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MULTIVARIATE ANALYSIS OF COTTON (*GOSSYPIUM* SPP) ACCESSIONS FOR AGRO-MORPHOLOGICAL AND FIBRE QUALITY TRAITS

Balarabe A.[1], *Yahaya, A.I.[2], Mohammed, S.B.[2], Mohammed, M.S.[2] and Iyad, A.M.[2]
[1] Department of Crop and Forestry, National Agricultural Extension and Research Liaison Services, Ahmadu Bello University, Zaria, Nigeria
[2]Department of Plant Science, Institute for Agricultural Research Samaru, Ahmadu Bello University, Zaria, Nigeria.
*Corresponding Author: +2348035951027, abdulyahyu@gmail.com

ABSTRACT
Genetic improvement of important agronomic, fibre yield and quality triats in cotton has been slow due to its narrow genetic base. Hence, the need to study and explore available germplasm resources to assess the variation among the cotton germplasm and to classify and identify the potential genotypes that can be used in the development of hybrid with the best agronomic, fibre yield and quality traits. One hundred cotton genotypes were evaluated from diverse sources in a 10 x 10 lattice design, replicated twice across two environments in 2020 wet season. Significant ($p \leq 0.05$) genotypic variation was observed for plant height, number of sympodial branches, number of bolls, seed cotton yield and the degree of pest attack. Principal component analysis (PCA) indicated that four components explained over 99.5% of the total variation of the data, with PC1 and PC2 accounting for 86.5% and 10% of the total variability, respectively. PC1 was associated with most of the traits except stand count at emergence, whereas PC2 was related to stand count at emergence. Cluster analysis produced three clusters, cluster 1 consisted of genotypes (n = 39) that had low values for most of the traits assessed except fibre fineness. Cluster 2 was composed of genotypes (n = 35) with high values of traits measured while cluster 3 had genotypes (n = 26) with the highest values of measured traits. Using rank summation index, genotypes VIR-7112-HG-69-15, VIR-7072, LA-213-SEA-ISLAND LEAF, BULGARIA-996 and 30858 with rank summation indices of 243, 270, 313, 330, 332 and 368 respectively were the best materials and potential genotypes for the development of hybrid with the best agronomic, fibre yield and quality traits, while LINIA-7010 ranked last with rank summation index of 712. It could be concluded that significant genetic variation exists for important agronomic and fibre quality traits like seed cotton yield, number of bolls, plant height, number of sympodial branches, fibre length and fibre fineness. Consequently, the selection of promising cotton genotypes based on the aforementioned traits will make genetic improvement further feasible.

Keywords: Genetic diversity, Cotton, Fibre yield, Principal Component Analysis, Cluster Analysis.

INTRODUCTION
Cotton (*Gossypium spp*), the king of apparel fibre, since times immemorial popularly called "White gold" is the world's most important fibre crop. It is native to tropical and subtropical regions of the world including America, India, and Africa (Kumar *et al*., 2017). The crop is the leading fibre crop grown in more than 80 countries (Shakeel *et al*., 2011) and serves as an important source of ingredients for livestock feed and oil, with a world consumption of approximately 27 million metric tons per year (Chen *et al*., 2007).

The average seed cotton yield is estimated at 600 kg/ha, while the global average is about 2,230 kg/ha. The low yield of Nigerian cotton compared to the world average is indeed a major constraint towards competitive cotton production in the country. Furthermore, Nigeria's average fibre lint yield is 190 kg/ha which is still low compared with the world's average of 644 kg/ha (USDA, 2019).

Genetic variation within cultivated cotton germplasm is narrow. Thus, genetic improvement of the crop for most of the desired traits is slow (Akter *et al.,* 2019). This calls for the need to explore cultivated, primitive and wild germplasm for novel traits that could be useful in genetic improvement of the crop.

In an attempt to broaden the genetic base of Nigeria's national cotton breeding programme, the Institute for Agricultural Research (IAR) Samaru, Ahmadu Bello University Zaria introduced 459 new cotton germplasm from the United States, comprising of both cultivated and wild species. Multivariate analysis has been useful in the identification the genetic diversity in different crop plants (Brown-Guedira *et al.,* 2000).

Considering the importance of genetic diversity, this study was conducted to unravel the variation among 100 cotton germplasm for earliness, yield and fibre quality with the view of identifying superior genotypes that can be utilized in further breeding programmes. In addition, the study aimed to **c**lassify the genotypes and identify the potential ones that can be used in the development of hybrid with the best fibre quality, seed cotton yield and earliness traits.

MATERIALS AND METHODS

Experimental Sites

The experiment was conducted in the 2020 cropping season at two locations; IAR Samaru Zaria, (latitude 25.154865 and longitude 56.854692). Samaru is in the northern guinea Savannah agro-ecological zone of Nigeria with an annual precipitation ranging between 800-1300 mm, and temperatures usually range from 10-36°C. The soil is mostly sandy loam (Kowal and Knabe, 1972). The second location was Malumfashi, Katsina State of Nigeria (latitude 11.761786 and longitude7.621555) in the Sudan Savannah agro-ecology of Nigeria with an annual precipitation ranging between 702.2 – 797.1mm and temperature range from 10- 33.7^0C. The soils are classified as typical Alfisols, loose and sandy (NAERLS, 2019).

Sources of the Genetic Materials

The genetic materials consisted of 100 exotic cotton germplasm sourced from USDA cotton repositories and other cotton elite/ landrace of Nigerian origin. The list comprised four major cultivated species (*Gossypium hirsutum (68),* G. *barbadense (23),* G. *arboreum (4)* and G. *herbaceum*) (5) and the three (3) checks (SAMCOT 8,9 and 10) are of Nigerian origin.

EXPERIMENTAL DESIGN

The experiment was laid out in a 10 x 10 balanced lattice design (Chomtee, 1999) with two replications. Each replication consisted of ten blocks each with ten genotypes. Each genotype was grown in one row plot of 10 m in length. A spacing of 90 by 45 cm inter and intra row was used.

Agronomic and Cultural Practices

All agronomic and cultural practices were carried out according to the IAR recommendations for cotton production (CDC, 2007). Three to four seeds were sown per hill. Thinning was done to reduce the population to two plants per stand at three weeks after sowing (WAS). Butachlor (active ingredient butachlor) was applied as a pre-emergence herbicide at the rate of 4 litres per hectare. Three supplemental hoe weeding were done at three, six and nine WAS.

Application of 65 kg/ha of nitrogen in the form of Urea (46% N) was applied. The application was split dose – with the first and second half applied at 3 and 8 WAS respectively. Phosphorus (P) was applied in the form of single super phosphate (P_2O_5) at the rate of 35 kg/ha whereas 30 kg/ha of potassium was also applied in the form of muriate of potash (K_2O). The P_2O_5 and K_2O were applied after the first weeding (at 3 WAS) into shallow grooves of 8-10 cm away from the plants. Lambda-cyhalothrin (Karate), a contact and systemic insecticide, was sprayed at 8 - 9 WAS at the rate of 400 ml/ha to control extra early pests (aphids and white flies)

Data Collection

From five randomly selected plants per entry across replications, data were collected on 11 agronomic and fibre quality traits. These included: stand count at emergence, stand count at harvest, plant height, days to 50% flowering, days to 50% square, number of sympodial branches per plant, number of bolls per plant, boll weight, seed cotton yield per plant, degree of pest attack, fibre length and micronaire.

Data Analysis

Analysis of variance (ANOVA) was carried out using the SAS software (SAS institute Inc. 2004). Comparisons between treatment means were done using Fisher's protected least significant difference (LSD) (Gomez and Gomez, 1984). The statistical model for the two environments was based on the linear model for lattice design.

Principal component analysis (PCA) was performed using R-software (version 4.1.0) that revealed the set of traits contributing to the observed variation in the data sets for the genotypes. Owing to differences in the unit of measurement of the traits assessed, the data were scaled before PCA analysis, using built-in R functions *prcomp* on the genotype means. In PCA, one of the most used criteria for solving the number of components to retain is the eigenvalue one criterion, also known as the Kaiser criterion (Kaiser, 1960). With this approach, components with an eigenvalue of greater than or equal to 1 were retained and used for further analyses and interpretation.

The quantitative data collected on different traits were standardized to zero mean and unit variance as per Sneath and Sokal (1973) and Manly (1986). Squared Euclidian distance between genotypes was calculated from the standardised data matrix by the unweighted pair group method using the arithmetic averages (UPGMA) method and cluster analysis was performed using K means clustering in R (Version 4.1.0).

RESULTS AND DISCUSSION

Variations in Agro-Morphological Characters of Diverse Cotton Genotypes Evaluated Across Locations

The existence of significant ($P \leq 0.05$) and highly significant differences in the traits as observed in the present study suggests the presence of considerable genetic variability among the cotton genotypes (Table 1). The genotypes varied significantly for all measured traits except fibre fineness (FFN). Genotype x Environment interaction was also signinficant for plant height (PHT), number of sympodial branches (NOS), number of bolls (NOB), degree of pest attack (DPA), boll weight (BW) and seed cotton yield (SCY). This implies that environment is contributing to the expression of these traits. Agronomic traits like the days to first flowering (DFF) varied from 54 – 68 days, number of sympodial branches (NOS) ranged from 6.2 - 48.0 while the number of bolls per plant (NOB) ranged from 16.8 - 106.2. Boll weight (BW) ranged from 1.9 - 5.8 g and seed cotton yield (SCY) from 108 - 1107 kg (Table 2).

However, the fibre quality traits like fibre fineness (FFN) ranged from 3.0 - 5.2 ug/inch while fibre length (FBL) ranged from 24 – 36 mm. The presence of wide variation among these genotypes could be exploited in breeding programmes to improve yield via selection and genetic recombination through crossing. Important parents can be identified for crossing to locally adapted varieties/landraces from these pools of genotypes with desired attributes. These observations are contrary to some earlier research reports such as that of Guang and Xiong-Ming, (2006), who reported the presence of a low level of genetic variability in cotton. The low variability might be due to the fact that the cotton genotypes evaluated in that study were obtained from a few sources while materials for this research were gotten from USDA's worldwide collection from different continents and regions of the world. Similarly, Iqbal *et al*. (2001) reported low genetic variability among cultivated upland cotton genotypes and attributed the genetic variance to the continuous utilization of the genetic sources in the breeding programme.

The results of the rank summation index (Table 2) showed that genotypes LONREN-1, VIR-7112-HG-69-15, VIR-7072, LA 213 SEA ISLAND LEAF, BULGARIA 996, 30858 and S-55 obtained from various origins were the best genotypes for all the traits studied, indicating that these genotypes are potential genetic materials for genetic improvement of cotton in Nigeria.

Principal Component Analyses and Loading of Variables for Diverse Cotton Genotypes

For the exploitation and maintenance of genetic diversity, genetic variance can be partitioned into principal components (PC). Four out of 10 PCs were selected with eigenvalue greater than 1 in this study (Table 3). The contribution of these PCs towards total variability was 99.52% which indicates that valuable information is present in the first four components. The PC-1 contributed maximum (86.52%), followed by PC-2 (10%), PC-3 (1.99 %) and PC-4(1%). The present results were in line with the findings of Saeed *et al*. (2014), Latif *et al*. (2015), Kaleri *et al*. (2015), Shah *et al*. (2018) and Kumari and Gunasikaran (2019), who separately reported that first two PCs play a key role in describing major variability contributors while investigating different traits (Saeed *et al* 2014).

Table 1: Mean squares from the analysis of variance for cotton genotypes evaluated for agro-morghological and quality traits across environments in 2020

Source of variation	DF	SCE	SCH	PHT	NOS	DFS	DFF	NOB	DPA	BW	SCY	FBL	FFN
Block (Env*Rep)	37	2.87**	2.16	14.60	3.63	0.98	0.91	13.51**	0.51*	1.05	4.05	0.09*	6.8*
Gen	99	19.85**	31.39**	928.65**	271.56**	40.85**	16.30*	509.41**	0.47*	2.36**	1683.08**	0.11*	32.9
Env	1	0.81	0.30	43077.00**	362.90**	0.00	0.00	2106.81**	0.00	161.29**	131232.31**	-	-
Rep	1	1.69	14.06**	99.20**	0.42	1.00	2.56	42.25*	0.72	6.76**	46.38**	0.05*	0.2*
Gen*Env	-	1.18	1.82	636.10**	4.06**	0.00	0.00	93.38**	0.49*	2.24**	1264.19**	-	-
Error	81	1.53	1.66	10.76	2.59	1.18	0.81	6.44	0.35	0.80	2.92	0.1	0.4

SCE = Stand count at emergence, SCH = Stand count at harvest, DFS = Days to the first square, NOB = Number of bolls, DFF = Days to first flowering, NOS = Number of sympodial branches, BW = Boll weight (g), SCY = Seed Cotton Yield (kg/ha), PHT = Plant height, FBL = Fibre length, FFN = Fibre fineness, DPA = Degree of pest attack. * indicate significance (P< 0.05), ** indicate significance (P<0.01). Gen = Genotypes, Rep= Replication, Env=Environments, Df =Degrees of freedom

Table 2: Best and worst performing cotton genotypes evaluated for agro-morphological and quality traits across environments in 2020

GENOTYPES	SCE (count)	SCH (count)	PHT (cm)	NOS (count)	DFS (days)	DFF (days)	NOB (count)	DPA	BW (g)	SCY (kg)	FFN (ug/inch)	FBL (mm)	RSI
Best 10													
LONREN-1	7.3	8.8	115.8	25.1	54	65	37.0	2.4	4.1	533	4.2	25	243
VIR-7112-HG-69-15	6.3	5.9	98.7	14.3	51	62	27.2	2.1	3.2	356	4.7	29	270
VIR-7072	8.7	7.2	108.6	18.4	53	63	31.5	2.3	3.7	464	4.9	31	313
LA 213 SEA ISLAND LEAF	8.9	12.1	130.3	33.5	59	66	47.7	2.8	4.8	769	4.7	34	330
BULGARIA 996	8.6	10.5	126.2	29.3	56	65	41.7	2.6	4.4	655	3.9	29	332
30858	10.2	13.1	140.0	36.3	60	66	52.4	3.0	5.1	971	5.1	33	368
S-55	9.5	16.5	149.0	48.0	63	68	106.2	3.2	5.8	1107	4.8	32	371
BPA 68 C3 4030	9.7	7.6	110.2	20.5	53	64	32.7	2.3	3.8	494	4.3	30	380
HAR U 585-12	6.6	11.6	129.2	33.1	58	66	46.3	2.8	4.7	761	4.7	30	389
VAR-DIGVIJAY	7.6	8.5	113.5	24.1	54	64	34.4	2.4	4.0	523	4.4	31	390
Worse 10													
CH 252	6.3	5.4	92.7	13.6	51	61	24.6	1.9	3.1	303	4.3	30	638
AUB BR7	11.4	11.8	130.0	33.2	58	66	46.8	2.8	4.7	761	4.1	31	639
UKA B2(72)193	6.2	5.8	98.1	14.0	51	61	27.1	2.1	3.1	347	3.9	29	650
TADLA 1	7.0	10.9	128.2	31.1	57	66	44.4	2.7	4.6	713	4.5	27	664
AUB BR OK-7	6.7	9.2	117.8	26.2	55	65	38.7	2.4	4.2	567	4.5	29	667
SAMCOT 8	5.4	7.4	109.3	19.6	53	64	31.9	2.3	3.7	477	4.9	26	670
VIR 7116	6.3	14.1	142.0	38.1	61	66	53.4	3.1	5.4	1034	4.5	32	677
VIR-7114 1780/N	7.7	7.1	107.0	17.8	53	63	31.4	2.2	3.6	447	3.8	28	694
S918-12302 RESEL	10.6	7.5	109.7	19.9	53	64	32.5	2.3	3.7	486	3.5	29	708
LINIA 7010	4.7	6.1	99.1	14.6	51	62	27.7	2.1	3.2	369	4.6	31	712
CV	15.17	14.79	2.87	6.69	1.99	1.41	6.85	24.76	22.59	30.4	4.38	29.4	
LSD	1.73	1.80	4.58	2.25	1.51	1.26	3.54	0.83	1.25	239	13.75	10.70	
MEAN	8.15	8.72	114.40	24.02	54.56	63.94	37.05	2.39	3.96	562.4	1.20	0.63	
RANGE	3.53-14.44	1.7-16.5	72.41-148.9	6.23-48.02	48.8-62.9	53.7-67.6	16.8-106.2	1.4-3.21	1.9-5.8	108-1107	3.00-5.19	24-36	

SCE = Stand count at emergence, SCH = Stand count at harvest, DFS = Days to the first square, NOB = Number of bolls, DFF = Days to first flowering, NOS = Number of sympodial branches, BW = Boll weight, SCY = Seed Cotton Yield, PHT = Plant height, FBL = Fibre length, FFN = Fibre fineness, DPA = Degree of pest attack. CV = coefficient of variation, LSD = least significant difference

Table 3: Eigenvalues of the four major principal components

Principal components (PC)	Eigenvalues	Contribution (%)	Cumulative (%)
PC1	8.7	86.5	86.5
PC2	1.0	10.0	96.5
PC3	0.2	1.99	98.5
PC4	0.1	1.00	99.52

Table 4: Major Principal Components contributing to variability in the data and loadings of the traits to the components, with loadings over 10 in bold.

Traits	PC1	PC2	PC3	PC4
SCE	0.0	**99.0**	0.8	0.0
SCH	**11.5**	0.0	0.0	0.7
PHT	**11.4**	0.0	2.3	0.9
NOS	**11.3**	0.0	1.8	1.7
DFS	**11.2**	0.1	4.1	**11.7**
DFF	**10.1**	0.3	**51.1**	**19.5**
NOB	**10.1**	0.4	**33.4**	**54.2**
DPA	**11.4**	0.0	2.2	0.8
BW	**11.5**	0.0	1.1	0.3
SCY	**11.3**	0.0	3.1	**10.3**

SCE = Stand count at emergence, SCH = Stand count at harvest, DFS = Days to first square, NOB = Number of bolls, DFF = Days to first flowering, NOS = Number of sympodial branches, BW = Boll weight, SCY = Seed Cotton Yield, PHT = Plant height, FBL = Fibre length, FFN = Fibre fineness, and DPA = Degree of pest attack.

All the traits that contributed to PC-1 recorded almost uniform factor loadings (10.1 - 11.5) which were all positive (Table 4). The genotypes that contributed highest to PC-1 were
UKA B1-(72)004 (6.46), B4 18 FERTLITY RESTORER GENE (3.66), DES 24/CASCOT BR-1 (4.47) and AUB NE-277 (3.81) (Table 5). Saeed *et al*. (2014) and Isong *et al*. (2017) reported that maximum contribution of sympodia per plant and yield was found in PC-1. Ameer *et al*. (2019) also confirmed the maximum contribution of yield was observed in PC-1 while contrary findings were reported by Shakeel *et al*. (2015). The traits in PC-2 recorded positive loadings all through but were insignificant except for SCE. Three genotypes out of the 100 were responsible for the most contribution to this component and these genotypes were LA 887 (6.83), MALVI/ANI-1 (5.38) and VIR-7106 CR-142-45-7 (5.05). This result is contrary to the finding of Jehanzeb *et al*., (2017) who reported maximum positive loading by plant height in PC 2. In PC-3 and PC-4, there was maximum positive factor loading for days to first flowering and the number of bolls per plant with (51.06), (19.45) and (33.4), (54.21) respectively. Only one genotype S-55 was responsible for the major contribution in PC-3 while S-55 and UKA-B1-(72)004 were the genotypes with most contributions in PC-4. Farooq *et al*. (2015), Shakeel *et al*., (2018) and Ghulam *et al*., (2021) also recorded positive loading for days to first flowering and negative loading for the number of bolls per plant. A useful technique for the exploration of genotypes for successful breeding strategies is PCA (Akhter *et al*., 2009; Nazir *et al*., 2013). The PCA expressed the magnitude of variability among the traits and this information may be exploited in further breeding programmes for improvement in yield contributing traits (Nazir *et al*., 2013).

Table 5: Loadings of Genotypes to the four Principal Components contributing most of the variation among cotton genotypes evaluated

Components	Genotypes	Scores
1	S-55	7.4
	UKA B1-(72)004	6.5
	DES 24/CASCOT BR-1	4.5
	AUB NE-277	3.8
2	LA887	6.8
	MALVI/ANI-1	5.4
	VIR-7106 CR-142-45-7	5.1
	STONEVILLE A	4.9
3	S-55	32.2
	UKA B1-(72)004	28
	AUB NE-277	2.1
4	S-55	54.1
	UKA B1-(72)004	11.5
	AUB NE-277	3.7

Table 6: Cluster analysis of cotton genotypes evaluated for agro-morphological and quality traits across environments in 2020

Traits	Cluster 1	Cluster 2	Cluster3
SCE (count)	7.95	8.21	10.26
SCH (count)	6.03	10.49	15.60
PHT (cm)	99.12	124.62	148.87
NOS (count)	15.67	29.49	46.12
DFS (days)	51.50	56.58	62.48
DFF (days)	62.06	65.23	67.45
NOB (count)	27.69	42.38	84.51
DPA	2.05	2.62	3.20
BW (g)	3.22	4.44	5.73
SCY (kg)	37.27	68.56	109.60
FFN (ug/inch)	4.30	4.44	4.22
FBL (mm)	2.93	2.95	3.06

SCE = Stand count at emergence, SCH = Stand count at harvest, DFS = Days to the first square, NOB = Number of bolls, DFF = Days to first flowering, NOS = Number of sympodial branches, BW = Boll weight, SCY = Seed Cotton Yield (kg/ha), PHT = Plant height (cm), FBL = Fibre length (ug/inch), FFN = Fibre fineness (mm), DPA = Degree of pest attack.

Clustering of Genotypes based on Agronomic and Fibre Traits in Cotton

The 100 genotypes were classified into three clusters. Cluster 1 was composed of 39 genotypes followed by cluster 2 and cluster 3 composed of 35 and 26 genotypes, respectively. Clusters 1 and 2 recorded highest values for FFN, thus will produce cotton genotypes with desirable fibre fineness trait (Table 6). Moreover, all the traits in cluster 3 were found to have the highest value for most traits except for FFN which was the lowest. In other words, cluster 3 was more divergent than the others since it recorded the highest value for all the traits that contributed directly and indirectly to yield improvement. Thus, hybridization amongst genotypes under cluster 3 is expected to produce genotypes with the highest heterotic values. Multivariate criterion defines the extent of differences amongst a pair of variables, thus it is essential to determine the plant trait responsible for the differences between genotypes and the respective contributions that the different traits make up to the total variability in the germplasm (Albert, 2014). Depending on the specific objective of hybridization, the selection of parents should consider the special advantage of each cluster and each genotype within a cluster (Chahal and Gasal, 2002). Therefore, based on the cluster analysis, cluster 2 may be used to improve and develop genotypes with higher FFN traits while cluster 3 could be used to incorporate the traits for earliness and high values of yield component traits. Cluster 1 may be further studied in breeding programmes for the development of higher-yielding cotton genotypes with desirable traits for earliness and yield.

CONCLUSION

The genotypes used in this study varied significantly for important agronomic, seed cotton yield and fibre quality traits, which are key in further genetic improvement. Four PCs were identified with eigen value >1 and have contributed to 99.52% of variability mainly due to four traits; SCE, NOB, SCY and DFF.

RECOMMENDATIONS

Based on PCA analysis days to first flowering, number of bolls per plant and seed cotton yield were identified as promising traits that can be selected for the improvement of adapted cultivars. For drought-prone environments, the cotton genotypes S-55 and UKA-B1-(72)004 from PC 3 and PC 4, respectively were early maturing can be used for hybrid development. The cotton genotype LONREN-1 observed to perform best based on rank summation index can be exploited for overall improvement of agro-morphological and fibre quality traits in future breeding programme or mass production.

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