Number of repetitions for evaluating technological traits in cotton genotypes

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ABSTRACT. With the changes in spinning technology, technological cotton traits, such as fiber length, fiber uniformity, fiber strength, fineness, fiber maturity, percentage of fibers, and short fiber index, are of great importance for selecting cotton genotypes. However, for accurate discrimination of genotypes, it is important that these traits are evaluated with the best possible accuracy. The aim of this study was to determine the number of measurements (repetitions) needed to accurately assess technological traits of cotton genotypes. Seven experiments were conducted in four Brazilian States (Ceará, Rio Grande do Norte, Goiás, and Mato Grosso do Sul). We used nine brown and two white colored fiber lines in a randomized block design with four replications. After verifying the assumptions of residual normality and homogeneity of variances, analysis of variance was performed to estimate the repeatability coefficient and calculating the number of repetitions. Trials with four replications were found to be sufficient to identify superior cotton genotypes for all measured traits except short fiber index with a selective accuracy >90% and at least 81% accuracy in predicting their actual value. These results allow more accurate and reliable results in future researches with evaluating technological traits in cotton genotypes.

Key words: *Gossypium hirsutum* L. r. *latifolium* Hutch.; Repeatability; Experimental planning

INTRODUCTION

Upland cotton (*Gossypium hirsutum* L. r. *latifolium* Hutch.) is one of the main economically important crops in Brazil (Farias et al., 2016). However, to become competitive in the global market, there is a need to identify genotypes with higher quality fiber. This is because the industry requires greater strength during spinning and bundling, and a decrease in the content of short fibers, greater uniformity of length, and amount of mature fibers are also desirable. All these traits are needed to increase the processing speed in the textile industry (Carvalho et al., 2015a,b).

To this end, trials with cotton genotypes assess common technological properties such as fiber length, fiber uniformity, fiber strength, fineness, fiber maturity percentage, and short fiber content (Carvalho et al., 2015c). The correct discrimination of genotypes is important in these trials so that the traits are evaluated accurately. Thus, to achieve the desired accuracy, it is important to properly dimension the trial plots' size and shape, the sample size, and the number of repetitions for each trait of interest (Torres et al., 2015).

Dimensioning the number of repetitions can be performed from data of genotype trials based on repeatability coefficient estimates obtained from analyses of variance (Cruz, 2006). In this scenario, the use of data of trial cultivar competition allows for estimation of the number of repetitions for a certain crop trait, thus enabling the optimization of human and financial resources (Torres et al., 2015).

Based on the coefficient of repeatability, scaling of the number of repetitions has been performed to evaluate agronomic traits of common bean (Cargnelutti Filho and Ribeiro, 2010), soybean (Cargnelutti Filho and Gonçalves, 2011), and maize (Cargnelutti Filho and Guadagnin, 2011). These studies generally revealed variability among traits in the number of repetitions required to obtain a given accuracy. However, the use of repeatability analysis for estimating the number of repetitions of technological cotton traits has been poorly studied. Thus, the aim of this study was to determine the number of measurements (repetitions) required for assessing the traits fiber length, fiber uniformity, fiber strength, fineness, fiber maturity, percentage of fibers, and fiber index of cotton genotypes.

MATERIAL AND METHODS

Seven trials were conducted in the States of Ceará (CE), Rio Grande do Norte (RN), Goiás (GO), and Mato Grosso do Sul (MS). Two trials were conducted in Barbalha-CE, in 2010 and 2011, using crops grown in the dry season with furrow irrigation over the crop cycle. In addition, two trials were conducted in Apodi-RN, in the same years, using crops grown in the rainy season, but with three 15-mm complementary irrigations, due to a dry period that occurred in the region. Finally, one trial was performed in Ipanguassú-RN in 2011, with sprinkler irrigation during the whole crop cycle, one trial was performed in Itaquiraí-MS in 2011, and another was performed in Santa Helena-GO in 2011. The last two trials were performed without irrigation.

Nine brown and two white colored fiber lines were used (BRS Rubi, a cultivar yielding brown fiber and BRS Aroeira, yielding white fiber). The trials were conducted in a randomized block design with four replications. The number of treatments and blocks used in this study is similar to previous studies that have aimed to scale the number of repetitions necessary to evaluate important agronomic traits (Cargnelutti Filho and Ribeiro, 2010; Cargnelutti Filho and Gonçalves, 2011; Cargnelutti Filho and Guadagnin, 2011). Each plot consisted of two 5.0-m lines spaced 1.0 m apart. The traits evaluated were fiber length (mm), fiber uniformity (%), fiber strength (gf/tex), fineness (micronaire), fiber maturity (%), percentage of fibers (%), and short fiber index (%). At harvest, 20 bolls from each sample unit were taken for the determination of these fiber traits, which was performed in a high volume instrument, an electronic device currently used for measuring fiber traits. The sample size used in this study (N = 20) is the same as that used in other experiments conducted with upland cotton (Carvalho et al., 2015a,b,c).

In each of the seven trials, data from traits evaluated followed a statistical model of a randomized block design given by:

$$Y_{ij} = \mu + \tau_i + \beta_j + \varepsilon_{ij} \qquad \text{(Equation 1)}$$

where Y_{ij} is the observed value of the response variable in plot ij, μ is the overall mean, τ_i is the genotype effect (i = 1, 2, ..., 11), β_j is the block effect (j = 1, 2, 3, 4), and $\mathcal{E}_{ij}$ is the effect of the supposed standard experimental error that is randomly distributed with mean 0 and common variance σ^2 (Storck et al., 2011). Lilliefors, Bartlett, and Tukey tests were carried out to verify the compliance of the assumptions of residual normality, homogeneity of variance, and additivity of the mathematical model, respectively. Based on the results of analysis of variance, we obtained estimates of mean square of the block (MS_B), mean square of the genotype (MS_G), mean square of the error (MS_E), and the F-test value for the genotype by

$$Fc = MS_G/MS_E \qquad \text{(Equation 2)}$$

Subsequently, the selective accuracy (SA) was estimated (Resende and Duarte, 2007) using the expression

$$SA = (1-(1/Fc))^{0.5} \qquad \text{(Equation 3)}$$

Finally, based on the obtained SA, the experimental accuracy was evaluated according to the class limits established by Resende and Duarte (2007).

Assessments were considered in each block as measurements within the same individual (genotype) and the repeatability coefficient (r) was estimated for each trait and trial by analysis of variance. In this study, r is the intraclass correlation coefficient for genotypes and is estimated by the expression:

$$r = \left[(MS_G - MS_E)/J\right]/\left[(MS_G - MS_E)/J + MS_E\right] \quad \text{(Equation 4)}$$

where *J* is the number of measurements or repetitions (Cruz and Regazzi, 1997; Cruz, 2006).

The J needed to predict the actual value of individuals (genotypes) based on the pre-set (0.80 and 0.85) genotypic determination coefficients (R^2) was calculated by the following expression (Cruz and Regazzi, 1997):

$$J = [R^2(1-r)]/[(1-R^2)r] \quad \text{(Equation 5)}$$

R^2, which represents the certainty of prediction of the real value of the selected genotypes, based on J measurements, was obtained by the expression:

$$R^2 = [J\,r]/[1 + r(J-1)] \quad \text{(Equation 6)}$$

where *J* is the number of measurements (J = 4 blocks, in this study) and *r* is the repeatability coefficient, as above (Cruz, 2006). The statistical analyses were all performed using the GENES software (Cruz, 2013) and Microsoft Office Excel.

RESULTS AND DISCUSSION

In the 49 cases analyzed (seven traits x seven trials), we found that the errors were normally distributed and the mathematical model was additive ($P > 0.05$; Table 1). In 46 cases (94%), we observed that the residual variances were homogeneous ($P > 0.05$). Hence, the high rate of non-violation of the assumptions residual normality, homogeneity of variance, and additivity of the mathematical model suggest that the F-test is suitable for determining the sources of variation (block and genotype) of analysis of variance (Storck et al., 2011). Among the 49 cases, the F-test revealed a significant block effect ($P \leq 0.05$) in only three cases (6%), which indicates that the blocks were homogeneous and that the completely randomized design was appropriate.

The means fiber length, fiber uniformity, fiber strength, fineness, fiber maturity, percentage of fibers, and short fiber index (Table 1) were similar to means previously obtained in other trials with cotton genotypes (Freitas et al., 2007; Araújo et al., 2013; Jerônimo et al., 2014; Carvalho et al., 2015a,b). This suggests suitability of this database for the proposed study, because it represents actual situations of field trials with cotton genotypes.

Regarding fiber length, fiber uniformity, fiber strength, fineness, fiber maturity, and percentage of fibers, there was a significant genotype effect ($P \leq 0.05$) in the seven tests. This suggests that it is possible to identify superior genotypes based on the genetic variability (Table 1). As for the short fiber index, we found significant genotype effects in all trials except for trials 2 and 4. Hence, the discrimination of genotypes based on this trait, using the F-test, may not be due to lack of genetic variability, but is more likely to be due to lower experimental accuracy (Resende and Duarte, 2007). In the 47 cases (96%) in which we found a significant genotype effect, the average SA, r, and R^2, based on four repetitions, were 0.93, 0.66, and 86.78%, respectively. In contrast, for trials 2 and 4, for which we did not detect significant genotype effects for short fiber index, the means of SA, r, and R^2 were 0.70, 0.31, and 60.35%, respectively. This reinforces the hypothesis that this lack of differences between genotypes is associated with lower experimental accuracy.

Table 1. Summary of analysis of variance, mean, selective accuracy (SA), and P values from the normality (Lilliefors), homogeneity of variance (Bartlett), and additivity (Tukey) tests for the seven cotton genotype traits, assessed in seven trials.

Sources of variation	d.f.	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7
Fiber length (mm)								
Block	3	0.50^{ns}	1.62^{ns}	0.05^{ns}	0.73^{ns}	0.12^{ns}	0.17^{ns}	0.52^{ns}
Genotype	10	5.74*	7.48*	14.13*	6.14*	8.01*	9.54*	3.70*
Error	30	0.96	0.72	1.09	0.49	0.29	1.50	0.56
Mean	-	27.89	28.33	25.75	27.37	28.64	25.67	28.45
SA^{1}	-	0.91	0.95	0.96	0.96	0.98	0.92	0.92
Normality	-	0.06	0.29	0.82	0.17	0.15	0.13	0.90
Homogeneity	-	0.06	0.40	0.83	0.27	0.47	0.93	0.99
Additivity	-	0.13	0.22	0.45	0.50	0.06	0.08	0.91
Fiber uniformity (%)								
Block	3	4.43^{ns}	1.97^{ns}	2.53^{ns}	0.24^{ns}	1.32^{ns}	12.94*	0.56^{ns}
Genotype	10	9.71*	7.62*	9.22*	8.06*	6.32*	11.19*	9.31*
Error	30	1.91	0.76	1.65	0.90	2.07	1.75	0.81
Mean	-	82.39	83.10	82.65	82.41	82.13	79.80	82.56
SA^{1}	-	0.90	0.95	0.91	0.94	0.82	0.92	0.96
Normality	-	0.10	0.83	0.95	0.22	0.10	0.07	0.25
Homogeneity	-	0.04	0.37	0.37	0.64	0.15	0.26	0.95
Additivity	-	0.44	0.07	0.43	0.31	0.29	0.16	0.12
Fiber strength (gf/tex)								
Block	3	5.58^{ns}	7.10^{ns}	2.57^{ns}	11.00^{ns}	1.82^{ns}	1.23^{ns}	1.06^{ns}
Genotype	10	27.19*	29.99*	33.93*	22.68*	34.25*	20.78*	45.68*
Error	30	3.82	4.23	2.34	3.89	5.34	2.75	5.51
Mean	-	27.03	26.99	24.61	26.57	28.12	23.10	27.19
SA^{1}	-	0.93	0.93	0.96	0.91	0.92	0.93	0.94
Normality	-	0.13	0.68	0.64	0.23	0.21	0.95	0.93
Homogeneity	-	0.70	0.58	0.89	0.08	0.21	0.88	0.92
Additivity	-	0.64	0.50	0.39	0.09	0.17	0.42	0.95
Fineness (micronaire)								
Block	3	0.07^{ns}	0.31*	0.15^{ns}	0.07^{ns}	0.03^{ns}	0.30*	0.11^{ns}
Genotype	10	1.15*	0.86*	1.45*	2.37*	1.55*	0.45*	1.43*
Error	30	0.10	0.33	0.05	0.14	0.05	0.07	0.13
Mean	-	4.92	4.67	4.01	4.56	3.15	3.28	4.29
SA^{1}	-	0.96	0.79	0.98	0.97	0.99	0.92	0.96
Normality	-	0.10	0.03	0.41	0.15	0.22	0.19	0.07
Homogeneity	-	0.59	0.00	0.51	0.12	0.11	0.47	0.09
Additivity	-	0.09	0.16	0.64	0.80	0.77	0.54	0.57
Fiber maturity (%)								
Block	3	0.52^{ns}	0.88^{ns}	0.97^{ns}	0.12^{ns}	0.87^{ns}	0.55^{ns}	0.75^{ns}
Genotype	10	6.57*	5.57*	9.27*	12.92*	11.22*	1.67*	11.74*
Error	30	0.80	0.83	0.50	1.78	0.40	0.48	0.98
Mean	-	88.14	87.14	85.27	86.69	83.48	83.73	86.70
SA^{1}	-	0.94	0.92	0.97	0.93	0.98	0.84	0.96
Normality	-	0.12	0.08	0.16	0.12	0.18	0.33	0.08
Homogeneity	-	0.88	0.46	0.82	0.08	0.42	0.12	0.07
Additivity	-	0.81	0.70	0.90	0.66	0.16	0.06	0.15
Percentage of fibers (%)								
Block	3	1.66^{ns}	1.33^{ns}	5.54^{ns}	2.61^{ns}	4.28^{ns}	0.57^{ns}	1.13^{ns}
Genotype	10	13.92*	6.91*	14.84*	21.26*	94.72*	8.83*	19.55*
Error	30	2.01	1.27	0.99	1.36	3.85	0.84	2.28
Mean	-	41.62	39.15	39.11	39.04	32.70	35.89	35.52
SA^{1}	-	0.93	0.90	0.97	0.97	0.98	0.95	0.94
Normality	-	0.06	0.11	0.12	0.09	0.19	0.09	0.07
Homogeneity	-	0.27	0.71	0.23	0.15	0.71	0.47	0.20
Additivity	-	0.22	0.31	0.07	0.19	0.41	0.36	0.08
Short fiber index (%)								
Block	3	3.81^{ns}	13.48^{ns}	1.85^{ns}	0.14^{ns}	1.38^{ns}	3.65^{ns}	4.20^{ns}
Genotype	10	13.41*	7.32 ^{ns}	8.79*	1.57*	14.23*	14.85*	5.79*
Error	30	3.27	7.10	1.42	1.57	4.68	2.36	2.10
Mean	-	5.76	4.69	10.05	2.67	6.15	12.93	3.83
SA^{1}	-	0.87	0.17	0.92	0.16	0.82	0.92	0.80
Normality	-	0.10	0.01	0.10	0.01	0.23	0.07	0.81
Homogeneity	-	0.66	0.02	0.13	0.01	0.25	0.88	0.15
Additivity	-	0.29	0.52	0.46	0.91	0.13	0.39	0.51

*Significant effect by F-test at 5% probability. ^{ns}Not significant; d.f. = degrees of freedom; ^{1}Class limits of experimental accuracy established by Resende and Duarte (2007): very high ($\text{SA} \geq 0.90$), high ($0.70 \leq \text{SA} < 0.90$), moderate ($0.50 \leq \text{SA} < 0.70$), and low ($\text{SA} < 0.50$).

SA scores higher than 0.90, which are equivalent to an R^2 of 81%, are typically targeted in trials because they provide very high experimental accuracy in discriminating the genotypes under evaluation (Resende and Duarte, 2007). In these seven trials with cotton genotypes, the SA ranged from 0.16 (short fiber index in trial 4) to 0.99 (fineness in trial 5), with a mean of 0.868 (Table 1). According to the class limits established by Resende and Duarte (2007), 41 cases showed very high experimental accuracy ($SA \geq 0.90$), six high accuracy ($0.70 \leq SA < 0.90$), and two cases low accuracy ($SA \leq 0.50$) (Table 1). Therefore, we can infer that there was variability in the experimental accuracy between traits and tests but that, in general, these traits were evaluated under satisfactory experimental conditions. The variability of experimental accuracy, based on SA between traits and tests, has been verified in studies with common bean (Cargnelutti Filho and Ribeiro, 2010), soybean (Cargnelutti Filho and Gonçalves, 2011), maize (Cargnelutti Filho and Guadagnin, 2011), and irrigated rice (Cargnelutti Filho et al., 2012) genotypes.

The r ranged from 0.31 (short fiber index in trials 2 and 4) to 0.89 (fineness in trial 5), with a mean of 0.65 (Table 2).

Table 2. Estimate of repeatability coefficients (r), genotypic determination coefficients (R^2), and number of measurements (repetitions) (J) associated with different R^2 of seven genotypic traits of cotton genotypes, assessed in seven trials.

Sources of variation	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Trial 6	Trial 7	Mean[1]
Fiber length (mm)								
r	0.55	0.70	0.75	0.74	0.87	0.57	0.87	0.72
R^2 (%)	83.24	90.39	92.25	92.10	96.32	84.28	95.19	90.54
J (R^2 = 0.80)	3	2	1	1	1	3	1	2
J (R^2 = 0.85)	5	2	2	2	1	4	1	2
Fiber uniformity (%)								
r	0.50	0.69	0.53	0.67	0.34	0.57	0.86	0.60
R^2 (%)	80.28	90.07	82.10	88.88	67.30	84.37	94.94	83.99
J (R^2 = 0.80)	4	2	3	2	8	3	1	3
J (R^2 = 0.85)	6	2	5	3	11	4	1	5
Fiber strength (gf/tex)								
r	0.60	0.60	0.77	0.55	0.58	0.62	0.76	0.64
R^2 (%)	85.94	85.89	93.10	82.86	84.41	86.74	90.27	87.03
J (R^2 = 0.80)	3	3	1	3	3	2	1	2
J (R^2 = 0.85)	4	4	2	5	4	3	2	3
Fineness (micronaire)								
r	0.72	0.29	0.88	0.80	0.89	0.57	0.73	0.70
R^2 (%)	91.23	61.98	96.68	93.95	97.08	84.26	88.80	87.71
J (R^2 = 0.80)	2	10	1	1	1	3	2	3
J (R^2 = 0.85)	2	14	1	1	1	4	2	4
Fiber maturity (%)								
r	0.64	0.59	0.81	0.61	0.87	0.38	0.85	0.68
R^2 (%)	87.84	85.12	94.58	86.25	96.40	71.38	94.53	88.01
J (R^2 = 0.80)	2	3	1	3	1	6	1	2
J (R^2 = 0.85)	3	4	1	4	1	9	1	3
Percentage of fibers (%)								
r	0.60	0.53	0.78	0.79	0.86	0.70	0.83	0.72
R^2 (%)	85.57	81.55	93.35	93.62	95.93	90.53	93.51	90.58
J (R^2 = 0.80)	3	4	1	1	1	2	1	2
J (R^2 = 0.85)	4	5	2	2	1	2	1	2
Short fiber index (%)								
r	0.44	0.31	0.57	0.31	0.34	0.57	0.70	0.48
R^2 (%)	75.62	60.35	83.88	60.35	67.11	84.09	87.31	76.67
J (R^2 = 0.80)	5	7	3	7	8	3	2	5
J (R^2 = 0.85)	7	10	4	10	10	4	2	7

[1] Mean of r (R^2 and J associated with different R^2, calculated based on mean of r).

The variability of r between traits and trials is particularly important in this study by representing different real situations that enable inferences regarding J, with overall applications. The variability of r and, subsequently, estimation of J between traits and trials were also observed in common bean (Cargnelutti Filho et al., 2009; Cargnelutti Filho and Ribeiro, 2010), soybean (Storck et al., 2009; Cargnelutti Filho and Gonçalves, 2011), maize (Cargnelutti Filho et al., 2010; Cargnelutti Filho and Guadagnin, 2011), and irrigated rice (Cargnelutti Filho et al., 2012).

The obtained R^2 from four repetitions ranged from 60.35% (short fiber index in trials 2 and 4) to 97.08% (fineness in trial 5) (Table 2). For each trait, the mean r from the seven trials may adequately represent the trials with cotton genotypes, and therefore, resizing the number of repetitions based on mean r would be an appropriate procedure. For this reason, to achieve an R^2 of 81% from means r of fiber length (0.72), fiber uniformity (0.60), fiber strength (0.64), fineness (0.70), fiber maturity (0.68), percentage of fibers (0.72), and short fiber index (0.48), would require 3, 3, 2, 2, 2, 2, and 5 repetitions, respectively.

The R^2 based on the mean r between the seven trials and for a fixed J of 4, was 0.9054, 0.8399, 0.8703, 0.8771, 0.8801, 0.9058, and 0.7667, respectively, for fiber length, fiber uniformity, fiber strength, fineness, fiber maturity, percentage of fibers, and short fiber index (Table 2). From this we may infer that four replications allow for detection of genotypic differences with a certainty of 90.54, 83.99, 87.03, 87.71, 88.01, 90.58, and 76.67% to predict the actual genotypic value for fiber length, fiber uniformity, fiber strength, fineness, fiber maturity, percentage of fibers, and short fiber index, respectively.

In general, for the seven traits, significant increases in R^2 were obtained with up to four repetitions ($J = 4$). For more than four repetitions, there was non-significant increases in R^2, which suggests a small gain in the predictive ability to detect the actual value of the genotype. For fiber length, an important trait in cotton genetic breeding programs (Carvalho et al., 2015a,b), we found that trials with four repetitions enable identification of superior cotton genotypes with 90.54% accuracy (higher than the desired accuracy of 81%). This level of accuracy is similar to that obtained for agronomic traits of other crops, such as common bean (Cargnelutti Filho et al., 2009), soybean (Storck et al., 2009), maize (Cargnelutti Filho et al., 2010), irrigated rice (Cargnelutti Filho et al., 2012) with 85, 80, 81, and 79% accuracy, respectively. Therefore, it can be inferred that the selective accuracy goals of 90% have been achieved in trials with common bean, soybean, maize, irrigated rice, and cotton using a number of repetitions lower than the six theoretically recommended by Resende and Duarte (2007). However, the use of a larger number of repetitions should be encouraged in order to maximize the experimental accuracy.

Conflicts of interest

The authors declare no conflict of interest.

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