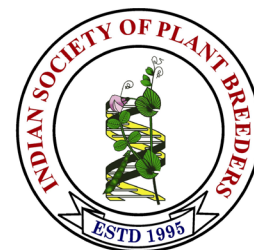# Electronic Journal of Plant Breeding

Research Article

Morphological characterization of *Gossypium arboreum* germplasm using qualitative descriptors

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Abstract
The study was undertaken to determine the Distinctness, Uniformity and Stability characteristics of fifty-seven germplasm accessions of Asiatic cotton. The experiment was carried out using Augmented Block Design during summer 2021. Based on dissimilarity matrix scores, the genotypes were divided into five distinct clusters. Cluster V had the most genotypes (29), while Cluster II had only one genotype (PDB 4). Seven principal components were defined through Principal Component Analysis (PCA), accounting for 84.43 per cent of the total variation. The first principal component (PC 1) was associated with boll tip (0.62) and boll shape (0.56). The second principal component (PC 2) was associated with boll color (0.43) and stem pigmentation (0.38). The trait boll colour had the highest diversity index (4.04). Petiole pigmentation (3.532) had the lowest diversity index, followed by leaf nectaries (3.640) and leaf pubescence (3.742). Genotypes namely PDB 4, CNA 1054, PDB 29, DWDA 1602, NDLA 3086 and MDL 2663 occupied convex of the hull and had the highest point among the variables.

Key words: DUS characters, Asiatic cotton, clusters, principal component analysis

INTRODUCTION

Gossypium spp. is widely cultivated for natural fibre and seed oil all over the world. *Gossypium* is a genus having 50 species, comprising five allotetraploids and 45 diploids widespread across the country (Fryxell, 1992; Wendel and Cronn, 2003). *Gossypium* species exhibit a wide range of phenotypical diversity and also a great deal of variation in both vegetative and flowering characteristics (Wendel and Cronn, 2003). The world's textile fibre is obtained only from four cotton species, comprising two tetraploid New World cotton and two diploid Old World Asian-African cotton. Depending upon the meiotic-chromosomal pairing pattern, the diploid *Gossypium* species are categorized into different genomes labelled as A-G and K genomes (Wendel and Cronn, 2003). The tetraploid species evolved from the hybridization of diploid *Gossypium*

herbaceum and *Gossypium arboreum* having A genome with diploid wild species of *Gossypium raimondii* and *Gossypium gossipioides* L. (Wendel, 1989). Descriptors of varieties of crop species are mandatory for characterisation of varietal identity, assessment of varietal purity and distinguishing the uniqueness of a novel variety from existing varieties for genetic resource documentation. The genotype was characterised using the national DUS test standards of the cotton crop for qualitative morphological features specified by the Protection of Plant Varieties and Farmers' Rights Act (2001), New Delhi.

In the crop improvement programme, seed cotton yield is the summation of all of its component attributes and

it can be strengthened by utilizing the positive effects of yield components. Genetic variation among the genotypes and its direct and indirect consequences were widely studied using the Principal Component Analysis, Correlation Analysis and Clustering approaches (Brown-Guedira *et al*., 2000). PCA was used to determine genetic variation among phenotypic variables, leading to the discovery of phenotypic diversity in cotton (Saravanan *et al*., 2006; Esmail *et al*., 2008; Li *et al*., 2008). With this in consideration, the proposed study was aimed at investigating genetically divergent genotypes by combining DUS morphological characteristics with associated agronomic features.

MATERIALS AND METHODS

The 57 diverse accessions of *Gossypium arboreum* were raised in Augmented Block Design in uniform environmental conditions at the Department of Cotton, Tamil Nadu Agricultural University, Coimbatore, India during summer, 2021. The spacing adopted was 75 x 45 cm and all the agronomic practices were followed. At the appropriate development phase, data on 15 qualitative attributes were recorded using the DUS general guidelines proposed by PPV & FRA, 2001. Leaf colour, leaf pubescence, leaf nectaries, leaf petiole pigmentation and leaf shape were documented based on a visual inspection of the fourth leaf from the top of the crop. The presence or absence of stem pigmentation, stem hairiness, flower attributes *viz*., petal colour, petal spot, stigma type and pollen colour, boll qualities *viz*., boll colour, boll shape, prominence of boll tip and boll surface were documented at corresponding stages of the crop. Five randomly selected plants were studied in each accession. Variation was absent for the characters *viz.,* boll surface, petal spot, pollen colour and stigma type, whereas the remaining eleven traits have shown variation under study.

Principal component analysis was used to examine the proportionate value of different variables in capturing genetic diversity in Asiatic cotton based on eleven qualitative characteristics. PCA identifies the variable or trait which is responsible for classifying the population into clusters/groups. The trait that contributes maximum variation is generally considered for selection (Santhy *et al*., 2020). PCA was worked out using PAST 3 standardized values (Hammer *et al*., 2001). The Eigenvalues linked with a factor were shown in descending order against the number of the factors in a scree plot. A Scree plot is used to visually assess which components are responsible for the majority of the total variations. Using XLSTAT 2021, the Pearson correlation coefficient for eleven qualitative attributes was computed and a correlation matrix was constructed for comparing various characteristics. Using the scores of dissimilarity matrix, hierarchical clustering (Ward, 1963) was worked out using the traits leaf colour, leaf pubescence, leaf nectaries, petiole pigmentation, leaf shape, stem hairiness, stem pigmentation, petal colour, boll colour, boll shape and boll tip.

RESULTS AND DISCUSSION

In contrast to quantitative characteristics, qualitative characteristics are extremely useful in the characterisation of germplasm since they are less impacted by the environment (Manivannan *et al.,* 2018). The qualitative characteristics have defined various phenotypic groups that may be used to categorise germplasm. Owing to these attributes, qualitative characteristics are used to define a significant number of DUS characters for plant cultivar registration. Only a portion of these qualitative characteristics are available in reality (Kruskal, 1978). The scores of all 57 genotypes were computed based on observations on eleven attributes (**Table 1**). Most of the genotypes contain green leaf (92.98%); exceptionally, genotypes *viz*., PA 832, CNA 1054, TDA 265 and JLA 1110 had a light green leaf. Three categories of leaf pubescence *viz*., sparse (42.10%), medium (29.82%) and dense (28.07%) were observed. Leaf nectaries were noticed in 26 genotypes (45.61%) and were absent in 31 genotypes (54.38%). Nectariless cotton is beneficial in reducing tarnished plant bug attacks as well as managing pink bollworm damage (Carty *et al.,* 1983). The trait petiole pigmentation was found in 18 genotypes (31.57%) and the remaining 39 genotypes had no pigmentation in their petiole. Among the genotypes, three categories of leaf shape were observed, a semi-digitate leaf was predominant in 47 genotypes (82.45%) compared to palmate (K12, MDL 2663, PA 785, RG 763, K11, FDX231, PA 840, LD 1019, RG 856) and digitate type leaf (NDLA3086). In stem hairiness traits, three classes were observed; 25 genotypes contained dense hairs, 23 genotypes were observed with medium hairs and nine genotypes contained sparse hairs. Stem pigmentation was observed in 37 genotypes (64.91%) and was absent in the remaining genotypes. Regarding petal colour, yellow flower was observed in 44 genotypes at a higher proportion (77.19%) than cream (14.03%) and white (7.01%), a distinctly pink colour flower was found in the PDB 4 genotype. The majority of the genotypes (98.24%) had green bolls, with only one genotype PDB 4, having red bolls. A higher frequency of elliptical bolls (63.15%) was observed as compared to ovate (28.07%) and round bolls (8.77%). There were two types of boll tips found: blunt and pointed, with pointed bolls (91.22%) predominating in 52 genotypes and blunt bolls were found in DWDA 1602, FDX 235, MDL 2663, NDLA 3086, and AKA 0262. All the genotypes contained pitted bolls, petal spots, yellow pollen and exerted stigma. Our findings were consistent with those of Manivannan *et al.* (2018), who discovered that traits *viz.,* boll shape, leaf shape and bract size distinguished 816 *G.arboreum* accessions in a higher order than other morphological traits.

PCA in cotton was also studied by Manivannan and Dharajothi (2019), who proposed transferring several associated variables into a few separate principal components which had explained much of the heterogeneity in the original collection. PCA is the most

Table1. Morphological description of *Gossypium arboreum* accessions

Descriptor Traits	Categories	Scores	Type of assessment	No. of genotypes	Frequency%
Leaf Colour	Light green	1	VS	4	7.01
	Green	2		53	92.98
Leaf Pubescence	Sparse	1	VS	24	42.10
	Medium	5		17	29.82
	Dense	9		16	28.07
Leaf Nectaries	Absent	1	VG	31	54.38
	Present	9		26	45.61
Petiole Pigmentation	Absent	1	VS	39	68.42
	Present	9		18	31.57
Leaf Shape	Palmate	1	VS	9	15.78
	Semi-digitate	2		47	82.45
	Digitate	3		1	1.75
Stem Hairiness	Sparse	3	VS	9	15.78
	Medium	5		23	40.35
	Dense	7		25	43.86
Stem Pigmentation	Absent	1	VS	20	35.08
	Present	9		37	64.91
Petal Colour	White	1	VS	4	7.01
	Cream	2		8	14.03
	Yellow	3		44	77.19
	Pink	4		1	1.75
Boll Colour	Green	3	VS	56	98.24
	Red	5		1	1.75
Boll Shape	Round	3	VG	5	8.77
	Ovate	5		16	28.07
	Elliptic	7		36	63.15
Boll Tip	Blunt	1	VG	5	8.77
	Pointed	9		52	91.22

VG-Visual assessment by a single observation of a group of plants or parts of plants

VS-Visual assessment by observations of individual plants or parts of plants

extensively used multivariate statistical approach for reducing data among independent and interdependent variables while satisfying the properties of the dataset that contribute far more to its variance by discarding higher-order principal components and maintaining lower-order ones. The principal component analysis of the qualitative data generated from 57 genotypes indicated five independent variable groups accounting for 68.72 per cent of total variation (Eigenvalue greater than one). When variables are inter-correlated, the PCA is an effective data reduction technique (Pearson, 1901). Seven principal components that accounted for 84.43% of the total variation were identified under principal component analysis. The PC contributed 17.37, 15.64, 13.31, 11.62, 10.77, 8.77 and 6.92 per cent of the total variation for the first to seventh components, respectively (**Table 2**). The morphological characters *viz.*, boll tip (0.62) and boll shape (0.56) belonged to the category of PC 1. The second principal component was related to boll colour (0.43) and stem pigmentation (0.38). The third principal component was associated with leaf shape (0.45) and stem pigmentation (0.39). PC 4 was correlated with leaf nectaries (0.52) and petal colour (0.43); PC 5 was related to stem pigmentation (0.37) and petiole pigmentation (0.35). Likewise, the sixth and seventh principal components were associated with leaf pubescence (0.56), leaf colour (0.49) and leaf shape (0.74) and leaf nectaries (0.47), respectively (**Table 3**).

A scatter plot (**Fig. 1**) incorporating PC1 and PC2 factor scores revealed a clear pattern of clustering between genotypes in the factor plane. The genotypes PDB 4, CNA 1054, PDB 29, DWDA 1602, NDLA 3086 and MDL 2663 occupied the convex of the hull, and these genotypes had the greatest point among the factors. Based on all the principal components, scatter plot and scree plot, the strongest variation was recorded for boll tip and boll shape, while moderate variability was registered for stem hairiness and leaf shape. All other morphological characteristics showed a low level of diversity (**Fig.2**).

Genotypes *viz.,* DAS 1021, JLA 1227, CNA 1039, PA 363, FDX 492 and CJHK 13132 were found to be in a separate group surrounding the origin. Agglomerative Hierarchical Clustering was worked out using Ward's approach for the traits leaf colour, leaf pubescence, leaf nectaries,

Table 2. Principal component analysis

Principal Component	Eigen value	Variance%	Cumulative variance%
PC1	1.91	17.37	17.37
PC2	1.72	15.64	33.01
PC3	1.46	13.31	46.32
PC4	1.27	11.62	57.95
PC5	1.18	10.77	68.72
PC6	0.96	8.77	77.50
PC7	0.76	6.92	84.43
PC8	0.57	5.19	89.62
PC9	0.54	4.92	94.54
PC10	0.37	3.38	97.93
PC11	0.22	2.06	100.00

Table 3. Principal component analysis of various traits

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11
BC	0.04	0.43	0.22	-0.01	-0.52	0.33	-0.07	-0.13	-0.29	0.50	0.00
BS	0.56	0.30	-0.02	-0.12	0.16	-0.22	0.16	-0.01	0.00	0.03	-0.68
BT	0.62	0.16	-0.05	-0.12	0.17	-0.07	0.03	0.15	-0.12	0.02	0.69
LC	-0.22	0.18	-0.19	-0.44	0.19	0.49	0.36	0.49	0.06	0.03	-0.05
LN	0.09	-0.01	-0.43	0.52	0.10	0.25	0.47	-0.34	0.20	0.25	0.07
LP	0.37	-0.16	0.31	0.01	-0.19	0.56	-0.12	-0.06	0.46	-0.37	-0.04
LS	-0.02	-0.19	0.45	-0.15	-0.29	-0.27	0.74	-0.05	0.02	-0.04	0.10
PC	-0.03	0.37	-0.13	0.43	-0.41	-0.23	0.02	0.55	0.26	-0.21	0.01
PP	-0.13	0.36	0.34	0.36	0.35	0.19	0.14	-0.08	-0.43	-0.46	0.00
SH	0.13	-0.41	0.34	0.38	0.23	0.08	-0.02	0.50	-0.12	0.43	-0.12
SP	-0.20	0.38	0.39	-0.02	0.37	-0.15	-0.08	-0.11	0.59	0.30	0.13

BC-Boll Colour, BS-Boll Shape, BT-Boll Tip, LC-Leaf Colour, LN-Leaf Nectaries, LP-Leaf Pubescence, LS-Leaf Shape, PC-Petal Colour, PP- Petiole Pigmentation, SH-Stem Hairiness, SP-Stem Pigmentation
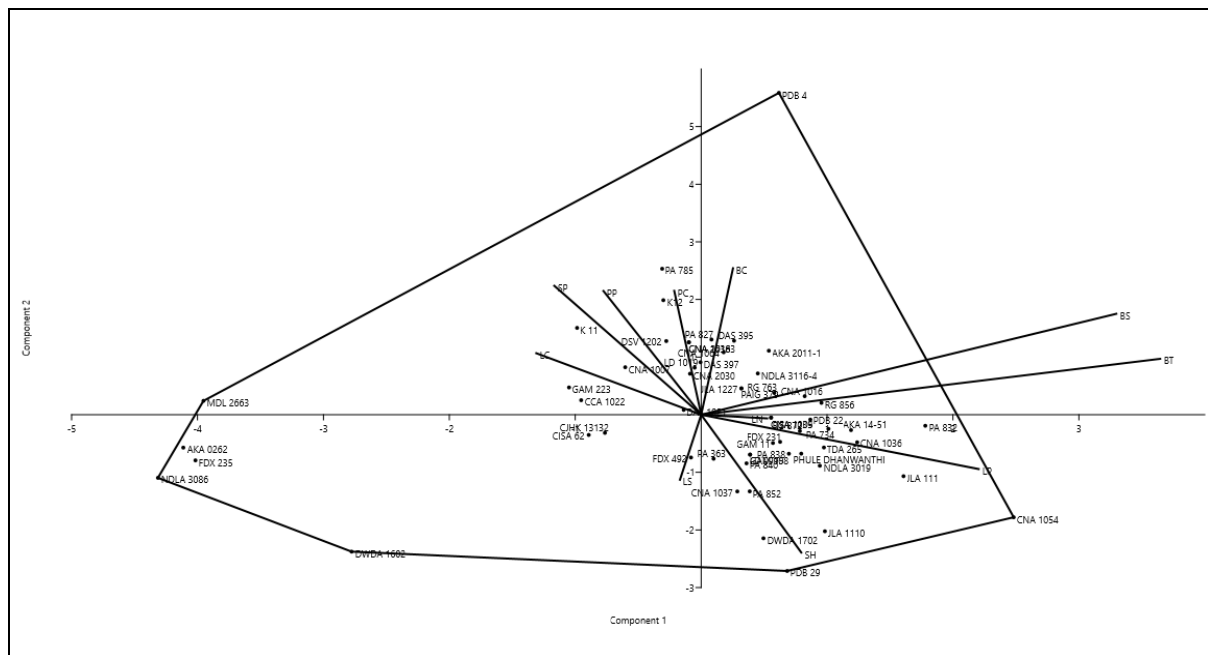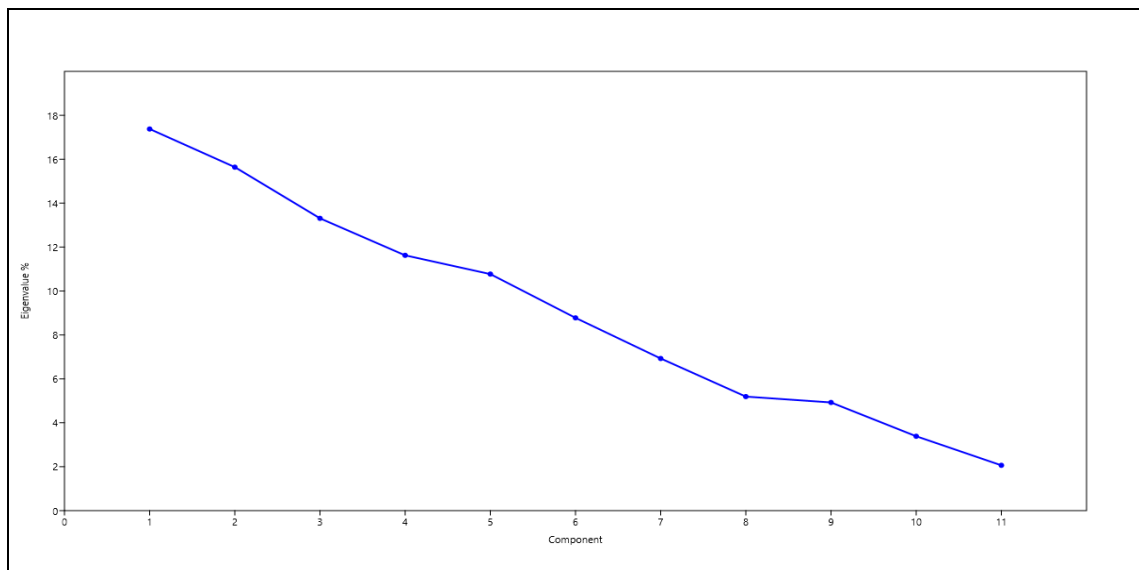

Fig.1.Scatter plot based on PC1 and PC2
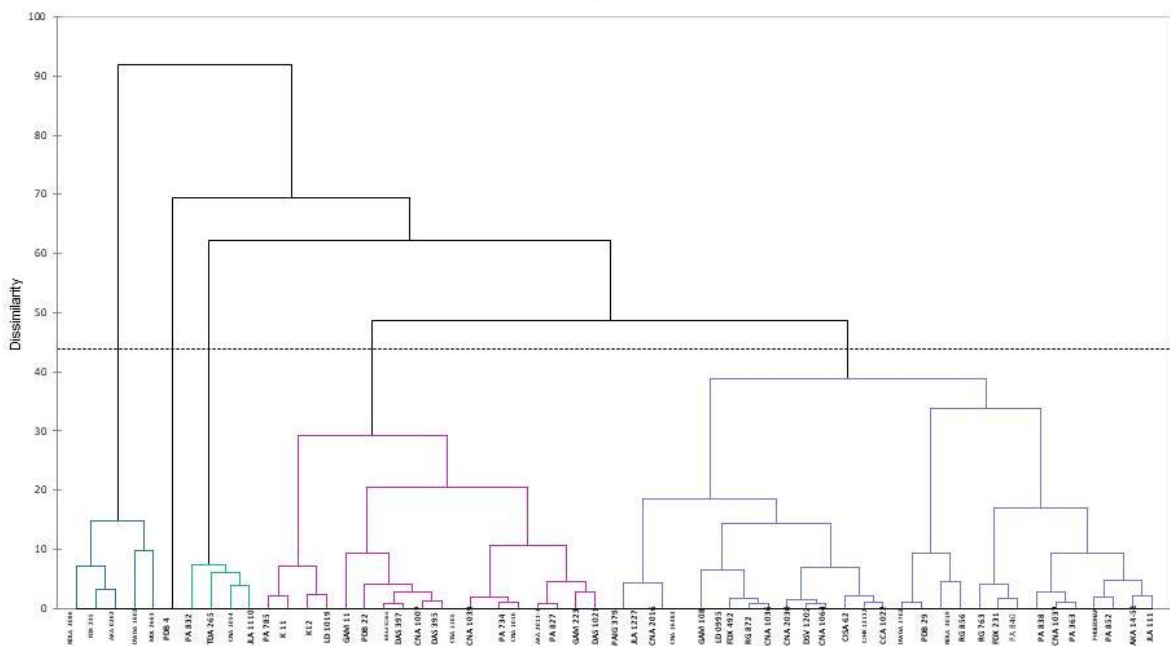
Fig. 2. Scree plot based on Eigen values

Fig. 3. Agglomerative Hierarchical Clustering pattern of *G. arboreum* accessions

petiole pigmentation, leaf shape, stem hairiness, stem pigmentation, petal colour, boll colour, boll shape and boll tip. A dissimilarity percentage of 44 per cent was observed which revealed five unique clusters based on standardised Euclidean distance (**Fig. 3**). The Pearson correlation coefficient was worked out among the qualitative traits (**Table 4**). Boll colour was shown to be positively and strongly linked to petal colour. Boll shape was discovered to be positively correlated with boll tip. Leaf colour was negatively correlated with stem hairiness. A positive association was observed between leaf pigmentation and stem hairiness. Petiole pigmentation was positively associated with stem pigmentation (**Fig. 4**). Shannon-Weaver diversity indices (H') were calculated

Table 4. Correlation between different traits

Traits	BC	BS	BT	LC	LN	LP	LS	PC	PP	SH	SP
BC	1.00	0.09	0.04	0.03	-0.12	0.18	0.04	0.27[b]	0.19	-0.23	0.09
BS		1.00	0.73[a]	-0.08	0.04	0.14	-0.04	0.05	0.01	-0.10	0.05
BT			1.00	-0.08	0.03	0.24	-0.11	-0.03	-0.05	0.03	-0.09
LC				1.00	-0.02	-0.13	-0.09	-0.11	0.03	-0.27[b]	0.08
LN					1.00	-0.01	-0.21	0.16	0.06	0.03	-0.21
LP						1.00	0.10	-0.14	-0.06	0.27[b]	-0.12
LS							1.00	-0.08	-0.04	0.13	0.01
PC								1.00	0.10	-0.15	0.04
PP									1.00	0.10	0.42[a]
SH										1.00	-0.07
SP											1.00

[a]-1% significance, [b]-5% significance, BC-Boll Colour, BS-Boll Shape, BT-Boll Tip, LC-Leaf Colour, LN-Leaf Nectaries, LP-Leaf Pubescence, LS-Leaf Shape, PC-Petal Colour, PP- Petiole Pigmentation, SH-Stem Hairiness, SP-Stem Pigmentation
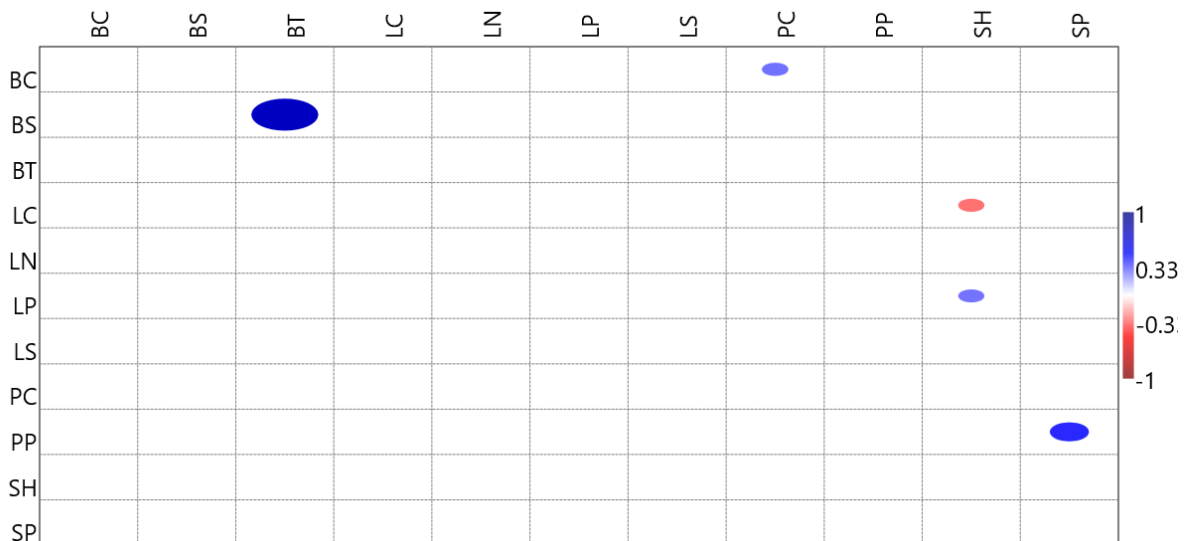

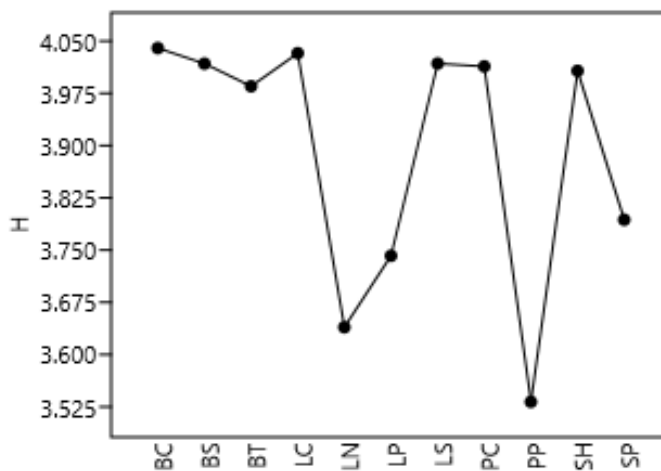**Fig.4.Correlation between different traits**

Fig.5. Shannon-Weaver diversity indices (H')

Table 5. Shannon–Weaver diversity indices (H') of different traits

Descriptors	Symbols	H'
Boll colour	BC	4.040
Boll shape	BS	4.018
Boll tip	BT	3.985
Leaf colour	LC	4.033
Leaf nectaries	LN	3.640
Leaf pubescence	LP	3.742
Leaf shape	LS	4.018
Petal colour	PC	4.014
Petiole pigmentation	PP	3.532
Stem hairiness	SH	4.007
Stem pigmentation	SP	3.794

for various characteristics (**Table 5**), with the trait boll color achieving the highest diversity index (4.040). The trait petiole pigmentation recorded the lowest diversity index (3.532), followed by leaf nectaries (3.640) and leaf pubescence (3.742). As indicated by this trend, lower indices discriminated against genotypes in a greater order than other variables (**Fig.5**). Manivannan and Waghmare (2020) and Rathinavel (2018) used clustering analysis for grouping cotton genotypes. Germplasm characterization plays a pivotal role in identifying and selecting genotypes for further genetic improvement. Based on our studies, NDLA 3086 of digitate leaves, PDB4 with pink flower and red boll, DWDA 1602, FDX 235, MDL 2663, NDLA 3086 and AKA 0262 with blunt boll tips, PA 832, CNA 1054, TDA 265 and JLA 1110 with light green leaves were easily distinguished and identified for further breeding work.

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