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BREEDING AND GENETICS

Nature of Genetic Divergence among Some Cotton Genotypes

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ABSTRACT

Inherent divergence and parenthood of germplasm could play an important role in genetic improvement of cotton. The present investigation was conducted to assess the genetic divergence among 14 local cotton genotypes and six exotic genotypes using multivariate Mahalanobis D^2 statistics and metroglyph analysis. The results showed highly significant differences among these genotypes for the studied quantitative characteristics. The Mahalanobis D^2 statistics showed that the dissimilarity coefficients were significant and highly significant, and ranged from 3.263 to 190.89, indicating a high amount of genetic divergence for these cotton genotypes. The metroglyph analysis grouped these genotypes into eight different clusters based on seven quantitative cotton characteristics. The intercluster D^2 values ranged from 11.381 to 178.902 among these groups, whereas the intracluster D^2 values ranged from 3.263 to 47.806. On the basis of this grouping, it was concluded that hybridization between genotypes of different clusters might be expected to give new genetic recombinants for the improvement of economic characteristics. This information could be utilized for hybridization between distinct genotypes to increase cotton genetic variability.

Genetic divergence is the basis for any crop improvement program. The knowledge of genetic variation existing in germplasm is an important and essential aspect for initiating any crop breeding program because hybrids between lines of diverse origin generally display greater heterosis than those between closely related parents. Cotton is no exception with greater heterosis being exhibited in interspecific crosses (*Gossypium hirsutum* L. x *Gossypium barbadense* L.) than in intraspecific crosses (Davis, 1978; Marani, 1967). Nassar (2013) reported evidence of heterosis within pairs of crosses between four Egyptian long-staple cultivars.

Assessing the amount of genetic divergence available for use in crop improvement can take many forms (Mohammadi and Prasanna, 2003). Pedigree analysis is one possibility, but coefficient of parentage per se has not been found to correlate necessarily with cultivar development success (Van Esbroeck and Bowman, 1998). Based upon the analysis of phenotypic data there are several available classification techniques; the simplest being taxonomic dichotomous keys. When metric data on phenotype is available, statistical tests can provide evidence of groupings (Sneath and Sokal, 1973). Genetic diversity as measured using molecular markers holds promise and heterotic qualitative trait loci have been identified (Jia et al., 2014; Mei et al., 2014), but low levels of intraspecific polymorphism have limited their utility. Success in using markers for genomic prediction in a set of diverse maize hybrids was marginal and underscored the value of quality phenotypic data as a useful predictor of progeny performance (Windjausen et al., 2012), reinforcing the earlier conclusion on the importance of quality over quantity of diverse germplasm (Van Esbroeck and Bowman, 1998) in plant improvement.

It is generally accepted that crosses between diffferent groups maximize genetic variability in the resulting progeny and allow for selection progress. One method used to quantify the genetic divergence in a given population is cluster analysis, and this has been used in cotton to select promising plants (Abd El-Baky, 2006; Abd El-Sayyed et al., 2006). Metroglyph analysis is a simple technique used for preliminary grouping of accessions (Anderson, 1957). With the help of this technique, one can predict genotypes that have high index scores and fall into different clusters to be crossed to produce maximum variability of good combinations of characteristics. Metroglyph analysis and index scoring have been used as tools to assess genetic variability within seven cotton cultivars (Khan et al. 2007), and to separate cotton genotypes based upon their reaction to biotic (Haidar et al., 2012) or abiotic stress (Aslam

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et al., 2013). In comparison to cluster analysis, the metroglyph technique has been found to give similar results, but is especially useful when a large number of genotypes need to be separated (Chandra, 1977).

The present study analyzed 20 cotton genotypes using Mahalanobis D^2 technique to estimate the dissimilarity coefficients and metroglyph analysis to classify cotton genotypes into different clusters. Quantification of divergence would be of help to develop better recombinants between groups and in choosing suitable genotypes for cotton breeding programs.

MATERIALS AND METHODS

The present investigation consisted of the selfed seeds of 20 cotton genotypes belonging to *G. barbadense*. Origin and pedigree of these genotypes are shown in Table 1. These genotypes were raised in a completely randomized block design with three replications during the 2012/2013 growing season at the Sakha Experimental Station, Kafr El-Sheikh, Agricultural Research Center, Egypt. Each genotype was sown in two rows 7 m in length spaced at 70 cm between rows and plants. Normal agronomic practices were followed for growing the cotton crop.

At maturity, 10 cotton plants were selected randomly for studying four yield parameters: boll weight in grams, seed cotton yield per plant in grams, lint yield per plant in grams, and lint percentage. Also, three fiber quality characteristics were tested at the Cotton Technology Laboratory, Cotton Research Institute, Agricultural Research Center, Giza, Egypt: fiber length as span length at 2.5% by digital fibrograph, fiber fineness as a micronaire value, and fiber strength as Pressley index.

Data were subjected to an analysis of variance (Steel et al., 1997), followed by calculation of genetic divergence using the Mahalanobis D^2 statistic with the genotypes grouped on the basis of minimum generalized distance as described by Mohammadi and Prasanna (2003) using Toucher's method (Rao, 1952). The inter- and intracluster distance among different groups and the contribution of each characteristic to total genetic divergence as the number of times that characteristic appeared first in ranking was calculated according to Singh and Chaudhary (1979).

Metroglyph analysis using the index score method was also applied (Anderson, 1957). A scatter diagram was plotted taking the two most variable characteristics: seed cotton yield as ordinate (X axis) and lint percentage as abscissa (Y axis). All other characteristics were represented as rays at different positions on the

Table 1. Origin and pedigree of the studied cotton genotypes

No.	Genotypes	Origin	Pedigree
1	Giza 75	Egypt	Giza 67 / Giza 69
2	Giza 85	Egypt	Giza 67 / C.B 58
3	Giza 86	Egypt	Giza 75 / Giza 81
4	Giza 89	Egypt	Giza 75 / Russian 6022
5	10229 / Giza 86	Egypt	10299 // Giza 75 / Giza 81
6	Giza 90	Egypt	Giza 83 / Dandara
7	Giza 90 / Australly	Egypt	Giza 83 / Dandara // Australly
8	Giza 83 // Giza 75 / 5844 /// Giza 80	Egypt	Giza 83 // Giza 75 / 5844 /// Giza 80
9	Giza 45	Egypt	Giza 28 / Giza 7
10	Giza 70	Egypt	Giza 59a / Giza 51b
11	Giza 87	Egypt	(Giza 77 / Giza 45) a
12	Giza 88	Egypt	(Giza 77 / Giza 45) b
13	Giza 92	Egypt	Giza 77 / Pima S7
14	Giza 93	Egypt	Unknown
15	Suvin	Indian	Sujata x Vincent
16	Early Pima	America	Unknown
17	Pima high yield	America	Unknown
18	Pima high percentage	America	Unknown
19	Pima S6	America	5934-23-2-6 / 5903-98-4-4
20	Pima S7	America	6614-91-93 / 6907-513-509-501

glyph. Each ray represented a particular characteristic obtained by dividing the range of variation into three equal classes giving the grades low, medium, and high for each characteristic. The length of ray assigned to the characteristic was dependent upon the index scores of genotype for that characteristic (1 for low value, 2 for medium, and 3 for the highest value). The glyph positions and rays were used to assess the variability pattern and correlated traits for assessment of their divergent groups. Each genotype was assigned a unique number and is represented as a glyph that is the intersection point of mean values of X and Y co-ordinates as described by Khan et al. (2007). The index values and the position of rays and arrows for the different characteristics are given in Table 2. The number assigned each cluster was allotted on the basis of the net index score of the cluster in ascending order.

RESULTS AND DISCUSSION

Mean values of the studied quantitative characteristics among 20 cotton genotypes are shown in Table 3. The analysis of variance using quantitative characteristics revealed that mean squares due to genotypes were highly significant for all the studied characteristics, indicating existence of considerable genetic divergence among these genotypes, reflecting their genetically diverse background, different geographical origins, and pedigrees (Table 4).

Among the genotypes, Giza 86 x 10229 (G5) had the highest index score for all individual characteristics except fiber length and fiber strength, which scored 2 (Table 3). These results suggest that genotypes having high index scores might be crossed to yield maximum variability for good combinations of characteristics. This information should be useful for the breeders interested in creating a desired level of variability for a specific engineering cross.

Genetic divergence was estimated by Mahalanobis D^2 statistic to calculate the genetic dissimilarity coefficients among these cotton genotypes. D^2 values ranged from 3.263 to 190.89, corresponding to all possible combinations among 20 cotton genotypes taking two genotypes at a time. These estimates were treated as Chi-square values, which showed that most dissimilarity coefficients were significant or highly significant. The genetic dissimilarity coefficient was highest between Giza 90 (G6) and Pima high yield (G17) and lowest between Giza 70 (G10) and Giza 88 (G12) (Table 5). These results reflect that the high D^2 value was due to genetic dissimilarity among genotypes, whereas the low D^2 value indicated genetic similarity among genotypes.

The contribution of each characteristic towards total genetic divergence among all combinations of the 20 cotton genotypes was counted as the number of times it appeared first in ranking (Fig. 1 and Table 6). This was used as a criterion for the contribution of each characteristic to the total genetic divergence. The contribution of each characteristic to the genetic divergence showed that seed cotton yield had the highest contribution to genetic divergence (64.211%) followed by fiber strength (19.474%), and was due to genetic dissimilarity among the genotypes for these characteristics. Lint percentage and fiber length were negligible (0.526%) to the total genetic divergence; this might be due to genetic similarity among the genotypes for these characteristics.

Table 2. Class intervals for the studied seven quantitative characteristics

Characteristic	Rang of means	Score I		Score II			Score III	
		Less than	Sign	From	To	Sign	Greater than	Sign
BW	2.49-3.56	2.87	○	2.87	3.09		3.09	
SCY	105.50-283.60	145.90		145.90	191.13		191.13	
LY	42.21-110.87	53.92	○	53.92	71.70		71.70	
L%	32.39-41.87	36.62		36.62	38.80		38.80	
FL	30.79-37.47	33.40	○	33.40	35.26		35.26	
FF	3.06-4.89	3.66	○	3.66	4.12		4.12	
FS	9.17-11.77	10.22	○	10.22	10.85		10.85	

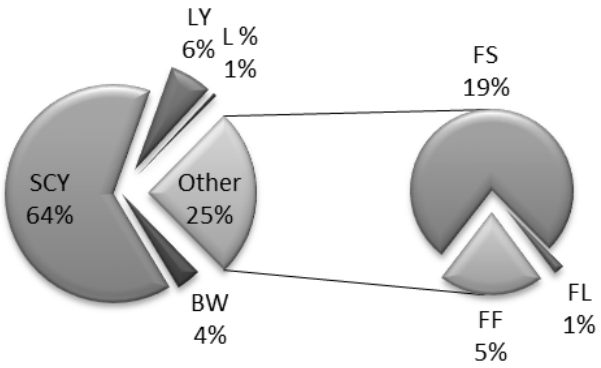

Figure 1. Contribution degree of each characteristic towards total genetic divergence among studied cotton genotypes. BW, boll weight; SCY, seed cotton yield; LY, lint yield; L%, lint percentage; FL, fiber length; FF, fiber fineness; and FS, fiber strength.

According to metroglyph analysis, the 20 cotton genotypes formed eight distinct clusters. Cluster I had the maximum number of genotypes with four; followed by clusters II, III, and IV with three genotypes; clusters V, VI, and VII with two genotypes; and cluster VIII with a unique genotype (Table 7). Cluster I consisted of four genotypes: G6, G7, G8, and G18 and had the highest index score of 47. Cluster VIII had a unique genotype, G13, with the minimum index score of 13. Haidar et al. (2012) used metroglyph analysis to classify 13 locally developed elite cotton genotypes and two exotic lines (*G. hirsutum*) into six clusters for some qualitative and quantitative characteristics.

Table 3. Phenotypic mean performance and index score for the studied quantitative characteristics among 20 cotton genotypes

Genotypes	BW	SCY	LY	L%	FL	FF	FS	Index score
G1	2.944 2	217.489 3	77.500 3	35.830 1	33.489 2	4.033 2	10.189 1	14
G2	2.918 2	197.983 3	72.067 3	36.279 1	33.694 2	3.756 2	10.017 1	14
G3	3.089 2	178.842 2	68.194 2	38.222 2	34.144 2	4.533 3	10.033 1	14
G4	3.369 3	265.933 3	94.856 3	35.799 1	32.844 1	4.889 3	9.700 1	15
G5	3.557 3	260.080 3	105.787 3	40.616 3	34.333 2	4.507 3	10.247 2	19
G6	2.487 1	105.507 1	42.207 1	40.127 3	31.833 1	3.927 2	9.173 1	10
G7	3.000 2	114.087 1	46.660 1	40.827 3	30.960 1	4.620 3	9.800 1	12
G8	3.047 2	140.487 1	57.193 2	40.677 3	30.793 1	4.067 3	10.067 1	13
G9	2.727 1	165.987 2	53.640 1	32.389 1	37.353 3	3.140 1	11.007 3	12
G10	2.847 1	132.053 1	46.607 1	35.361 1	35.993 3	3.833 2	11.187 3	12
G11	3.124 3	155.860 2	54.467 2	34.979 1	37.198 3	3.367 1	11.233 3	15
G12	2.813 1	129.220 1	46.420 1	35.814 1	37.473 3	3.420 1	11.320 3	11
G13	3.247 3	118.993 1	43.800 1	36.828 2	34.893 2	3.527 1	11.400 3	13
G14	2.787 1	169.820 2	63.347 2	37.438 2	36.820 3	3.060 1	11.773 3	14
G15	2.972 2	148.713 2	44.047 1	37.286 2	32.067 1	3.767 2	10.420 2	12
G16	2.983 2	156.789 2	61.700 2	39.243 3	32.950 1	4.128 3	10.200 2	15
G17	3.060 2	283.611 3	110.867 3	38.981 3	33.044 1	4.456 3	10.111 1	16
G18	2.833 1	125.417 1	52.550 1	41.871 3	36.133 3	3.200 1	11.550 3	13
G19	3.176 3	151.900 2	59.933 2	39.839 3	34.189 2	3.733 2	10.111 1	15
G20	2.667 1	151.600 2	54.333 2	35.825 1	36.400 3	3.873 2	11.073 3	14
Mean	2.982	168.519	62.809	37.711	34.330	3.892	10.531	
LSD at 0.05	0.213	38.378	13.220	1.288	0.749	0.222	0.374	
LSD at 0.01	0.283	51.042	17.582	1.713	0.996	0.295	0.497	

Table 4. Mean squares from analysis of variance for the studied quantitative characteristics among 20 cotton genotypes

Source	df	BW	SCY	LY	L%	FL	FF	FS
Replications	2	0.010	506.866	22.830	1.021	0.175	0.042	0.177
Genotypes	19	6.253**	24825.504**	3529.185**	992.960**	821.728**	10.980**	77.499**
Error	38	0.017	552.320	65.533	0.622	0.210	0.018	0.052

*,** Significant at 0.05 and 0.01, respectively.

Table 5. Genetic divergence estimated by Mahalanobis D^2 among the studied 20 cotton genotypes

Genotypes	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G17	G18	G19	G20
G1	.00																			
G2	20.25	.00																		
G3	**39.83**	19.64	.00																	
G4	**51.47**	**71.68**	**91.10**	.00																
G5	**51.36**	**70.80**	**89.50**	13.40	.00															
G6	**117.5**	**97.27**	**77.80**	**168.90**	**167.00**	.00														
G7	**108.1**	**87.82**	**68.30**	**159.40**	**157.00**	9.789	.00													
G8	**79.82**	**59.62**	**40.10**	**131.00**	**129.00**	**38.08**	28.43	.00												
G9	**57.00**	**37.32**	20.60	**108.20**	**107.00**	**62.31**	**53.46**	27.86	.00											
G10	**90.89**	**70.72**	**51.60**	**142.30**	**141.0**	27.71	19.50	15.48	**34.81**	.00										
G11	**65.91**	**45.82**	27.18	**117.30**	**116.00**	**52.40**	**43.39**	17.86	10.49	25.11	.00									
G12	**93.67**	**73.50**	**54.30**	**145.10**	**143.00**	25.20	17.33	17.70	**37.62**	3.26	27.84	.00								
G13	**104.10**	**83.91**	**64.60**	**155.50**	**154.00**	14.50	8.217	25.98	**48.28**	13.49	**38.49**	10.92	.00							
G14	**49.89**	**29.73**	10.86	**101.20**	**99.80**	**67.99**	**58.62**	**30.80**	**11.63**	**41.38**	16.74	**44.02**	**54.49**	.00						
G15	**76.50**	**56.71**	**38.60**	**127.70**	**127.00**	**43.36**	**34.93**	15.93	**21.05**	17.43	13.86	20.44	**29.87**	29.03	.00					
G16	**62.81**	**42.59**	23.04	**114.10**	**112.00**	**54.89**	**45.34**	17.11	**14.75**	**29.41**	9.543	**32.05**	**41.95**	13.94	19.53	.00				
G17	**74.13**	**94.05**	**113.00**	24.07	24.16	**190.00**	**181.00**	**152.80**	**131.00**	**164.60**	**139.70**	**167.30**	**177.70**	**123.40**	**150.5**	**136.2**	.00			
G18	**95.63**	**75.41**	**55.80**	**146.90**	**144.00**	23.04	14.00	16.78	**41.69**	11.05	**31.29**	9.520	12.04	**45.91**	25.57	**32.98**	**168.6**	**.00**		
G19	**68.02**	**47.79**	28.23	**119.30**	**117.00**	**49.73**	**40.22**	12.25	17.46	24.41	8.92	26.93	**36.80**	18.67	16.54	5.39	**141.2**	**27.68**	**.00**	
G20	**69.91**	**49.74**	**30.77**	**121.30**	**120.00**	**48.11**	**39.02**	13.70	14.85	21.02	4.47	23.76	**34.32**	20.42	11.64	10.28	**143.6**	**26.94**	**7.32**	**.00**

bold Significant at 0.05 and 0.01 (X^2 at 0.05 and 0.01 for 18 degrees of freedom = 28.87 and 34.81, respectively).

Table 6. Contribution degree of each characteristic towards total genetic divergence among studied cotton genotypes

Characteristic	BW	SCY	LY	L%	FL	FF	FS	Total
First ranking	7	122	12	1	1	10	37	190
Contribution %	3.684	64.211	6.316	0.526	0.526	5.263	19.474	100

Table 7. Cluster number, cluster index scores and cotton genotypes included in each group following Metroglyph technique

Cluster No.	Genotypes No.	Cluster index scores
I	6,7,8,18	47
II	1,2,4	43
III	9,11,20	41
IV	3,14,15	40
V	5,17	35
VI	16,19	30
VII	10,12	23
VIII	13	13

The scatter diagram used seed cotton yield as X-axis and lint percentage as Y-axis using the values of each genotype as shown in Fig. 2. Each axis was divided into three categories based on range of variation, low for seed cotton yield (105.5-145.904 g/plant), medium (145.905-91.133 g/plant), and high (191.134283.6 g/plant). Also, lint percentage divided to low (32.389-36.621 %), medium (36.622-38.802 %), and high (38.803-41.871 %) categories. The scatter diagram grouped all the genotypes into eight clusters. The other studied characteristics were plotted on the scatter diagram. Each characteristic is represented by a glyph or rayed glyph. The length of the rays indicates the mean value of the studied characteristic. A long ray indicates a high mean value and a short ray indicates the medium or mean value. A glyph with no rays represents a low mean value.

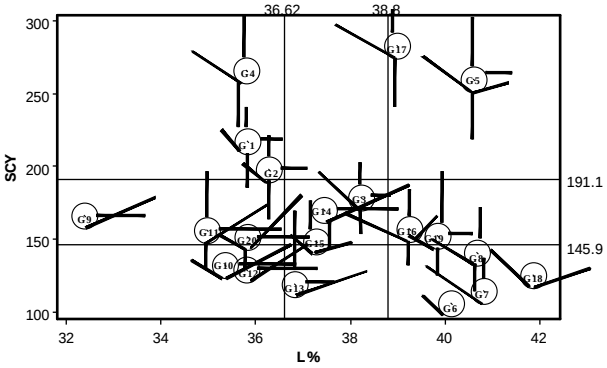

Figure 2. Metroglyph scatter diagram showing groups formed from cotton genotypes.

Mahalanobis intercluster distance values ranged from 11.381 between clusters III and VI and 178.902 between clusters I and V. The intercluster D^2 values were highly significant between all clusters except between clusters III and VI; clusters VII and VIII were not significant, exhibiting less genetic divergence. These clusters, which are closer to each other, would not be expected to yield transgressive segregants or display heterosis. The intracluster D^2 values ranged from 3.263 for cluster VII to 47.806 for cluster II. Most of these clusters did not exhibit genetic variability (Table 8).

D^2 values were found to be effective measures of genetic divergence and potentially useful for cotton breeding programs due to their focus on genetic versus geographic diversity (Thiyagu et al., 2011). There is some indication of this in our results when looking at the genotypes from America (G16-G20) which did not cluster together, possible as a consequence of local selection since their acquisition. Concurrently, Thiuagu et al. (2011) used 66 SSR markers to assess genetic diversity and postulated the utility of molecular marker data on the selection of parents for cotton improvement. While we lacked the facilities to undertake molecular marker work, our results indicate that both genetic divergence analysis and performance data can be used to choose parents for crossing. Hybridization between clusters I or II (maximum score index) with clusters

Table 8. Average intra- (Diagonal values) and inter- (Above diagonal values) cluster divergence D^2 and D (square roots of D^2 values in the parenthesis) values of eight groups for 20 cotton genotypes for seven quantitative characteristic

Clusters	I	II	III	IV	V	VI	VII	VIII
I	21.686**	110.624**	45.363**	47.114**	178.902**	35.029**	17.941**	15.187*
	(4.657)	(10.518)	(6.735)	(6.864)	(13.375)	(5.919)	(4.236)	(3.897)
II		47.806**	74.748**	65.834**	42.991**	75.781**	123.634**	114.543**
		(6.914)	(8.646)	(8.114)	(6.557)	(8.705)	(11.119)	(10.703)
III			9.938	19.644**	175.435**	11.381	43.503**	40.366**
			(3.152)	(4.432)	(13.245)	(3.374)	(6.596)	(6.354)
IV				26.194**	117.314**	19.997**	38.219**	49.682**
				(5.118)	(10.831)	(4.472)	(6.182)	(7.049)
V					24.169**	161.084**	154.266**	165.971**
					(4.916)	(12.692)	(12.420)	(12.883)
VI						5.396	161.084**	39.379**
						(2.323)	(12.692)	(6.275)
VII							3.263	12.209
							(1.806)	(3.494)
VIII								0.000
								0.000

***, ** Significant at 0.05 and 0.01 (X^2 at 0.05 and 0.01 for 6 degrees of freedom = 12.59 and 16.81, respectively).**

VII or VIII (minimum score index) is expected to give better progenies. This information could be useful for breeders interested in creating a desirable level of variability for a specific characteristic and thus would be helpful in identifying and engineering the crosses. The metroglyph analysis would be a suitable technique for grouping genotypes into different clusters based on their genetic dissimilarity background. Khan et al., (2007), Shakeel et al., (2011), and Haidar et al. (2012) found this technique to be suitable for preliminary classification of a large number of germplasm into distinct clusters depending on dissimilarity background. The information furnished herein would be helpful to the breeder in the selection of superior genotypes that might be improved directly or utilized as parents in a hybridization program for the development of future varieties.

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