



Genetic diversity and correlation network approach on cotton genotypes in southern of Minas Gerais¹

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ABSTRACT

The cultivation of Cotton (*Gossypium hirsutum*) is trending in an upward expansion at Minas Gerais state, Brazil. Due this growth, the breeding program has been demanded for the knowledge about the genetic diversity and correlation between morpho-agronomic traits, to start interesting crosses. The objective of this work was to study the genetic diversity and the correlation network between morpho-agronomic traits of cotton genotypes. Two cotton accessions and five commercial cultivars: FM993, FMT701, FM910, DP604 and DP604BG were evaluated for 16 traits. The results revealed divergence between the genotypes. Unweighted pair-group method arithmetic average (UPGMA) analysis allocated the genotypes in three clusters, with DP604 and Accession 2 being the most divergent. The correlation network helped to visualize the association between traits, presenting a negative correlation between anthracnose with plant height (-0.465), plant vigor (-0.486) and main root length (-0.437). Also, even with some genotypes presenting a higher number of reproductive branches, the correlation for this trait with number of bolls showed a high significant value (0.68). Thus, the genotypes showed wide genetic diversity for the composition of future crosses in cotton breeding programs. The correlation network allowed the visualization of a medium to high correlation pattern for the morpho-agronomic traits.

Keywords: *Gossypium hirsutum* L.; mahalanobis generalized distance; morpho-agronomic traits; multivariate analysis; clustering method.

INTRODUCTION

The Brazilian cotton sector is the most recent in comparison to other agricultural sectors and extremely promising when considering the capital invested during planting and management and the amount withdrawn with the harvest. Cotton production in Brazil has been increasing due to improved domestic prices, making cotton an attractive alternative to corn and soybeans (Muhammad *et al.*, 2019). Brazil is the fourth largest producer in the world, with an

estimated final production of 2.93 million tons in 2019/20, 4.2% higher than the previous harvest (Conab, 2020).

The crop is from the genus *Gossypium* spp., which encompasses more than 50 species worldwide (Wendel & Grover, 2015). Among these, four species have been domesticated and exploited economically: *G. hirsutum* L. and *G. barbadense* L. (allopolyploid species, originated in the Americas), and *G. arboreum* L. and *G. herbaceum* L. (diploid species, originated in Africa and Asia respectively)

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(Morello *et al.*, 2015). However more than 90% of the annual cotton crop worldwide is *Gossypium hirsutum* L. (USDA, 2019), this specie produces a higher fiber yield and survive better on harsh environments (Hu *et al.*, 2019).

Due to the large number of species, the genus *Gossypium* has wide genetic variability (Wendel & Grover, 2015). However, over the years cotton breeders often select consolidated cultivars on the market as parentals, favoring a narrow genetic basis and hybridization between genetically similar individuals (Rahman *et al.*, 2012). Thus, understanding the genetic diversity is essential when selecting parents for crop breeding (Sharma *et al.*, 2018; Hu *et al.*, 2019).

The correlation network is an approach implemented to facilitate the results interpretation by breeders and, is a bidimensional network representation of a p dimensional correlation matrix. These analyses allow the detection of important structures and complex statistical patterns that are hard to extract by other means (Silva *et al.*, 2016). Previous studies show the effectiveness and multidisciplinary of the method when comparing multiple factors (Kumar & Deo, 2012; Saba *et al.*, 2014; Pearce *et al.*, 2015).

Once consolidated as a major cotton-growing state, Minas Gerais saw this agronomic practice be replaced by coffee growing in a large part of its territory. Nowadays, cotton cultivation is mostly concentrated in the northern and northwestern regions of Minas Gerais, and in the Triângulo Mineiro and Alto Paranaíba region. Among the advantages of the cultivation in the state, the main highlight is the large concentration of cotton mills in the state and the extremely favorable logistics for the acquisition of inputs and the flow of production. The crop also appears as an alternative for crop rotation with soybeans and corn, requiring improvement programs aimed productive cultivars adapted to the regions of interest (Conab, 2020). In face of that and, with the lack of past studies for the region (Machado *et al.*, 2002; Silva *et al.*, 2014), the present work was performed with the hypothesis that potential genotypes for cotton cultivation in the south of Minas Gerais state can be selected or obtained by breeding, enhancing the number of economical crops for the region.

Thus, the objective of the present study was to estimate the correlation between morpho-agronomic traits and to determine the genetic diversity between cotton (*G. hirsutum* L.) genotypes cultivated in the south of Minas Gerais, Brazil. Moreover, to identify a link between the

traits for selection and possible contrasting cultivars and / or accessions to use as candidates to start a cotton breeding program for the region.

MATERIAL AND METHODS

A field experiment was carried out in an experimental area of the Departamento de Agricultura, at Universidade Federal de Lavras, in Lavras, MG, Brazil (21°13'36.47"S, 44°57'40.35"W, at 975 m altitude), from November 2016 to April 2017. According to the Koppen classification, the region climate is classified as Cwa, mesothermal with mild summers and winter droughts (Sá Júnior *et al.*, 2012). The soil of the experimental area is classified as Oxisol with clay texture (Embrapa, 2013). Before running experiments, a composed soil sampling of the experimental area was collected and the chemical characteristics of the soil was properly corrected, by means of liming, plastering and fertilization recommended for the crop.

Two cotton (*G. hirsutum*) accessions (1 and 2) (from the Departamento de Agricultura germplasm bank, of the Universidade Federal de Lavras) and five commercial cultivars: FM993, FMT701, FM910, DP604 and DP604BG were disposed in a Randomized Complete Block Design (RCBD) experiment, with three replicates. The experimental unit consisted of 40 plants allocated in five meters, and a spacing of one meter between lines, resulting in a useful experimental plot of five m².

Five plants from each plot were evaluated 170 days (first open boll) after sowing for sixteen quantitative traits: plant height (PH, cm), number of leaves (NL), number of nodes (NN), internode length (IL, cm), number of vegetative branches (NVB), number of reproductive branches (NRB), length of vegetative branches (VBL, cm), length of reproductive branches (RBL, cm), number of flowers / flower buds (NFL), number of bolls per plant (NA), number of secondary roots (NSR), main root length (RL, cm), width (LW, cm) and length of leaves (all leaves were evaluated) (LL, cm); and two qualitative traits: plant vigor (PV) and reaction to anthracnose [*Colletotrichum gossypii* Southworth (teleomorph *Glomerella gossypii*)] (ANT). For plant vigor, a visual score from 1 to 5 was used. For reaction to anthracnose, an score from 1 to 5 was used, adapting the diagrammatic scale proposed by Araújo & Suassuna (2008) for cotton ramulosis, where: (1) plants without symptoms; (2) plants with necrotic spots on the younger leaves; (3) plants with necrotic spots on the leaves

and up to three super numerary branches; (4) plants with necrotic spots on the leaves, with more than three super numerary branches and size reduction; (5) plants with necrotic spots on the leaves, shortening of internodes, intense over budding and size reduction. Data were subjected to the analysis of variance ($p < 0.05$), and means were grouped by Scott-Knott clustering test (Scott & Knott, 1974).

For multivariate analysis, we estimated the genetic distances between the genotypes using the Mahalanobis Generalized Distances method, using the data from five plants of each plot. The genetic distances were submitted to the UPGMA clustering method, obtaining a dendrogram. Finally, a phenotypic correlation network was also performed to obtain patterns of link between all traits. The analyses were performed by software R (R Development Core Team, 2019) and the software Genes, version 2016 (Cruz, 2016) in integration with the software R. Thus, the integration of the correlation network used the “Qgraph” package (Epskamp *et al.*, 2012).

RESULTS AND DISCUSSION

Twelve traits showed significant differences between the genotypes studied. The coefficients of variation (CV) ranged from 9.07 to 29.31%, indicating good experimental precision, similar to previous studies (Santos *et al.*, 2017; Teodoro *et al.*, 2019). The morpho-agronomic traits with significant differences are most those related with vegetative growth, also reflecting the results for plant vigor. This allows selecting plants with high potential for growth and development characteristics considering that most of the studies with cotton genotypes are aimed on yield and fiber characteristics (Teodoro *et al.*, 2018; 2019; Miranda *et al.*, 2020).

The averages of the measured traits, with significant values for the ANOVA test ($p < 0.05$), ranged from 89.00 to 121.8 cm for plant height, 20.1-22.9 for number of nodes, 4.3-5.5 for internode length, 8.6-13.6 for number of reproductive branches, 3.3-5.8 for number of vegetative branches, 27.6-35.9 for reproductive branch length, 18.2-25.2 for vegetative branch length, 3.6-4.8 for plant vigor, 9.8-14.0 for number of secondary roots, 11.3-12.7 for leaf length, 11.3-12.7 for leaf width, and 1.7-3.3 for anthracnose (Table 1).

It is also interesting to point that the DP604BG cultivar is essentially derived from DP604 cultivar. In face of that, most of the results between the two cultivars are similar, showing that the transgenic event (Bollgard - resistant to

lepidopteran insects) did not affect the plants performance significantly. The FM993 cultivar presented the higher results for all traits evaluated. Both accessions presented lower values when compared with the cultivars on most of the traits except for internode length and vegetative branch length, where the Accession 2 was grouped with the cultivars with higher values (Table 1).

There is strong competition for assimilates between vegetative and reproductive structures over the growth season and thus, the preference to diversion of assimilates to different plant parts determines the survival and development of specific plant parts (Tariq *et al.*, 2017). In the present work, despite the genotypes were grouped in three distinct groups for the number of reproductive branches, the number of bolls per plant did not present statistical differences, showing that the genetic materials with fewer reproductive branches compensated this with more bolls per branch. This result illustrates that even when the genotypes showed different vegetative growths, the responses for production were similar.

The same can be observed on the vegetative development by looking at the number of vegetative branches results, where the genotypes were also divided in three groups. However, the number of leaves per plant maintained the same for all genotypes, showing that the plants with less vegetative branches compensated producing more leaves per branch. The presence of a satisfactory number of leaves is necessary to guarantee the interception of sufficient photosynthetically active radiation, since the efficiency of photosynthesis is crucial for the final production in all species (Constable & Bange, 2015).

Calculating the Mahalanobis distance (Table 2), it was possible to identify those genotypes more divergent. For the present study, Accession 2 and DP604 were the two genetic materials with the highest value of distance (6135,11). The Mahalanobis distance is based on the calculation of a sample covariance matrix, reflecting the genetic relationship among initial genotypes in a breeding program.

Based on the genetic distances, genotypes were grouped using the UPGMA clustering method to produce a dendrogram (Figure 1). The cut-off point was established at 16.67% of the maximum fusion point, allowing the formation of three groups. The group I was formed by four cultivars (FM993, DP604, DP604BG and FMT701); the Accession 2 was allocated on group II; and the Accession 1 and FM910 cultivar stayed on group III. Hybridization between distant groups should

Table 1: Morpho-agronomic traits averages of genotypes (cultivars and accessions of *Gossypium hirsutum* L).

Genotypes	Traits ⁽¹⁾											
	PH	NN	IL	NRB	NVB	RBL	VBL	PV	NSR	LL	LW	ANT
FM993	121.8 A	22.5 A	5.5 A	13.1 A	5.8 A	35.9 A	25.2 A	4.8 A	14.0 A	12.7 A	13.5 A	1.8 A
FM910	115.3 B	22.9 A	5.0 A	13.4 A	4.5 B	31.4 B	23.8 A	4.3 B	12.6 A	11.2 B	12.3 B	2.0 A
DP604BG	104.2 C	21.9 A	4.7 B	13.6 A	4.6 B	31.0 B	22.0 A	4.2 B	12.0 B	12.3 A	13.2 A	1.7 A
DP604	100.0 C	20.1 B	5.1 A	11.4 B	4.0 B	29.9 B	22.5 A	4.3 B	11.8 B	12.3 A	13.2 A	2.0 A
FMT701	93.2 D	20.6 B	4.9 A	10.4 B	4.8 B	27.9 B	18.5 B	3.7 C	9.8 B	12.0 A	13.0 A	2.2 B
Accession 1	89.0 D	20.5 B	4.3 B	11.2 B	3.3 C	27.6 B	18.2 B	3.6 C	10.9 B	11.3 B	11.9 B	3.3 B
Accession 2	109.1 B	21.2 B	5.1 A	8.6 C	3.4 C	28.5 B	21.9 A	4.3 B	13.8 A	11.6 B	12.4 B	2.9 B
Maximum	121.8	22.9	5.5	13.6	5.8	35.9	25.2	4.8	14.0	12.7	13.5	3.3
Minimum	89.0	20.1	4.3	8.6	3.3	27.6	18.2	3.6	9.8	11.2	11.9	1.7
Means	104.7	221.4	44.9	111.7	44.3	330.3	221.7	44.2	112.1	111.9	112.8	22.3
CV (%)	10.7	9.94	13.8	21.7	24.5	21.7	28.2	12.2	29.3	10.8	9.07	27.7

⁽¹⁾ Values followed by the same capital letter in column does not differ statistically by the Scott-Knott test ($p < 0.05$). CV: Coefficient of variation; PH: Plant height (cm); NN: Number of nodes; IT: internode length (cm); NRB: Number of reproductive branches; NVB: Number of vegetative branches; RBL: Reproductive branch length (cm); VBL: Vegetative branch length (cm); PV: Plant vigor; NSR: Number of secondary roots; LL: Leaf length (cm); LW: Leaf width (cm); ANT: reaction to anthracnose.

Table 2: Genetic distance between genotypes estimated by the Mahalanobis Generalized Distances method.

Genotypes	Accession 1	Accession2	DP604	DP604BG	FM910	FM993	FMT701
Accession 1	0	1405.71	1820.85	1175.87	84.80	1393.12	824.83
Accession 2		0	6135.11	4980.92	969.90	5329.51	4242.35
DP604			0	26.28	2305.62	89.56	231.59
DP604BG				0	1589.83	94.15	60.33
FM910					0	1801.55	1224.40
FM993						0	106.56
FMT701							0

should result in the greatest hybrid vigor and highest number of useful segregating by the increasing of genetic variability (Carvalho *et al.*, 2017). On the other hand, crosses between genetically similar individuals as in DP604 × FM993 and DP604BG × FMT701 can be discarded from a possible diallel since those genetically related parents tend to share many genes and alleles and consequently producing descendants with low levels of heterozygosity when crossed (Miranda *et al.*, 2020).

Empirical cotton breeding was based upon the concept of selecting single and best high-yielding progeny from the segregating populations (Mubarik *et al.*, 2020). However, in the last decades new objectives and consequently new characters started to be outstanding depending on the

objective of the contemporary agriculture. Moreover, yield is the result of combined effect of several metric traits and environment (Handi *et al.*, 2017). That is why the study of the relationships between the morpho-agronomic traits is of great importance. In the phenotypic correlation network (Figure 2), there was a correlation within each trait evaluated above 0.3 and a maximum correlation of 0.85 (Pearson correlations). As expected, the reaction to anthracnose (ANT) showed a negative correlation with important vegetative traits such as plant height (-0.465, $p < 0.05$), plant vigor (-0.486, $p < 0.05$) and main root length (-0.437, $p < 0.05$). This is due to the effect of the disease on the plant development since this pathogen reduce growth and development of cotton seedlings (Hyde *et al.*, 2009).

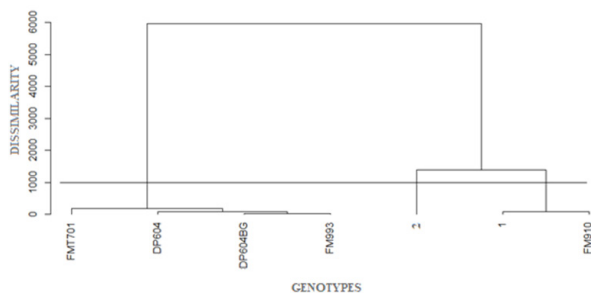


Figure 1: Dendrogram illustrating the analysis of *Gossypium hirsutum* L. genotypes (5 commercial cultivars and 2 accessions) by Unweighted Pair-Group Method Arithmetic Averages (UPGMA) obtained with the Mahalanobis generalized distance.

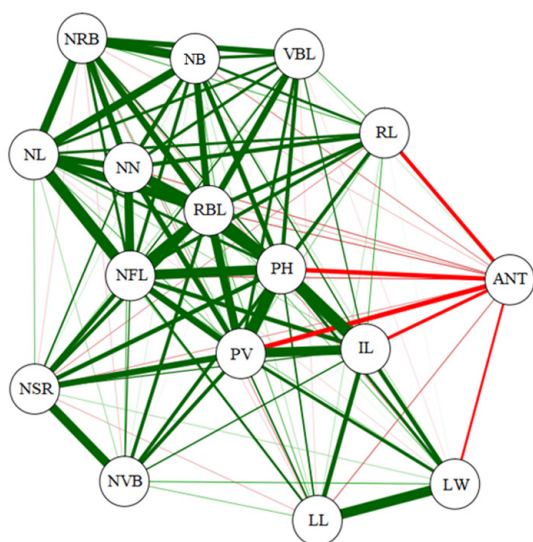


Figure 2: Phenotypic correlation network of cotton genotypes (*G. hirsutum* L.) traits. Red and green lines represent negative and positive correlations, respectively. Line width is proportional to the strength of the correlation. Variables evaluated in the network: plant height (PH), number of nodes (NN), internode length (IL), number of reproductive branches (NRB), number of vegetative branches (NVB), mean length of reproductive branch (RBL), mean length of vegetative branch length (VBL), plant vigor (PV), number of secondary roots (NSR), leaf length (LL), leaf width (LW), root length (RL), number of leaves (NL), number of flowers (NFL), number of bolls per plant (NB), reaction to anthracnose (ANT). $n = 21$.

When analyzing both the vegetative growth and plant vigor, it is clear the association pattern between those traits, so depending on the character of interest, breeders can focus the selection on fewer variables since there are significant correlations between them. Plant height was centralized on the analysis showing that larger individuals presented a higher number of nodes (0.596, $p < 0.05$) and consequently more branches.

Previous studies showed a strong negative correlation

between plant height and some important fiber characteristics such as mean boll weight, fiber percentage, fiber maturity, elongation and fiber uniformity (Teodoro *et al.*, 2018, 2019; Miranda *et al.*, 2020). Thus, genotypes with reduced size can be considered valuable for possible crosses in a cotton breeding program (Teodoro *et al.*, 2018), such as the Accession 1 and FMT701 cultivar used on the present study. It is also important to point that for the present study the genotypes did not show statistical differences for number of bolls, even with differences between number of reproductive branches. The correlation for this association was significant (0.68, $p < 0.05$), thus genotypes with more reproductive branches were less productive considering the number of bolls between individuals. Although root length did not differentiate between genotypes in the present study, according to Erande *et al.* (2014) root length had the highest and positive direct effect on cotton yield. Thus, it is an important trait to be considered in a breeding program. The correlation network helped visualizing the association between groups (vegetative and reproductive growth) and graphically demonstrated the importance of all variables/traits. Silva *et al.* (2016) studying pepper (*Capsicum spp.*), found out that the use of correlation network increased the effectiveness of genotypic selection. This is due to the fact that the use of this methodology helps to visualize the formation and association of groups of variables and to measure the importance of each one of them (Rosado *et al.*, 2017).

Broadening genetic base and exploitation of genetic diversity is the basic objective in any breeding program with an objective to obtain hybrids (Shakeel *et al.*, 2015). Most of the studies on cotton genetic diversity are focused on quantitative characters such as yield, fiber quality, fiber yield, neglecting many times phenotypic characteristics. Although, integrating as many characteristics as possible, as focused on the present study, is essential to provide a better basis for evaluating strategies, increasing the variability for the breeding programs (Carvalho *et al.*, 2017).

CONCLUSIONS

The genotypes present genetic diversity and can be useful for the composition of future crosses in cotton breeding programs. The Accession 2 and cultivar DP604 are the most divergent.

The correlation network allows the visualization of a medium to high correlation pattern for the reproductive and vegetative morpho agronomic traits and negative correlations of the reaction to anthracnose with the others.

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