigen value more than one). This is evident from the extraction of four lower-order principal components from the PCA, which is explained by the presence of high number of independent variables. The PCA is highly effective as a data reduction tool, when variables are inter-correlated (Pearson, 1901). These independent components therefore would be used for effective classification of the genotypes.

PCA identified five principal components were accounted for a cumulative variation of 86.36%. The first principal component accounted for 40.37%, second for 17.43%, third for 11.32%, fourth for 9.75% and fifth for 7.50% of total variation. First principal component (PC 1) was correlated with boll shape (0.99) and bract size (0.10). Second principal component (PC 2) was associated with leaf shape (0.98) and bract size (0.11). PC 3 was correlated with bract size (0.74) and boll size (0.24). Forth PC (PC 4) was associated with nectarines (0.82) and bract size (0.57). Fifth PC (PC 5) was related with leaf lobbing (0.97). Based on all the principal components, maximum variation was recorded for boll shape (39.96 %), moderate variability was recorded for leaflet shape (25.75 %) and bract size (11.49). Low amount of variability observed for nectarines (7.99%), leaf lobbing (7.25%) and boll size (2.71%), however very less variability observed among remaining traits (Table 4). Based on the four PCs, scatter and scree plots depicted the variability.

Pearson (1901) correlation coefficient was employed among the qualitative traits(Table 5). Bract size was positive and significantly correlated with boll size and boll shape and leaf size. Petal color was significantly correlated in positive with leaf color, but negatively correlated with nectaries. Boll size was positive and significantly correlated with leaf size, leaf shape and leaf lobbing, however, negatively associated with boll size, nectaries and plant type. Positive association was observed between boll shape with plant type and stem colors, the same trait was negatively correlated with leaf lobbing. Leaf size is negatively associated with necatries. Leaf shape was negatively associated with leaf lobbing and leaf color. Leaf color was negatively correlated with stem color.

A scatter plot (Fig. 1) drawn using PC1 and PC2 factor scores showed clear pattern of grouping between the genotypes in the factor plane. Convex of the hull occupied by the genotypes namely A 32, AC 30, AC 32, AC 543, AC 544 and 78/1A, these genotypes showed the highest point among the factors. Genotypes namely AC 17, AC 622-SP1, AC 36 and C 1 were clustered around the origin as a distinct group. There were six distinct clusters found in the biplot plane. PCA was used to characterize 26 *G.arboreum* genotypes based on fibre attributes namely fibre length, strength, micronaire and ginning out turn (Iqbal *et al.,*2015). Grouping was done based on standardized Euclidean distance and un-weighted paired group method using arithmetic average (UPGMA) clustering method showed six distinct clusters (Fig. 2).

Shannon weaver diversity indices (H’) were worked out for various traits (Table 6), Maximum diversity index of 6.70 was recorded for the trait stem color. The trait leaf shape showed lowest diversity index (6.472), followed by boll shape (6.492) and nectar (6.570). This pattern indicates that lower indices discriminated the genotypes in higher order than other traits as evidenced (Fig.3).

This study is conducted for comparing the morphological characteristics of *G.arboreum* accessions. The data obtained showed that germplasm resources present a wide range of diversity for morphological traits. The investigations were also very useful in choosing the most precious accessions for further breeding programmes.

Among the traits flower color, leaf color and stem color are highly useful in distinguishing the entire germplasm accessions. Petal color is very much useful in identifying seven genotypes having purple color petal with red spot (CJDC-93-32, W 5-65-1258, 30810, AC 3370, Yaganti, AKA5 and AKA7) and distinctly two genotypes having yellow petal without any spot (Sanguineum / G 26 and Vira-6). Among green purple predominated stems, eight genotypes were purple (AC 622-SP2, Gao 16 CB-9, H 209, H 460, PBN 48, Sanguineum / G 26, 320-1 and 1173 WR) and only two genotypes were expressed green stem color(AC38 and AC 216). In the same way, 12 genotypes were having purple color leaves (AC 622-SP2, AC 727-SP1, Gao 16 CB-9, H 52-473, H 209, H 460, PBN 48, Sanguineum / G 26, Vira-6, 320-1 1173 WR and Desi 10) in comparison with all other accessions.

In the present regime of Intellectual Property Rights, these morphological traits are very much useful for DUS (distinctiveness, uniformity and stability) characterization in plant variety registration and protection of breeder's rights. The present study clearly emphasis the potential of morphological traits employed as markers in characterization and utilization of plant genetic resource in cotton.

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Table 1. Morphological description of *desi* cotton (*G.arboreum*) accessions

Descriptor Trait	**Category**	**No of Genotypes**	**Frequency (%)**	**Descriptor Trait**	**Category**	**No of Genotypes**	**Frequency (%)**
Stem color (SC)	Purple	8	0.98	Petal color(PC)	Yellow with red spot	807	98.9
	Purple Green	806	98.7		White with red spot	7	0.85
	Green	2	0.24		Yellow spotless	2	0.24
Leaf size(LSZ)	Small	45	5.51	Boll shape(BSP)	Ovate	354	43.4
	Medium	729	89.3		Round	5	0.61
	Large	42	5.14		Conical	457	56.0
Leaf lob(LB)	2 to 3	2	0.24	Boll size(BSZ)	Small	10	1.20
	3	3	0.36		Medium	691	84.7
	3 to 4	3	0.36		Large	115	14.1
	3 to 5	137	16.8	Nectaries(NE)	Nectar	723	88.6
	4 to 5	18	5.69		Nectariless	93	11.4
	5	641	78.5	Plant type(PT)	Spreading	25	3.10
	5 to 6	1	0.12		Semi spreading	791	96.9
	5 to 7	11	1.34				
Leaf shape(LSP)	Deeply palmated long lobes	612	75.0				
	Deeply palmated lobes	204	25.0				
Leaf color(LC)	Purple	12	1.47				
	Green	804	98.5				
Bract size(BS)	Small	5	0.61				
	Medium	418	51.2				
	Large	393	48.1				

Table 2. Principal component analysis of different traits

Principal component	Eigen value	Proportion of variation	Cumulative
1	3.98	40.37	40.37
2	1.72	17.43	57.79
3	1.12	11.32	69.12
4	0.96	9.75	78.87
5	0.74	7.50	86.36

Table 3. Principal components of various traits

Traits	PC 1	PC 2	PC 3	PC 4
Bract size	0.10	0.11	0.74	0.57
Petal color	0.00	-0.01	0.03	-0.04
Boll size	-0.03	0.08	0.24	0.06
Boll shape	0.99	-0.06	-0.06	-0.03
Leaf size	0.00	0.06	0.15	-0.03
Leaf shape	0.05	0.98	-0.16	0.01
Leaf lobbing	-0.04	-0.06	0.21	0.02
Leaf color	-0.01	-0.01	0.01	-0.01
Nectaries	-0.02	-0.10	-0.54	0.82
Plant type	0.01	0.01	-0.01	-0.03
Stem color	0.01	0.01	0.00	0.01

Table 4. Qualitative traits towards variability

Traits	Variability contribution (%)
Boll shape	39.96
Leaf shape	25.45
Bract size	11.49
Nectaries	7.99
Leaf lobbing	7.25
Boll size	2.71
Plant type	1.63
Leaf size	1.21
Stem color	0.79
Petal color	0.77
Leaf color	0.75

Table 5. Correlation among different traits

	BR	**PC**	**BSZ**	**BSP**	**LSZ**	**LSP**	**LOB**	**LC**	**NE**	**PT**	**SC**
BR	1.00	0.01	0.16[a]	0.14 [a]	0.10 [b]	0.06	0.04	0.01	-0.06	-0.03	0.05
PC		1.00	-0.02	0.00	-0.02	-0.04	-0.01	0.21 [a]	-0.11 [a]	0.03	-0.08 [b]
BSZ			1.00	-0.08 [b]	0.09 [b]	0.07 [b]	0.07 [b]	-0.04	-0.08 [b]	-0.09 [b]	0.04
BSP				1.00	0.00	0.04	-0.08 [b]	-0.06	-0.03	0.07 [b]	0.08 [b]
LSZ					1.00	0.06	0.01	0.06	-0.10 [b]	0.04	-0.02
LSP						1.00	-0.07 [b]	-0.07 [b]	-0.05	0.03	0.06
LOB							1.00	-0.03	-0.03	-0.05	-0.03
LC								1.00	-0.04	0.02	-0.57 [a]
NE									1.00	-0.06	0.01
PT										1.00	-0.02
SC											1.00

[a]-1% significance, [b]-5% significance, BR Bract size, PC Petal color, BSZ Boll size, BSP Boll shape, LSZ Leaf size, LSP Leaf shape, LOB Leaf lobbing, LC Leaf color, NE Nectaries, PT Plant type, SC Stem color

Table 6. Shannon–Weaver diversity indices (H') of various traits

Traits	**Symbol**	**H'**
Bract size	BR	6.670
Petal color	PC	6.668
Boll size	BSZ	6.680
Boll shape	BSP	6.492
Leaf size	LSZ	6.676
Leaf shape	LSP	6.472
Leaf lobbing	LOB	6.691
Leaf color	LC	6.686
Nectaries	NE	6.570
Plant type	PT	6.695
Stem color	SC	6.702
Average		6.630
Sd		0.02

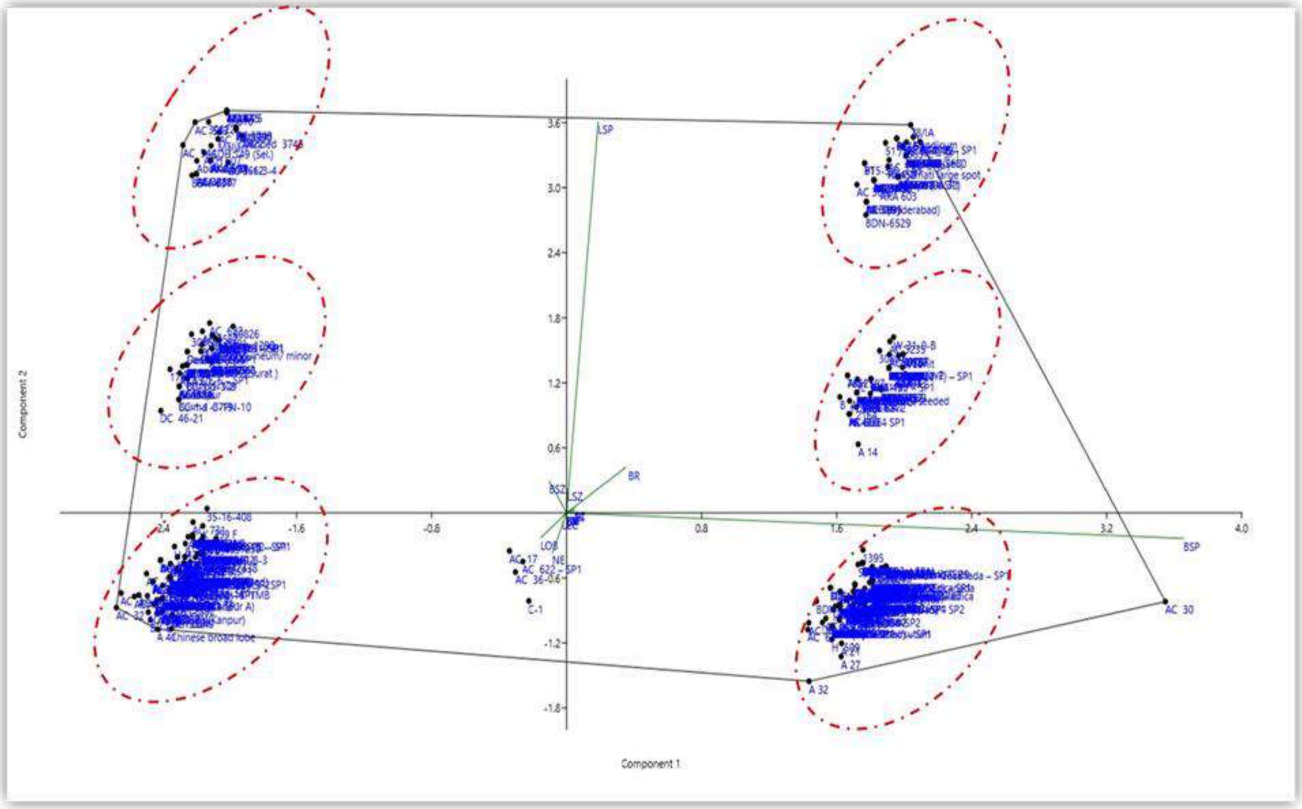

Fig. 1. Scatter plot based on PC1 and PC2 of qualitative characters

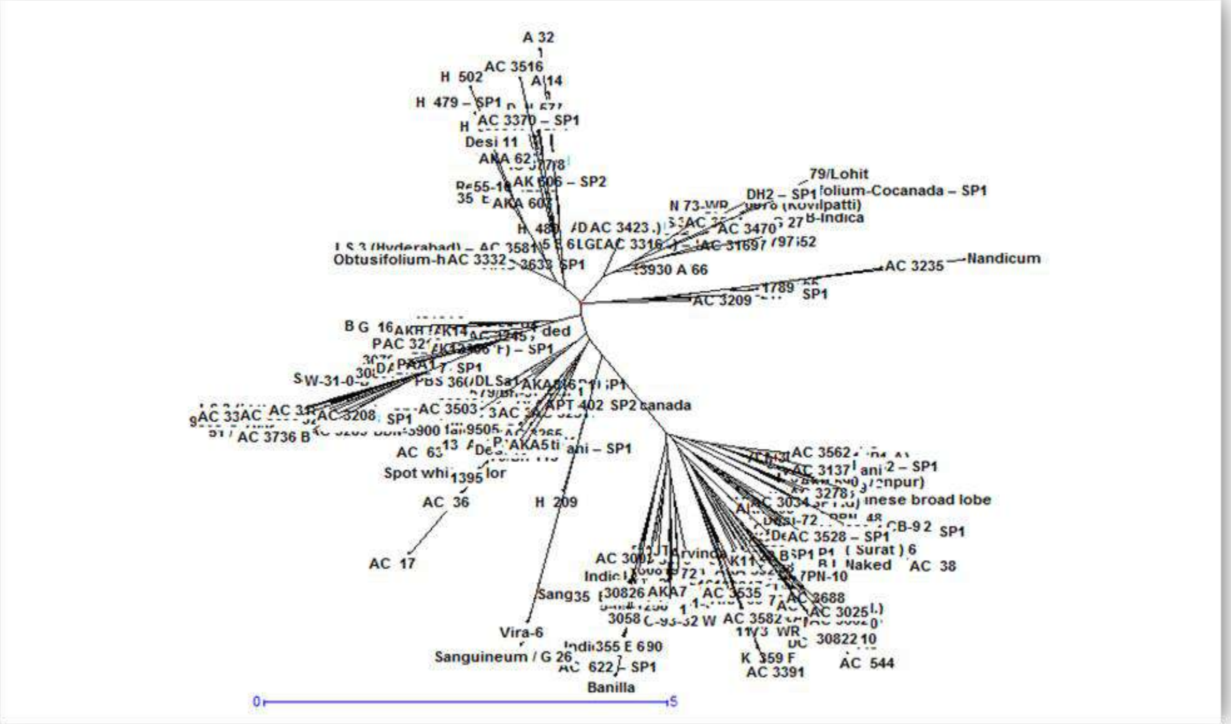

Fig. 2. Clustering pattern of *desi* cotton (*G.arboreum*) accessions.

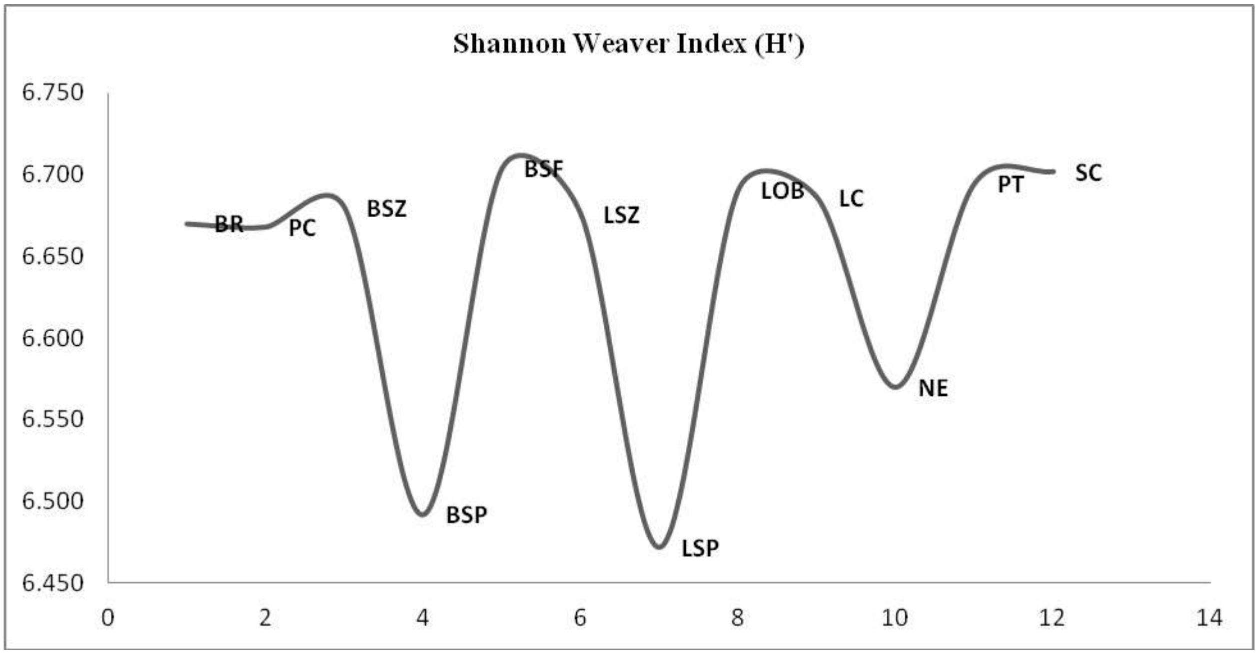

Fig. 3. Shannon–Weaver diversity indices (H') of various traits.