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Diversity studies in extant diploid cotton genotypes with seed image and fiber characteristics

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ABSTRACT : An experiment was conducted to evaluate the diploid cotton genotypes for diversity, using geometry of seeds measured through an image analysis system, fiber properties, and yield contributing traits. Seeds of twenty diploid genotypes were used in this study with 22 parameters. The traits *viz.,* area, width, width X, boll weight, length, height Y, perimeter, and lint weight may be directly used for selection of genotypes with a significant positive association with seed index. In the principal component analysis, the first five components showed Eigen value > 1, with a contribution of 86.83 per cent of variability, the remaining components contribute 13.17 per cent. The first component was attributed by the traits like length, width x, area, boll weight, radial variance, height Y, seed index, lint weight, perimeter, and width may be useful for yield improvement. In the scatter plot, the genotypes like G.Cot.17, G.Cot.21, AKH 081, AKA5, Digvijay, and Ragavendra were found at the vortex of the polygon and showed distinct from other genotypes and they were highly diversified. The cluster analysis grouped the twenty genotypes into five clusters. Among the clusters, cluster II was the largest having 12 genotypes and it was clustered into two groups consists of 9 and 3 genotypes. Cluster V formed the solitary cluster and highly distinct from the rest of the genotypes.

Key words : Clustering, correlation, image analysis, principal components

Cotton is one of the most important commercial cash crops cultivated worldwidefor fiber. The genus *Gossypium* contains 45 diploid and 6 allotetraploid species which spread across temperate and tropical regions of Africa, Australia, Central and South America, Arabia, Indian subcontinent, the Galapagos and Huawei (Fryxell, 1992). There are four domesticated species of cotton, two tetraploid cultivars from America, *Gossypium hirsutum* (L.) and *G. barbadense*, and two diploid cultivars from Africa and Asia, namely *G. arboreum* and *G. herbaceum* (Wendel *et al.,*2002). Based on chromosomal affinity studies, species of the genus *Gossypium* are grouped into eight (A-G & K) diploid genomes (Beasley, 1940; Endrizzi *et al.*, 1985; Stewart, 1994). Members of each genome group have morphological resemblance but they rarely form hybrids with species of other genome groups.The *G. arboreum* might be evolved from *G. herbaceum* because *G. arboreum* differs from *G. herbaceum* only at single reciprocal chromosomal translocation (Gerstel, 1953; Hutchinson, 1954). The visually assayable morphological descriptors are being employed in the process of varietal characterization and seed quality regulations. Grow out test is employed to determine/confirm the genetic purity of the crop species. The morphological descriptor has a traditional significance due to its immediate visual access and does not require any equipment for the assay. However, this approach demand huge land resources, a laborious process and time-consuming, also very much depend on the degree of expertise of the observer. The majority of the descriptors require subjective decisions on minor distinctive ness due to the interaction of cultivars with the environment. To minimize or eliminate the influence of the environment on the genotype or a variety an alternate direct approach is the most desirable. A computerized system that captures and process morphological information of seed image is attempted as a technique for identification of variety as well distinctiveness.

Varietal characterization through image analysis technique was successfully developed by studying the geometry of seeds *viz.,* area, perimeter, length, width, elongation, centroid, circularity, average radial, radial variance, CMRV and shape factor. The seed imaging technique is one such system which offers more prospect to the researcher and also allow to study the seed surface features more closely and hence offer a chance to increase the available character set. Image analysis enables to handle and process large amounts of seed material for examination in a relatively short time (Barn and Baitley, 1987). Thus, it has a potential use in wide range of tasks such as determining the cultivar identity of seed lots, testing of the distinctness of new cultivars for the award of breeder's rights and cultivar registration (Keefe and Draper, 1986). In these processes knowledge on the association of different traits is important which facilitates for more accuracy and desirable decision making. The studies on diversity are also important for evolving the genotypes that need to meet the future demands. The rationale behind the present study was to use the geometry of seed image of diploid cottons as a tool to find out the diversity among the genotypes.

MATERIALS AND METHODS

The study was conducted using the seeds of diploid cotton varieties AKH 081, G.Cot.21, AKA8401, G.Cot. 23, AKA 5, Jawahar Tapti, AKA 7, Jayadhar, Aravinda, PA 183, Digvijay, PA 255, G.Cot.13, Ragavendra, G.Cot.15, Sujay, G.Cot.17, Veena, G. Cot. 19, Y 1. Seeds were sown in the field for maintenance breeding, from this crop true to type bolls were harvested for the measurement of boll weight (g/boll), lint weight (g/boll) and seed Index (g). From the lint separated from these bolls, fiber properties such as fiber length (2.5% span length), maturity (%), uniformity (%), fiber fineness (micronaire), Fiber strength (g/tex), elongation (%) were recorded using High Volume Instrument (Premier) under ICC mode. The seeds were defuzed, cleaned, and seeds with complete shapes used for capturing the images. For image characteristics, images of 25 seeds were taken in every replication of individual genotypes. The geometry of seed Image was measured using Delta-T© (Delta-Instrument Device- Cambridge, UK) image analysis system by running custom-written software 'winDIAS' (Nick and Jekins, 2000).

In every replication, 25 seeds were placed on lighting hood in such a way that the embryo axis of seed facing Image Analysis System and the longitudinal axis of the seed running parallel to the surface of the camera lens. Seeds were viewed with a video camera (DSP surveillance color CCD camera CVS 200/3300) using transmitted light so that a binary image of the silhouette of the seed was recorded by the winDIAS. The image of the support was removed by software after image grabbing in the computer that leaves an image of the object consists of five rows and five columns for geometric data measurements.

Data measurement : Before going for actual measurement, calibration done by placing a transparent plastic ruler on the lighting hood illuminated from below. A ruler was aligned diagonally across the field of view, and the focus was adjusted to sharpen the image. Again aperture adjustment was made until optimum color and contrast achieved. Input length is given in centimeter.

Descriptors like area, perimeter, length, and width, from the menu object meter, were selected. After setting, the image was grabbed using image grabber, and the color threshold was done until the entire area was highlighted. For logging the data, the measurement button was pressed; the entire data was extracted each time by clicking entire objects. Data were viewed from the review module, and the data of each parameter were summed up for average value in the win DIAS. The seed parameters measured were as below Area, Perimeter, Length: Distance between two points marked on screen using the mouse (or) diameter of the smallest circumscribed circle that will fit around an object, Width: Width is measured in horizontal X-axis, Elongation: It is the ratio of the width and length $E = \frac{W}{l}$

Centroid: It is the most central point or centre of gravity of the object (measured from the top left-hand corner of the screen). winDIAS calculates it using an algorithm based on the following equation.

$$\overline{Y} = \frac{\frac{1}{6} * \sum[(Y2\,i + 1 + Y2\,i + 1 * Yi + Y2\,i)\,(Xi + 1 - Xi)]}{\frac{1}{2}(X\,i + 1 + X)(Yi - Yi + 1)}$$

$$\bar{x} = \frac{1/6 * \sum[(X2\,i + 1 + X2\,i + 1 * Xi + X2\,i)\,(Yi + 1 - Yi)]}{\frac{1}{2}(Y\,i + 1 + Y)(Xi - Xi + 1)}$$

Circularity is the square root of the ratio of the actual area of the object to the area of a circle with the same circumscribed diameter.

$$\mathbf{C} = \sqrt{\frac{\mathbf{A}}{\mathbf{Ap}}}$$

Where, A is the actual area of the object and A_p is he area of a circle with a diameter equal to the circumscribed diameter of length of the object. Radial variance: It is square of the standard deviation of all distances measured from the centroid to each perimeter point.

$$\text{RV} = \frac{\sum(Rj - \bar{R})2}{n - 1}$$

CMRV: It is the correlation of the Average Radial and Radial Variance.

$$\text{CMRV} = \frac{\text{RV}*100}{\overline{\text{R}}}$$

Shape factor (S factor): Shape factor is the ratio of the actual perimeter to that of a circle with the same area.

$$\text{S} = \frac{P}{Pc}$$

Where P is the perimeter of the object and Pc is the perimeter of a circle with the same area as the object. Pc is calculated as follows.

$$(Pc = 2\ (\pi * A)^{0.5}$$

Where, A is the actual area of the object.

Table 1. Variability parameters of seed characters, fiber quality and yield component traits among the diploid extant cotton genotypes

Characters	**Mean**	Minimum	Maximum	Range	Standard deviation
Area	**39.99**	31.30	60.76	29.46	6.37
Perimeter	**38.21**	32.97	52.45	19.48	6.06
Cent. X	**55.53**	24.19	146.75	122.56	29.07
Cent. Y	**68.87**	30.77	130.28	99.51	22.21
Length	**9.60**	8.23	13.17	4.94	0.94
Width	**6.01**	5.07	6.93	1.86	0.50
Rad. Var.	**20.19**	13.22	42.24	29.02	6.15
CMRV	**1.00**	31.32	160.65	129.33	34.18
Circularity	**0.74**	0.67	0.82	0.15	0.04
Elongation	**0.63**	0.51	0.75	0.24	0.06
S factor	**1.71**	1.39	2.28	0.89	0.23
Width X	**8.29**	7.20	10.25	3.05	0.66
Height Y	**7.33**	6.29	9.85	3.56	0.71
Fiber length (2.5% span length)	**23.13**	19.70	26.90	7.20	2.01
Maturity (%)	**0.88**	0.78	0.94	0.16	0.04
Uniformity (%)	**48.54**	46.40	51.13	4.73	1.20
Fiber fineness (micronaire)	**5.23**	4.25	6.80	2.55	0.64
Fiber strength (g/tex)	**20.75**	19.00	22.17	3.17	0.93
Elongation (%)	**6.38**	5.90	7.00	1.10	0.32
Boll weight (g/boll)	**1.60**	1.11	2.67	1.56	0.30
Lint weight (g/boll)	**0.88**	0.55	1.74	1.19	0.25
Seed Index (g)	**7.21**	5.61	9.29	3.68	0.88

The correlation, principal component analysis were done using the SPSS 16.0 software. The data on 22 morphological traits for all genotypes were subjected to multivariate hierarchical cluster analysis using the computer software NTSYS pcv2.02i (Rohlf, 1998). The mean values were subjected to hierarchical cluster analysis performed by an un-weighted pair-group arithmetic average method (Sneath and Sokal, 1973) using sequential agglomerative hierarchical nested cluster analysis (SHAN) program. A tree diagram was constructed using the TREEPLOT program of NTSYS pc.

RESULTS AND DISCUSSION

The variation among the genotypes recorded using descriptive statistics is presented in table 1. The trait CMRV showed the greatest variation among the genotypes with range of 31.32 to 160.65 followed by centroid X ranging from 24.19 to 146.75. The trait fiber maturity showed the least variation of 0.78 to 0.94.

The traits such as area, width X, width, boll weight, length, height Y, radial variance, perimeter, and lint weight showed a significant positive correlation with seed index (Table 2). Therefore these traits may directly be considered in the selection process. With reference to inter-correlation, the trait area had a significant positive inter-correlation with length, width, radial variance, width X, height Y, perimeter, boll weight, and lint weight. The perimeter had a positive inter-correlation with seed length, width, radial variance, shape factor, width, height Y, boll weight. The seed length had a positive significant inter-correlation with radial variance, width X, height Y, boll weight, and lint weight. The seed width had a positive inter-correlation with circularity, elongation, width X, height Y, and elongation (%). The radial variance had a significant correlation with width X, height Y, boll weight, and lint weight. Width X with height Y, boll weight and lint weight; Height Y with boll weight and lint weight; fiber maturity with fiber fineness and boll weight with lint weight.

The Principal Component Analysis is a powerful tool to obtain parental lines for a successful breeding program (Akter *et al.*, 2009). In the principal component analysis, the most commonly used criterion for resolving the number of components is the Eigen value criterion. Five components depicted the Eigen value more than 1, which contributes 86.83 per cent variability and the remaining components contributed 13.17 per cent of the variability. The traits like length (0.940), width X (0.912), area (0.902), boll weight (0.898), radial variance (0.849), height Y (0.834), seed index (0.832), lint weight (0.788), perimeter (0.678) and width (0.554) together contributed 41.124 per cent and had shown a considerable positive factor loadings on PC 1 (Table 3 and Fig.1). For the second component, elongation (0.887), elongation per cent (0.815), circularity (0.791) and width (0.762) contributes 20.928 per cent of variability.

The traits like centroid Y and circularity were the major contributors of PCA 3. The component one is mainly attributed by the seed length, width, area, boll weight, radial variance, height, seed index, and lint weight indicating that these characters contributed the maximum to the total genetic diversity in the population which will be useful for the development of yield improvement in diploid cotton. Draper and Travis

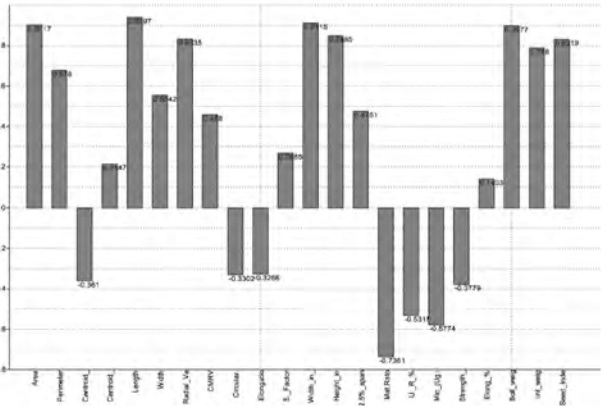

Fig. 1 : PCA factor loadings for component one of diploid cotton.

Table 2. Correlation coefficient between seed parameters, fiber properties and yield contributing traits in extant diploid cotton genotypes

Characters	Area	Perimeter	CentroidX	CentroidY	Length	Width	Radial variance	CMRV	Circularity	Elongat.	S factor	WidthX	HeightY
Area	1.000	0.518*	-0.224	0.376	0.853**	0.755**	0.698**	0.428	-0.007	-0.077	0.028	0.854**	0.897**
Perimeter		1.000	-0.044	-0.132	0.603**	0.486*	0.751**	0.114	-0.287	-0.099	0.869**	0.699**	0.499*
Centroid X			1.000	-0.143	-0.361	0.049	-0.101	-0.275	0.357	0.396	0.083	-0.355	0-.164
Centroid Y				1.000	0.344	0.063	0.130	-.025	0-.071	-0.242	-0.372	0.174	0.447*
Length					1.000	0.358	0.845**	0.398	-0.525*	-0.557*	0.214	0.849**	0.894**
Width						1.000	0.362	0.252	0.554*	0.564**	0.129	0.621**	0.501*
Radial variance							1.000	0.188	-0.441	-0.387	0.473*	0.713**	0.783**
CMRV								1.000	-0.106	-0.166	-0.119	0.428	0.317
Circularity									1.000	0.953**	-0.335	-0.233	-0.249
Elongat.										1.000	-0.074	-0.201	-0.331
S factor											1.000	0.324	0.065
Width X												1.000	0.668**
Height Y													1.000

Contd..

Table 2. Correlation coefficient between seed parameters, fiber properties and yield contributing traits in extant diploid cotton genotypes

Characters	Fiber length	Fiber maturity	Fiber uniformity	Fiber fineness	Fiber strength	Fiber elongation (%)	Boll weight	Lint weight	Seed index
Area	0.313	-0.740**	-0.372	-0.567**	-0.416	0.185	0.707**	0.556*	0.838**
Perimeter	-0.034	-0.602**	-0.135	-0.526*	-0.140	0.416	0.471*	0.390	0.510*
Centroid X	-0.375	0.036	0.247	-0.007	0.377	0.032	-0.436	-0.430	-0.278
Centroid Y	0.188	0.027	-0.061	0.334	-0.395	-0.417	0.198	0.196	0.117
Length	0.407	-0.545*	-0.523*	-0.331	-0.360	-0.072	0.870**	0.823**	0.638**
Width	-0.013	-0.741**	-0.024	-0.739**	-0.269	0.605**	0.237	0.005	0.793**
Radial variance	0.274	-0.553*	-0.425	-0.358	-0.208	0.057	0.730**	0.685**	0.552*
CMRV	0.109	-0.212	-0.290	-0.160	-0.486*	0.039	0.472*	0.424	0.412
Circularity	-0.273	-0.155	0.392	-0.293	0.019	0.458*	-0.523*	-0.683**	0.145
Elongat.	-0.339	-0.213	0.441	-0.390	0.118	0.567**	-0.537*	-0.700**	0.148
S factor	-0.223	-0.273	0.057	-0.286	0.090	0.374	0.137	0.132	0.102
Width X	0.357	-0.604**	-0.465*	-0.545*	-.0474*	0.175	0.744**	0.615**	0.798**
Height Y	0.267	-0.647**	-0.329	-0.361	-0.274	0.022	0.730**	0.654**	0.637**
Fiber length	1.000	-0.271	-0.692**	-0.258	-0.009	-0.350	0.633**	0.626**	0.373
Fiber maturity		1.000	0.171	0.894**	0.001	-0.473*	-0.575**	-0.412	-0.798**
Fiber uniformity			1.000	0.218	0.220	0.098	-0.514*	-0.492*	-0.355
Fiberfineneee				1.000	-0.047	-0.627**	-0.389	-0.200	-0.760**
Fiberstrengh					1.000	-0.109	-0.236	-0.135	-0.424
Fiber elongation						1.000	-0.145	-0.323	0.428
Boll weight							1.000	0.965**	0.676**
Lint weight								1.000	0.459*
Seed index									1.000

Table 3. Principal components of seed characters, fiber properties and yield components of extant diploid cotton genotypes

	Principal components				
Characters	1	2	3	4	5
Length	0.940	-0.240	-0.031	0.147	0.100
Width X	0.912	0.094	0.011	0.113	-0.128
Area	0.902	0.193	0.277	0.135	0.165
Boll weight	0.898	-0.301	-0.007	-0.174	-0.002
Radial variance	0.849	-0.040	0.113	0.206	0.350
Height Y	0.834	-0.070	-0.320	0.146	0.205
Seed index	0.832	0.395	0.247	-0.096	-0.096
Lint weight	0.788	-0.501	-0.098	-0.173	0.028
Maturity ratio	-0.736	-0.513	0.043	0.232	-0.197
Perimeter	0.678	0.289	-0.600	0.240	-0.020
Uniformity ratio	-0.531	0.338	-0.097	0.394	0.172
Elongation	-0.327	0.887	0.232	-0.083	0.045
Elongation (%)	0.140	0.815	-0.167	0.033	-0.328
Circularity	-0.330	0.791	0.478	-0.059	0.097
Width	0.554	0.762	0.246	0.096	0.076
Fiber fineness	-0.577	-0.662	0.084	0.421	0.024
S Factor	0.269	0.224	-0.866	0.201	-0.111
Centroid Y	0.215	-0.336	0.545	0.440	0.445
Fiber length	0.475	-0.385	0.245	-0.653	0.065
Fiber strength	-0.378	0.046	-0.438	-0.540	0.461
Centroid X	-0.361	0.350	-0.196	0.034	0.529
CMRV	0.458	-0.063	0.299	0.085	-0.523
Eigen values	9.05	4.60	2.44	1.61	1.40
Variability (%)	41.12	20.93	11.09	7.32	6.37
Cumulative variability (%)	41.12	62.05	73.14	80.46	86.83

Table 4. Clustering of extant diploid cotton genotypes

Clusters	Groups	No. of genotypes	Name of genotypes
I	1	1	AKH 081
	2	2	AKA 8401, Aravinda
II	1	9	AKA 5, Jayadhar, G.Cot.17, Y1, AKA 7, PA 183, .Cot.19, PA 255, G.Cot.13
	2	3	G.Cot. 21, Jawahar Tapti, Sujay
III	1	2	G.Cot. 23, Veena
IV	1	2	G.Cot. 15, Ragavendra
V	1	1	Digvijay

(1985) separated seeds of 49 different crops and weed species based on their shape in combination with length factor using the image processing system. Myers and Edsall (1989) reported the possibility to discriminate against the Australian wheat varieties using machine vision procedures. Jingbin, *et al.*, (2012) conducted a study for the variety identification of delinted cotton seeds using the color and shape parameters based on BP neural network.

The scatter plot was drawn between the principal components 1 and 2, which explain the arrangement of grouped genotypes in a factor plane (Fig. 2). The distance of the genotypes from the base of the spread-out plot to the outer side of the polygon showed the longest distance from others, which generates greater genetic diversity. The genotypes like G.Cot.17, G.Cot.21, AKH081, AKA5, Digvijay, and Ragavendra were found at the vortex of the polygon and showed distinct from other genotypes and they are highly diversified ones. Malik *et al.,* 2013 has also reported that the cotton genotypes which are placed at the vortex of the polygon showing the longest distance from the base depicted the greatest genetic variability and closer genotypes are found at the origin.

In the cluster analysis, 20 genotypes were grouped into five clusters (Table 4, Fig.3). Among them, cluster II is the largest having 12 genotypes and it is clustered into two groups consists of 9 and 3 genotypes. Followed to this cluster I contained three genotypes. The cluster V forms the solitary cluster having Digvijay. The genotype in the cluster V is distinct and highly divergent with other genotypes and distinguishable.

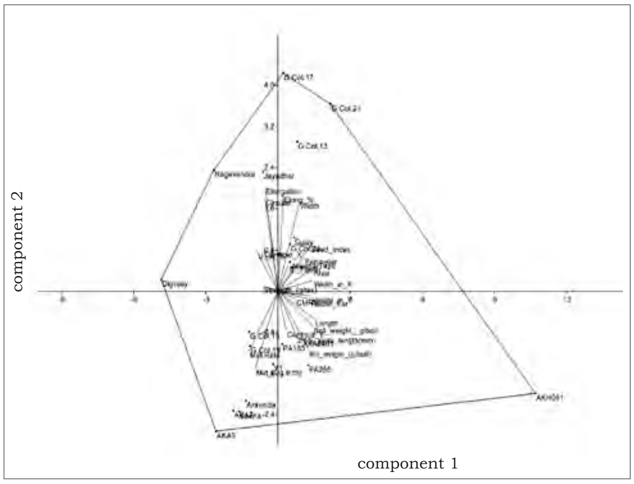

Fig. 2. Scatter plot for extant diploid cotton genotypes with component 1 and 2

Conclusion

Machine vision has been utilized for cultivar description, characterization, and identification of varieties using seeds and plant parts (Draper and Travis, 1984). Finch-Savage (1986) measured the seedling vigor of some vegetables using an image analysis system and reported the positive relationship of the results with seed vigor and field performance. The traits like area, width X, width, boll weight, length, height Y, radial variance, perimeter, and lint weight showed a significant positive association with seed index. These traits are used as selection criteria for a breeding program to develop high yielding cotton varieties. The first five principal component analysis had shown Eigen value > 1, which contributes 86.83 per cent variability. The first component is attributed mainly by the traits like seed length, width, height, and seed index which will be useful for the development of yield improvement in cotton. In the scatter diagram, the genotypes like G.Cot. 17, G.Cot. 21, AKH 081, AKA 5, Digvijay, and Ragavendra have shown distinct from other genotypes and are highly diversified. The cluster

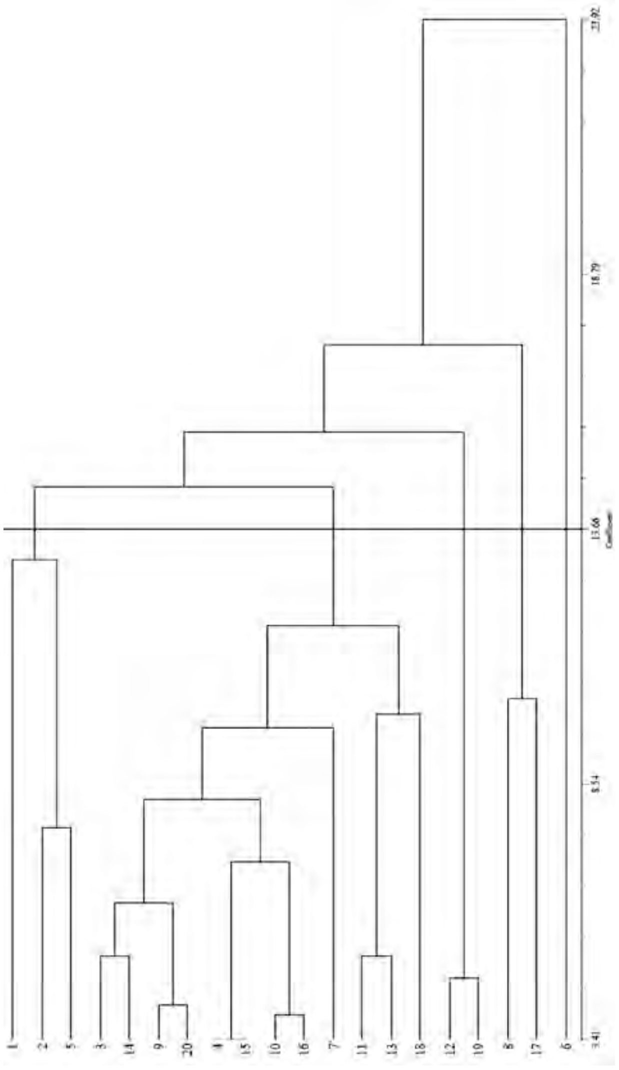

Fig. 3 : Dendrogram based on clusters of various traits in extant diploid cotton genotypes

analysis grouped the genotypes into five clusters with cluster II is the largest containing 12 genotypes. The cluster V was solitary and the genotype distinguishable. The seed image analysis not only useful for varietal identification in cotton and also for identifying the admixed seeds in a seed lot.

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