CC BY

Research Article

Sample size for morphological traits of cotton cultivars[1]

Mikael Brum dos Reis[2], Alberto Cargnelutti Filho[2],
Leonardo Cesar Pradebon[2], Lais Cherobini[2], Manoela Conterato Della Pace[2], Debora Luiza Pozzebon[2]

ABSTRACT

The adequate sample size helps in the experimental precision and in the efficient use of resources in research with cotton. This study aimed to determine the sample size and understand the dissimilarities between traits and between cultivars of cotton at the stages of first visible flower bud and full maturity. Nine uniformity trials were carried out with the cultivars IMA2106GL, IMA5801B2RF, IMA5802B2RF, TMG31B3RF, TMG44B2RF, IMA5045WS3, TMG51WS3, TMG91WS3, and 21064WS3. At the stage of first visible flower bud, the main stem height, first productive branch height, main stem diameter, and number of leaves, nodes, and primary branches were evaluated; and, at the full maturity stage, in addition to the first five traits, the number of productive branches. Assuming a mean estimation error of 5 % and a confidence level of 95 %, the number of plants needed to measure the traits is 14 for main stem height, and number of leaves and nodes; 38 for main stem diameter and number of primary branches; and 126 for first productive branch height and number of productive branches.

KEYWORDS: *Gossypium hirsutum* L., experimental sizing, dissimilarity matrix.

RESUMO

Tamanho de amostra para caracteres morfológicos de cultivares de algodoeiro

O tamanho de amostra adequado auxilia na precisão experimental e no uso eficiente de recursos em estudos com algodoeiro. Objetivou-se determinar o tamanho de amostra e compreender as dissimilaridades entre caracteres e entre cultivares de algodoeiro nos estádios de primeiro botão floral visível e maturação plena. Foram conduzidos nove ensaios de uniformidade, compostos pelas cultivares IMA2106GL, IMA5801B2RF, IMA5802B2RF, TMG31B3RF, TMG44B2RF, IMA5045WS3, TMG51WS3, TMG91WS3 e 21064WS3. No estádio de primeiro botão floral visível, avaliaram-se os caracteres altura da haste principal e do primeiro ramo produtivo, diâmetro da haste principal e o número de folhas, nós e ramificações primárias; e, no estádio de maturação plena, além dos primeiros cinco caracteres, o número de ramos produtivos. Assumindo-se um erro de estimação da média de 5 % e grau de confiança de 95 %, são necessárias 14 plantas para mensurar os caracteres altura da haste principal e número de folhas e de nós; 38 para diâmetro da haste principal e número de ramificações primárias; e 126 para altura do primeiro ramo produtivo e número de ramos produtivos.

PALAVRAS-CHAVE: *Gossypium hirsutum* L., dimensionamento experimental, matriz de dissimilaridade.

INTRODUCTION

Cotton is one of the main agricultural commodities in Brazil, which leads the world market as a top exporter of lint cotton and reached 3.026 million metric tons in 2025 (CEPEA 2025).

Studies have used the morphological traits main stem height and diameter (Ferreira et al. 2018, Rossi et al. 2020); number of nodes, vegetative and productive branches (Zaman et al. 2021); and leaves (Wu et al. 2023), in order to understand the responses of the crop to environmental and management conditions, and, consequently, improve practices, with a view to maximizing cotton seed and lint yields.

For data that follow the normal distribution, it is possible to determine the sample size using the standard deviation, the critical value of the Student's *t*-distribution, and the estimation error (Bussab & Morettin 2023). Reduced sample sizes may lead to inaccurate estimates of means (Bussab & Morettin 2023), whereas excessively large samples entail

[1] Received: Feb. 20, 2026. Accepted: June 08, 2026. Published: June 30, 2026. DOI: 10.1590/1983-40632026v5685261.
[2] Universidade Federal de Santa Maria, Centro de Ciências Rurais, Departamento de Fitotecnia, Santa Maria, RS, Brazil.
E-mail/ORCID: eng.mikaelbrum@gmail.com/0000-0003-4226-690X; alberto.cargnelutti.filho@gmail.com/0000-0002-8608-9960; leonardopradebon@gmail.com/0000-0001-7827-6312; laischerobini@gmail.com/0000-0002-6431-4653; manoelaconterato@gmail.com/0009-0001-1063-9938; debora.pozzebon@acad.ufsm.br/0009-0001-8186-5916.
Editor: Luis Carlos Cunha Junior/Data Availability Statement: Research data are only made available by authors upon request.

unnecessary financial and operational costs, without proportional gains in precision (Singh & Masuku 2014).

For studies involving a set of variables, cluster analyses allow them to be aggregated according to the similarities of their characteristics (Hair Junior et al. 2009). The Tocher's clustering method has been employed in studies with the morphological traits plant height and number of branches. For example, Bhimate et al. (2019) and Rizwan et al. (2021) analyzed 36 and 45 cotton genotypes in their studies, respectively, and identified the formation of distinct clusters among cotton genotypes. In the same way, it is expected that it will be possible to identify similarities between the sample size of morphological traits and between cultivars of cotton.

Therefore, this study aimed to determine the sample size for estimating the means of the morphological traits of cotton and investigate the dissimilarities between traits and between cultivars at the stages of first visible flower bud and full maturity.

MATERIAL AND METHODS

Nine uniformity trials were conducted with cotton at the Universidade Federal de Santa Maria (29º43'28"S, 53º43'12" W, and altitude of 95 m). According to the Köppen's classification, the climate of the region is Cfa, humid subtropical (Alvares et al. 2013). The soil is classified as Argissolo Vermelho Distrófico arênico (Ultisol) (Santos et al. 2025), corresponding to a Typic Hapludult (USDA 2022).

After harrowing the soil, basal fertilization was carried out with the NPK (05-20-20) fertilizer, broadcast at a dose of 500 kg ha^{-1}. Top-dressing fertilization was split and applied at three phenological stages, according to Marur & Ruano (2001). At the V3 stage, 133 kg ha^{-1} of urea and 68 kg ha^{-1} of potassium chloride were applied, and, in each of the stages B1 and F1, 66.5 kg ha^{-1} of urea and 34 kg ha^{-1} of potassium chloride.

Each uniformity trial was composed of eight 20-m-long rows, with spacing of 1 m between rows and 0.125 m between plants in the row, totaling a usable area per trial of 160 m^2. In each uniformity trial, 160 plants were randomly marked, spaced 1 m apart, arranged in a grid of 1 × 1 m, totaling 1,440 plants.

Five cotton cultivars (IMA2106GL, IMA5801B2RF, IMA5802B2RF, TMG31B3RF, and TMG44B2RF) were sown on Oct. 14, 2024, and four other cotton cultivars (IMA5045WS3, TMG51WS3, TMG91WS3, and 21064WS3) were sown on Nov. 09, 2024. On Jan. 29, 2025, due to a dry period, water supplementation was carried out in the trials through the application of a 30-mm irrigation depth, using a drip line, evenly distributed in all trials.

Morphological traits were evaluated in the 1,440 plants at two different times: first visible flower bud (B1 stage), defined as the time when at least 50 % of the plants in the trial had the first visible flower bud; and at full maturity, corresponding to the moment of harvest.

In the first evaluation, the following morphological traits were evaluated: main stem height (cm), first productive branch height (cm), main stem diameter (mm), and number of leaves, nodes, and primary branches. In the second evaluation, carried out at full maturity, in addition to the previously mentioned traits (except for number of primary branches), the number of productive branches was included in the evaluation.

The traits were classified according to the nature of the measurement, with main stem height, first productive branch height, and main stem diameter considered as measurement traits; and number of leaves, nodes, primary branches, and productive branches considered as counting traits. The main stem height was determined by measuring the distance between the base of the plant and the insertion point of the last expanded leaf of the main stem; the first productive branch height by measuring the distance between the base of the plant and the insertion point of the first branch with flower bud and/or flower; and the main stem diameter was measured at the height of the cotyledon nodes, with a digital caliper (502.150BL model, with a resolution of 0.01 mm).

The number of leaves was counted along the main stem, from the cotyledon nodes (parallel nodes of the main stem), considering as true leaf the one whose midrib was at least 2.5 cm long (Marur & Ruano 2001); the number of nodes along the main stem, considering as the first node the first two parallel nodes visible, followed by the second, third and other alternate nodes visible along the branch; the number of primary branches along the main stem, by counting the axillary branches, including both vegetative and productive branches; and the number of productive branches by counting the primary

branches inserted along the main stem with flower bud, flower or fruit.

For each uniformity trial and trait, hypothesis tests were performed to check whether the data followed normal distribution, using the Lilliefors test and the data randomness test by the run test (Campos 1983). Each trial was composed of a matrix of 20 rows and 8 columns. The run test was performed by starting the walk in row 1, column 1, advancing to row 1, column 8, returning through row 2, from column 8 to column 1, proceeding in row 3, going from column 1 to column 8, and so on, until ending in row 20, column 1.

For each trait, based on the data of the 160 plants sampled in each trial and evaluation, the following parameters were calculated: mean, coefficient of variation, skewness, kurtosis and sample size (n), using the equation $n = [(t_{\alpha/2}\ s)/e]^2$ (Bussab & Morettin 2023), where: $t_{\alpha/2}$ is the critical value of the Student's *t*-distribution, corresponding to the area on the right equal to $\alpha/2$; that is, it is the value of *t* which satisfies $P(t > t_{\alpha/2}) = \alpha/2$, with $\alpha = 5$ % of error probability, and n - 1 degrees of freedom (n = 160 plants); s the estimate of the sample standard deviation; e the estimation errors (semi-amplitudes of the confidence interval), equal to 1 %, 2 %, 3 %, ..., 15 % of the mean estimate, with a confidence level (1 - α) of 95 %. The estimation error was calculated as a percentage of the mean estimate (m), using the equation $100[(t_{\alpha/2}\ s)/(\sqrt{\eta}\ m)]$ (Bussab & Morettin 2023), considering n = 160.

In order to investigate the dissimilarities in sample sizes, multivariate analysis was performed to estimate the Euclidean distance. Dissimilarity measures of morphological traits and between cultivars were obtained for the stages of first visible flower bud and full maturity. Cluster analysis was performed using the Tocher's method. The cophenetic correlation coefficient, which expresses the degree of representativeness of the distance matrix by the cluster diagram (Cruz et al. 2014), was calculated.

The analyses were performed in the Microsoft Excel and R software (R Core Team 2025), using the MultivariateAnalysis (Azevedo 2025) and ggplot2 (Wickham 2016) packages.

RESULTS AND DISCUSSION

All evaluated cultivars, except 21064WS3, showed an early cycle (Fuzatto 1999), which lasted 133 days (sowing on Oct. 14, 2024) and 131 days (sowing on Nov. 09, 2024), whereas the 21064WS3 cultivar had an average cycle of 154 days.

The water requirement of the crop varies from 650 to 700 mm (Bezerra et al. 2010). However, in all trials, rainfall was below this range. The cultivars sown on Oct. 14, 2024, reached 61 % of the water requirement, whereas those sown on Nov. 09, 2024, reached 63 %. The 21064WS3 cultivar obtained 78 % of the water requirement, due to its longer cycle. The average daily temperatures ranged from 18.45 ºC (Apr. 2025) to 26.39 ºC (Feb. 2025), remaining within the ideal limit of 18 to 32 ºC for cotton cultivation (Beltrão & Oliveira 2011).

According to Pimentel-Gomes (2009), the coefficient of variation (CV; %) is classified as low (< 10 %), medium (10 to 20 %), and high (> 20 %). Based on this classification, the CV values indicated a low variability for the traits main stem height (7.17 %), number of leaves (7.02 %), and number of nodes (7.16 %); medium variability for main stem diameter (12.97 %) and number of primary branches (12.08 %); and high variability for first productive branch height (22.37 %) and number of productive branches (20.73 %) (Tables 1 and 2).

These CV values indicate low variability between the measurement and counting morphological traits evaluated in the cotton cultivars, since, for both, there was an equivalent distribution of traits at the coefficient of variation levels. For the cultivars, the CV values were medium, ranging from 10.53 % (IMA5801B2RF) to 13.69 % (TMG44B2RF), demonstrating similarity in plant development, since similar dispersion values were observed among the cultivars.

Skewness coefficients ranged from -0.83 to 1.10 (mean of -0.02), and kurtosis coefficients from -0.49 to 2.15 (mean of 0.32), remaining mostly close to zero. These values demonstrate that the data distributions show little deformation in relation to the normal distribution curve, since the greater magnitudes of these values reflect a greater distance from the normal distribution (Bussab & Morettin 2023).

Randomness was met in 81.63 % of the measurement traits and 85.18 % of the counting traits. Normality was met in 89.79 % of the measurement traits, but not in the counting traits. Considering the Central Limit Theorem, according to which, for samples larger than 30 observations, the sample mean distribution tends to approach the normal

Table 1. Descriptive statistics of the measurement morphological traits main stem height (MSH; cm), first productive branch height (FPBH; cm), and main stem diameter (MSD; cm) of nine cotton (*Gossypium hirsutum* L.) cultivars evaluated at the stages of first visible flower bud (first evaluation) and full maturity (second evaluation).

Cultivar	Mean	CV (%)	Skw	Kur	Ran	Nor	Mean	CV (%)	Skw	Kur	Ran	Nor
	MSH - first evaluation						MSH - second evaluation					
IMA2106GL	78.58	7.70	-0.57	0.03	0.00	0.12	95.84	5.89	-0.53	0.67	0.40	0.08
IMA5801B2RF	85.09	5.88	-0.23	-0.25	0.00	0.54	110.81	5.15	-0.32	-0.26	0.00	0.31
IMA5802B2RF	74.83	8.03	-0.20	-0.33	0.02	0.14	99.98	5.62	-0.28	0.00	0.65	0.31
TMG31B3RF	77.05	8.37	-0.56	-0.05	0.28	0.17	100.13	6.18	-0.26	0.14	0.37	0.39
TMG44B2RF	75.06	9.38	-0.77	0.32	0.06	0.02	101.84	7.77	-0.71	0.29	0.31	0.07
TMG5045WS3	84.22	6.68	0.06	-0.16	0.06	0.77	89.85	7.31	0.22	0.01	0.00	0.49
TMG51WS3	75.36	6.36	0.19	-0.33	0.57	0.38	79.07	6.48	-0.04	-0.41	0.06	0.72
TMG91WS3	80.89	8.03	-0.12	-0.04	0.12	0.73	84.54	7.43	-0.26	0.27	0.12	0.49
21064WS3	82.44	8.07	0.34	-0.45	0.00	0.13	94.25	8.68	0.43	0.51	0.02	0.73
	FPBH - first evaluation						FPBH - second evaluation					
IMA2106GL	17.43	23.72	0.11	-0.49	0.01	0.35	-	-	-	-	-	-
IMA5801B2RF	22.60	22.70	1.10	1.51	0.84	0.00	-	-	-	-	-	-
IMA5802B2RF	13.43	24.51	0.29	0.19	0.07	0.12	-	-	-	-	-	-
TMG31B3RF	12.41	26.67	0.35	-0.03	0.90	0.04	-	-	-	-	-	-
TMG44B2RF	19.01	28.41	0.66	0.57	0.73	0.08	-	-	-	-	-	-
TMG5045WS3	19.86	24.64	0.16	-0.48	0.27	0.20	21.65	24.37	0.54	0.42	0.08	0.18
TMG51WS3	24.67	16.52	0.14	0.45	0.09	0.10	26.39	15.15	0.07	0.14	0.17	0.16
TMG91WS3	22.26	19.86	0.47	0.76	0.63	0.15	24.01	18.29	0.54	0.24	0.85	0.06
21064WS3	21.62	23.09	0.33	0.17	0.68	0.11	23.16	22.89	0.71	1.25	0.72	0.04
	MSD - first evaluation						MSD - second evaluation					
IMA2106GL	14.42	11.37	0.30	0.77	0.20	0.71	17.51	13.17	0.55	1.40	0.70	0.43
IMA5801B2RF	13.86	10.64	0.26	0.55	0.15	0.86	17.56	12.27	0.41	1.09	0.02	0.30
IMA5802B2RF	14.70	11.68	-0.14	-0.01	0.11	0.23	17.97	14.19	0.25	-0.03	0.11	0.77
TMG31B3RF	14.41	13.46	-0.28	0.56	0.44	0.89	18.47	12.25	-0.01	0.28	0.27	0.81
TMG44B2RF	13.68	14.86	-0.69	1.34	0.06	0.25	18.08	14.66	-0.18	0.32	0.11	0.93
TMG5045WS3	14.06	15.41	0.51	0.41	0.97	0.89	15.30	14.22	0.12	-0.15	0.38	0.89
TMG51WS3	12.20	13.33	0.59	0.43	0.61	0.04	13.20	11.59	0.31	-0.01	0.37	0.59
TMG91WS3	13.05	12.53	0.58	0.94	0.15	0.61	14.04	12.13	0.57	1.02	0.74	0.92
21064WS3	14.15	13.20	0.07	0.06	0.17	0.86	15.52	12.43	0.24	0.06	0.56	0.81

CV (%): coefficient of variation; Skw: skewness; Kur: kurtosis; Ran: randomness (p-value); Nor: normality (p-value).

distribution (Bussab & Morettin 2023), and aligned with the skewness and kurtosis coefficients close to zero, the sample size was determined as a function of the Student's *t*-distribution.

At the stage of first visible flower bud, the main stem height had the smallest sample sizes (6 plants ≤ n ≤ 14 plants). For main stem diameter, the value was approximately three times higher (18 plants ≤ n ≤ 38 plants), and the first productive branch height required the largest sample sizes (43 plants ≤ n ≤ 126 plants), reflected by its high variability (CV from 16.52 to 28.41 %). For the counting traits, the number of leaves and nodes showed low dissimilarity and sample size ranging from 4 to 13 plants. The number of primary branches showed a greater variability (CV = 12.09 %), requiring a sample size of 16 to 47 plants. At the stage of full maturity, a reduction in sample size was observed for main stem height (5 plants ≤ n ≤ 12 plants), main stem diameter (21 plants ≤ n ≤ 34 plants), and first productive branch height (36 plants ≤ n ≤ 93 plants). In contrast, the number of leaves and nodes increased, requiring 5 to 14 plants, whereas the number of productive branches had the highest values (41 plants ≤ n ≤ 99 plants) (Tables 3 and 4).

The larger sample size for first productive branch height and number of productive branches is associated with the high variability of these traits in cotton, evidenced by the CV values between 15.15 and 28.41 % for first productive branch height and between 16.06 and 25.16 % for number of productive branches. Similar results were reported by Ferreira

Table 2. Descriptive statistics of the counting morphological traits number of leaves (NL), nodes (NN), primary branches (NPriB), and productive branches (NProB) of nine cotton (*Gossypium hirsutum* L.) cultivars evaluated at the stages of first visible flower bud (first evaluation) and full maturity (second evaluation).

Cultivar	Mean	CV (%)	Skw	Kur	Ran	Nor	Mean	CV (%)	Skw	Kur	Ran	Nor
	NL - first evaluation						NL - second evaluation					
IMA2106GL	15.06	6.73	-0.57	0.16	0.80	0.00	17.51	5.58	-0.22	0.74	0.74	0.00
IMA5801B2RF	15.11	5.03	-0.54	0.84	0.21	0.00	17.72	7.02	0.19	0.97	0.00	0.00
IMA5802B2RF	15.37	6.43	-0.20	-0.04	0.56	0.00	17.84	6.84	0.35	-0.01	0.99	0.00
TMG31B3RF	15.54	6.77	-0.43	0.02	0.47	0.00	17.61	7.29	0.32	0.06	0.16	0.00
TMG44B2RF	15.17	8.53	-0.57	0.17	1.00	0.00	17.27	7.59	-0.29	0.55	0.57	0.00
TMG5045WS3	15.53	6.00	0.52	0.35	0.76	0.00	19.76	9.29	-0.30	-0.18	0.01	0.00
TMG51WS3	14.81	6.07	-0.25	0.44	0.97	0.00	18.74	8.39	0.19	-0.39	0.52	0.00
TMG91WS3	15.27	6.39	0.05	0.08	0.03	0.00	19.28	8.33	-0.34	0.54	0.20	0.00
21064WS3	16.19	5.59	-0.07	-0.08	0.24	0.00	23.31	8.42	-0.76	1.39	0.34	0.00
	NN - first evaluation						NN - second evaluation					
IMA2106GL	14.34	6.91	-0.66	0.58	0.21	0.00	17.49	5.63	-0.20	0.66	0.52	0.00
IMA5801B2RF	14.16	5.46	-0.20	1.50	0.26	0.00	17.71	7.03	0.21	0.96	0.00	0.00
IMA5802B2RF	14.57	6.47	-0.18	-0.25	0.37	0.00	17.83	6.98	0.25	0.06	0.99	0.00
TMG31B3RF	14.66	7.54	-0.45	0.01	0.36	0.00	17.57	7.54	0.23	0.10	0.16	0.00
TMG44B2RF	14.27	9.06	-0.62	0.27	0.43	0.00	17.24	7.82	-0.30	0.33	0.57	0.00
TMG5045WS3	15.53	5.95	0.54	0.39	0.99	0.00	19.76	9.29	-0.30	-0.18	0.01	0.00
TMG51WS3	14.81	6.07	-0.25	0.44	0.97	0.00	18.74	8.39	0.19	-0.39	0.52	0.00
TMG91WS3	15.27	6.39	0.05	0.08	0.03	0.00	19.28	8.33	-0.34	0.54	0.20	0.00
21064WS3	16.19	5.59	-0.07	-0.08	0.24	0.00	23.31	8.42	-0.76	1.39	0.34	0.00
	NPriB - first evaluation						NProB - second evaluation					
IMA2106GL	10.94	11.22	-0.83	2.15	0.97	0.00	7.96	22.66	0.03	-0.23	1.00	0.01
IMA5801B2RF	12.18	11.96	0.05	-0.25	0.13	0.00	7.96	22.70	0.14	0.41	0.69	0.00
IMA5802B2RF	10.99	10.46	-0.10	1.27	0.88	0.00	8.23	21.42	-0.30	-0.28	1.00	0.00
TMG31B3RF	11.75	12.52	0.17	0.37	0.04	0.00	9.49	21.85	0.21	0.73	0.13	0.00
TMG44B2RF	11.08	17.35	0.37	0.93	0.23	0.00	8.95	25.12	-0.30	0.48	0.21	0.01
TMG5045WS3	12.02	10.11	-0.27	0.21	0.78	0.00	9.98	19.67	0.10	0.05	0.24	0.01
TMG51WS3	10.13	12.03	-0.02	0.04	0.02	0.00	8.98	16.06	-0.45	0.75	0.93	0.00
TMG91WS3	10.29	12.06	0.08	0.62	0.32	0.00	8.70	16.65	0.46	0.47	0.26	0.00
21064WS3	11.31	11.05	-0.27	-0.30	0.92	0.00	10.90	20.48	-0.21	-0.38	0.86	0.01

CV (%): coefficient of variation; Skw: skewness; Kur: kurtosis; Ran: randomness (p-value); Nor: normality (p-value).

et al. (2018), who found a greater variability for number of productive branches (CV = 16.78 %), when compared to main stem height (CV = 8.74 %), and by Silva et al. (2019), who observed a greater variability for first productive branch height (CV = 18.15 %), if compared to main stem diameter (CV = 9.55 %). Cargnelutti Filho et al. (2009) observed the same relationship in soybean, with larger sample sizes for number of primary branches (32 plants) and first productive branch height (8 plants), when compared to number of nodes (5 plants) and main stem height (3 plants), considering an estimation error of 20 % of the mean of the traits.

This has also been found in showy rattlebox (*Crotalaria spectabilis* Roth), for which the main stem height trait had a CV of 12.25 % and a sample size of 24 plants, whereas the number of primary branches had a CV of 50.91 % and a sample size of 408 plants, considering the same estimation error of 5 % of the mean (Toebe et al. 2017). In white lupine (*Lupinus albus* L.), the main stem height had a CV of 12.54 % and a sample size of 12 plants, lower than those observed for main stem diameter, which had a CV of 22.55 % and a sample size of 38 plants for an estimation error of 15 % of the mean (Burin et al. 2014).

As in soybean, showy rattlebox and white lupine, this response was observed in the cotton crop, for which traits with greater variability required larger sample sizes, whereas those with lower variability required smaller sample sizes. A progressive increase was observed in the variability

Table 3. Sample size for the measurement morphological traits main stem height (MSH; cm), first productive branch height (FPBH; cm), and main stem diameter (MSD; cm) of nine cotton (*Gossypium hirsutum* L.) cultivars evaluated at the stages of first visible flower bud (first evaluation) and full maturity (second evaluation).

Cultivar	Mean estimation error (%)																	
	1	3	5	7	9	11	13	15	error	1	3	5	7	9	11	13	15	error
	MSH - first evaluation									MSH - second evaluation								
IMA2106GL	232	26	10	5	3	2	2	2	1.20	136	16	6	3	2	2	1	1	0.92
IMA5801B2RF	136	16	6	3	2	2	1	1	0.92	104	12	5	3	2	1	1	1	0.80
IMA5802B2RF	252	28	11	6	4	3	2	2	1.25	124	14	5	3	2	2	1	1	0.88
TMG31B3RF	274	31	11	6	4	3	2	2	1.31	150	17	6	4	2	2	1	1	0.97
TMG44B2RF	344	39	14	8	5	3	3	2	1.47	236	27	10	5	3	2	2	2	1.21
TMG5045WS3	174	20	7	4	3	2	2	1	1.04	209	24	9	5	3	2	2	1	1.14
TMG51WS3	158	18	7	4	2	2	1	1	0.99	164	19	7	4	3	2	1	1	1.01
TMG91WS3	252	28	11	6	4	3	2	2	1.25	216	24	9	5	3	2	2	1	1.16
21064WS3	254	29	11	6	4	3	2	2	1.26	295	33	12	7	4	3	2	2	1.36
	FPBH - first evaluation									FPBH - second evaluation								
IMA2106GL	2,196	244	88	45	28	19	13	10	3.70	-	-	-	-	-	-	-	-	-
IMA5801B2RF	2,011	224	81	42	25	17	12	9	3.55	-	-	-	-	-	-	-	-	-
IMA5802B2RF	2,345	261	94	48	29	20	14	11	3.83	-	-	-	-	-	-	-	-	-
TMG31B3RF	2,775	309	111	57	35	23	17	13	4.16	-	-	-	-	-	-	-	-	-
TMG44B2RF	3,148	350	126	65	39	27	19	14	4.44	-	-	-	-	-	-	-	-	-
TMG5045WS3	2,368	264	95	49	30	20	15	11	3.85	2,316	258	93	48	29	20	14	11	3.80
TMG51WS3	1,065	119	43	22	14	9	7	5	2.58	895	100	36	19	12	8	6	4	2.36
TMG91WS3	1,539	171	62	32	19	13	10	7	3.10	1,305	145	53	27	17	11	8	6	2.86
21064WS3	2,080	232	84	43	26	18	13	10	3.61	2,044	228	82	42	26	17	13	10	3.57
	MSD - first evaluation									MSD - second evaluation								
IMA2106GL	505	57	21	11	7	5	3	3	1.78	677	76	28	14	9	6	5	4	2.06
IMA5801B2RF	442	50	18	10	6	4	3	2	1.66	588	66	24	12	8	5	4	3	1.92
IMA5802B2RF	532	60	22	11	7	5	4	3	1.82	786	88	32	17	10	7	5	4	2.22
TMG31B3RF	707	79	29	15	9	6	5	4	2.10	586	66	24	12	8	5	4	3	1.91
TMG44B2RF	861	96	35	18	11	8	6	4	2.32	839	94	34	18	11	7	5	4	2.29
TMG5045WS3	927	103	38	19	12	8	6	5	2.41	789	88	32	17	10	7	5	4	2.22
TMG51WS3	694	78	28	15	9	6	5	4	2.08	524	59	21	11	7	5	4	3	1.81
TMG91WS3	613	69	25	13	8	6	4	3	1.96	574	64	23	12	8	5	4	3	1.89
21064WS3	680	76	28	14	9	6	5	4	2.06	603	67	25	13	8	5	4	3	1.94

of the traits main stem height, number of nodes, main stem diameter and number of primary branches, in this order, accompanied by a corresponding increase in sample size.

The dissimilarity in sample size for the traits between the stages ranged from 9.63 for main stem height to 17.75 for first productive branch height. The number of leaves and nodes had the lowest sample size dissimilarities ($0.85 \leq d \leq 13.41$), whereas the first productive branch height had the greatest dissimilarities, when compared to the other traits, with values ranging from 56.28 for number of productive branches to 249.72 for number of leaves (Table 5). IMA2106GL and TMG31B3RF were the cultivars that had the closest sample sizes relative to the others (27.78 and 16.67 %, respectively). In contrast, TMG51WS3 and TMG44B2RF had the largest sample size distances, with 61.11 and 38.89 %, respectively (Table 6). These results demonstrate that the sample size varies between morphological traits and between cultivars, reinforcing the need for considering these factors in sample sizing.

The traits main stem height, main stem diameter, and number of leaves, nodes, and primary branches were grouped together (C1), whereas the first productive branch height (C2) and number of productive branches (C3) formed distinct clusters (Figure 1). This result demonstrates that the dissimilarity of the sample sizes for the morphological traits was not influenced at the two phenological stages evaluated. This behavior has also been observed in pearl millet, for which the sample sizes estimated in the evaluations carried out from 40 days after sowing (DAS) were close

Table 4. Sample size for the counting morphological traits number of leaves (NL), nodes (NN), primary branches (NPriB), and productive branches (NProB) of nine cotton (*Gossypium hirsutum* L.) cultivars evaluated at the stages of first visible flower bud (first evaluation) and full maturity (second evaluation).

Cultivar	Mean estimation error (%)																	
	1	3	5	7	9	11	13	15	error	1	3	5	7	9	11	13	15	error
	NL - first evaluation									NL - second evaluation								
IMA2106GL	177	20	8	4	3	2	2	1	1.05	122	14	5	3	2	2	1	1	0.87
IMA5801B2RF	99	11	4	3	2	1	1	1	0.79	193	22	8	4	3	2	2	1	1.10
IMA5802B2RF	162	18	7	4	2	2	1	1	1.00	183	21	8	4	3	2	2	1	1.07
TMG31B3RF	179	20	8	4	3	2	2	1	1.06	208	24	9	5	3	2	2	1	1.14
TMG44B2RF	285	32	12	6	4	3	2	2	1.33	225	25	9	5	3	2	2	1	1.19
TMG5045WS3	141	16	6	3	2	2	1	1	0.94	337	38	14	7	5	3	2	2	1.45
TMG51WS3	144	16	6	3	2	2	1	1	0.95	275	31	11	6	4	3	2	2	1.31
TMG91WS3	160	18	7	4	2	2	1	1	1.00	271	31	11	6	4	3	2	2	1.30
21064WS3	123	14	5	3	2	2	1	1	0.87	277	31	12	6	4	3	2	2	1.31
	NN - first evaluation									NN - second evaluation								
IMA2106GL	187	21	8	4	3	2	2	1	1.08	124	14	5	3	2	2	1	1	0.88
IMA5801B2RF	117	13	5	3	2	1	1	1	0.85	193	22	8	4	3	2	2	1	1.10
IMA5802B2RF	164	19	7	4	3	2	1	1	1.01	191	22	8	4	3	2	2	1	1.09
TMG31B3RF	222	25	9	5	3	2	2	1	1.18	222	25	9	5	3	2	2	1	1.18
TMG44B2RF	320	36	13	7	4	3	2	2	1.41	239	27	10	5	3	2	2	2	1.22
TMG5045WS3	139	16	6	3	2	2	1	1	0.93	337	38	14	7	5	3	2	2	1.45
TMG51WS3	144	16	6	3	2	2	1	1	0.95	275	31	11	6	4	3	2	2	1.31
TMG91WS3	160	18	7	4	2	2	1	1	1.00	271	31	11	6	4	3	2	2	1.30
21064WS3	123	14	5	3	2	2	1	1	0.87	277	31	12	6	4	3	2	2	1.31
	NPriB - first evaluation									NProB - second evaluation								
IMA2106GL	491	55	20	11	7	5	3	3	1.75	2,004	223	81	41	25	17	12	9	3.54
IMA5801B2RF	559	63	23	12	7	5	4	3	1.87	2,011	224	81	42	25	17	12	9	3.54
IMA5802B2RF	427	48	18	9	6	4	3	2	1.63	1,791	199	72	37	23	15	11	8	3.35
TMG31B3RF	612	68	25	13	8	6	4	3	1.95	1,862	207	75	38	23	16	12	9	3.41
TMG44B2RF	1,175	131	47	24	15	10	7	6	2.71	2,462	274	99	51	31	21	15	11	3.92
TMG5045WS3	400	45	16	9	5	4	3	2	1.58	1,509	168	61	31	19	13	9	7	3.07
TMG51WS3	565	63	23	12	7	5	4	3	1.88	1,007	112	41	21	13	9	6	5	2.51
TMG91WS3	568	64	23	12	8	5	4	3	1.88	1,082	121	44	23	14	9	7	5	2.60
21064WS3	476	53	20	10	6	4	3	3	1.72	1,637	182	66	34	21	14	10	8	3.20

Table 5. Sample size dissimilarity matrix for an estimation error of 5 % of the mean for the morphological traits of nine cotton (*Gossypium hirsutum* L.) cultivars at the stages of first visible flower bud[1] and full maturity[2].

Trait	MSH[1]	MSH[2]	FPBH[1]	FPBH[2]	MSD[1]	MSD[2]	NL[1]	NL[2]	NN[1]	NN[2]	NPriB[1]
MSH[2]	9.63										
FPBH[1]	241.65	248.51									
FPBH[2]	184.39	182.22	17.75								
MSD[1]	54.67	59.63	192.42	124.57							
MSD[2]	53.19	59.47	190.91	135.25	17.22						
NL[1]	9.14	8.40	249.72	192.58	62.10	60.92					
NL[2]	11.28	8.70	243.36	174.87	53.47	53.84	13.18				
NN[1]	8.08	9.36	248.02	192.67	60.81	59.62	2.38	13.41			
NN[2]	10.84	8.95	242.72	174.87	52.90	53.26	13.17	0.85	13.26		
NPriB[1]	48.13	54.77	199.60	156.28	27.45	26.74	55.09	50.76	53.32	50.09	
NProB[2]	183.88	190.76	66.86	56.28	137.15	133.46	191.73	185.82	190.06	185.22	141.48

MSH: main stem height; FPBH: first productive branch height; MSD: main stem diameter; NL: number of leaves; NN: number of nodes; NPriB: number of primary branches; NProB: number of productive branches.

Table 6. Sample size dissimilarity matrix (5 % error of the mean estimate and 95 % confidence level) of nine cotton (*Gossypium hirsutum* L.) cultivars at the stages of first visible flower bud (lower diagonal) and full maturity (upper diagonal).

Cultivar	1	2	3	4	5	6	7	8	9
1		6.03	11.22	9.36	22.76	26.08	45.20	41.83	20.22
2	10.02		13.01	6.95	23.49	25.72	44.44	41.29	19.12
3	6.71	15.81		9.53	30.03	15.90	36.66	33.09	14.03
4	25.09	33.28	20.15		28.78	19.55	37.76	34.50	28.71
5	49.69	56.10	46.67	28.46		42.31	65.35	61.62	52.69
6	18.89	25.02	16.22	21.08	45.56		61.32	44.89	15.84
7	46.08	39.22	52.02	68.65	87.85	53.35		16.91	40.95
8	26.84	20.84	32.87	49.68	70.08	36.24	19.61		22.21
9	9.10	11.50	12.49	12.39	37.79	14.92	52.76	37.13	

Cultivar 1: IMA2106GL; 2: IMA5801B2RF; 3: IMA5802B2RF; 4: TMG31B3RF; 5: TMG44B2RF; 6: IMA5045WS3; 7: TMG51WS3; 8: TMG91WS3; 9: 21064WS3.

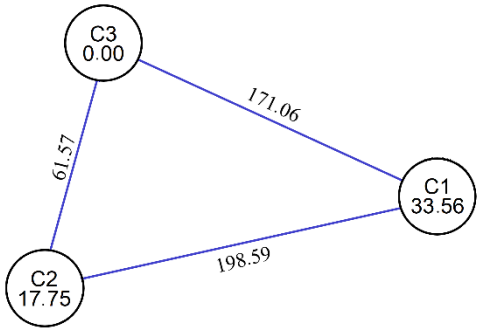

Figure 1. Distances within and between clusters for the sample size (5 % error of the mean estimate and 95 % confidence level) of the morphological traits of cotton (*Gossypium hirsutum* L.) evaluated at the stages of first visible flower bud and full maturity. C1: main stem height and diameter, and number of leaves, nodes, and primary branches; C2: first productive branch height; C3: number of productive branches. Cophenetic correlation coefficient (CCC) = 0.9415; $p \leq 0.003$.

to each other and lower than those required at the beginning of the cycle, at 26 and 33 DAS (Kleinpaul et al. 2017).

The dissimilarity of the sample size among the cultivars showed the same behavior as the traits. There was no dissimilarity in sample sizes between the two evaluated stages, since the same cultivars were gathered in the same clusters at both stages (Figure 2).

In all clusters, the cophenetic correlation coefficient was ≥ 0.8514 ($p \leq 0.005$), indicating a high representativeness of the original dissimilarity matrix through diagrams formed by the Tocher's method. The results obtained in this study should be interpreted as a reference, since differences in experimental conditions may alter sample size estimates depending on factors such as cultivar, environment, management, and research objectives.

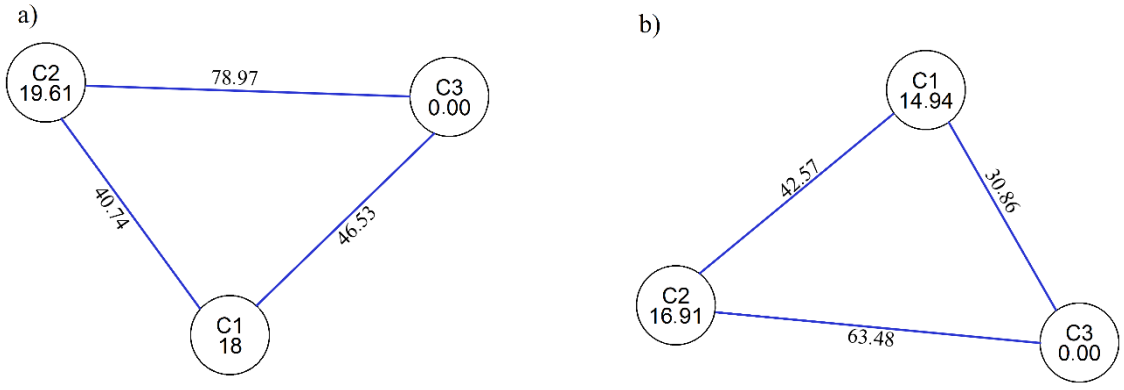

Figure 2. Distances within and between clusters for sample size (5 % error of the mean estimate and 95 % confidence level) among nine cotton (*Gossypium hirsutum* L.) cultivars evaluated at the stages of first visible flower bud (a) and full maturity (b). C1: IMA2106GL, IMA5801B2RF, IMA5802B2RF, TMG31B3RF, IMA5045WS3, 21064WS3; C2: TMG51WS3, TMG91WS3; C3: TMG44B2RF. a) Cophenetic correlation coefficient (CCC) = 0.8514; $p \leq 0.005$; b) CCC = 0.9118; $p \leq 0.007$.

CONCLUSIONS

1. The number of plants needed to measure the evaluated traits was 14 for main stem height, and number of leaves and nodes; 38 for main stem diameter and number of primary branches; and 126 for first productive branch height and number of productive branches, for cotton cultivars at the stages of first visible flower bud and full maturity;
2. There was no significant dissimilarity of sample sizes between the morphological traits of measurement and counting, among cotton cultivars and between the stages of first visible flower bud and full maturity.

ACKNOWLEDGMENTS

To the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; process nº 304878/2022-7), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes; Finance Code 001), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS), for providing research grants.

REFERENCES

ALVARES, C. A.; STAPE, J. L.; SENTELHAS, P. C.; GONÇALVES, J. L. M.; SPAROVEK, G. Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, v. 22, n. 6, p. 711-728, 2013.

AZEVEDO, A. M. *MultivariateAnalysis*: pacote para análise multivariada. 2025. Available at: https://cran.r-project.org/web/packages/MultivariateAnalysis/refman/MultivariateAnalysis.html. Access on: Jan. 01, 2026.

BELTRÃO, N. E. M.; OLIVEIRA, M. I. P. (ed.). *Ecofisiologia das culturas de algodão, amendoim, gergelim, mamona, pinhão-manso e sisal.* Brasília, DF: Embrapa Informação Tecnológica, 2011.

BEZERRA, J. R. C.; AZEVEDO, P. V. D.; SILVA, B. B. D.; DIAS, J. M. Evapotranspiração e coeficiente de cultivo do algodoeiro BRS-200 Marrom, irrigado. *Revista Brasileira de Engenharia Agrícola e Ambiental*, v. 14, n. 6, p. 625-632, 2010.

BHIMATE, M.; DESHMUKH, S.; RATHOD, D.; JADHAV, P.; UJJAINKAR, V. Assessment of genetic diversity in cotton (*Gossypium hirsutum* L.) genotypes using morphological traits. *Multilogic in Science*, v. 8, n. 1, p. 142-147, 2019.

BURIN, C.; CARGNELUTTI FILHO, A.; TOEBE, M.; ALVES, B. M.; FICK, A. L. Dimensionamento amostral para a estimação da média e da mediana de caracteres de tremoço branco (*Lupinus albus* L.). *Comunicata Scientiae*, v. 5, n. 2, p. 205-212, 2014.

BUSSAB, W. O.; MORETTIN, P. A. *Estatística básica*. 10. ed. rev. e ampl. São Paulo: Saraiva, 2023.

CAMPOS, H. *Estatística experimental não-paramétrica*. 4. ed. Piracicaba: ESALQ/USP, 1983.

CARGNELUTTI FILHO, A.; EVANGELISTA, D. H. R.; GONÇALVES, E. C. P.; STORCK, L. Tamanho de amostra de caracteres de genótipos de soja. *Ciência Rural*, v. 39, n. 4, p. 983-991, 2009.

CENTRO DE ESTUDOS AVANÇADOS EM ECONOMIA APLICADA (CEPEA). *PIB do agronegócio brasileiro*. 2025. Available at: https://www.cepea.esalq.usp.br/br/pib-do-agronegocio-brasileiro.aspx. Access on: Feb. 01, 2026.

CRUZ, C. D.; CARNEIRO, P. C. S; REGAZZI, A. J. *Modelos biométricos aplicados ao melhoramento genético*: v. 2. 3. ed. rev. e ampl. Viçosa: UFV, 2014.

FERREIRA, M. M.; TARTAGLIA, F. L.; FULANETI, F. S.; ALENCAR, C. C.; SANTOS, E. M.; ALVES, G. S. Crescimento e produtividade do algodoeiro herbáceo submetido à adubação orgânica. *Revista Brasileira de Agropecuária Sustentável*, v. 8, n. 2, p. 52-61, 2018.

FUZATTO, M. G. Melhoramento genético do algodoeiro. *In*: CIA, E.; FREIRE, E. C.; SANTOS, W. J. *Cultura do algodoeiro*. Piracicaba: Potafos, 1999. p. 15-34.

HAIR JUNIOR, J. F.; BLACK, W. C.; BABIN, N. J.; ANDERSON, R. E.; TATHAM, R. L. *Análise multivariada de dados*. 6. ed. São Paulo: Bookman, 2009.

KLEINPAUL, J. A.; CARGNELUTTI FILHO, A.; ALVES, B. M.; BURIN, C.; NEU, I. M. M.; SILVEIRA, D. L.; SIMÕES, F. M. Tamanho de amostra para estimação da média de caracteres de milheto em épocas de avaliação. *Revista Brasileira de Milho e Sorgo*, v. 16, n. 2, p. 251-262, 2017.

MARUR, C. J.; RUANO, O. A. A reference system for determination of cotton plant development. *Revista Brasileira de Oleaginosas e Fibrosas*, v. 5, n. 2, p. 313-317, 2001.

PIMENTEL-GOMES, F. *Curso de estatística experimental*. 15. ed. Piracicaba: FEALQ, 2009.

R CORE TEAM. *R*: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2025. Available at: https://www.R-project.org/. Access on: Feb. 07, 2025.

RIZWAN, M.; FAROOQ, J.; FAROOQ, M.; SARWAR, A.; ALI, A.; ILAHI, F.; ASIF, M.; SARWAR, G. Quantitative studies in upland cotton (*Gossypium hirsutum* L.) using multivariate techniques. *Pakistan Journal of Agricultural Research*, v. 34, n. 1, p. 113-120, 2021.

ROSSI, A. C. M.; SOUZA, E. R. C.; SILVA, M. G. Reguladores de crescimento na cultura do algodão (*Gossypium hirsutum* L.). *Research, Society and Development*, v. 9, n. 9, e821997951, 2020.

SANTOS, H. G.; JACOMINE, P. K. T.; ANJOS, L. H. C.; OLIVEIRA, V. Á.; LUMBRERAS, J. F.; COELHO, M. R.; ALMEIDA, J. A.; ARAUJO FILHO, J. C.; LIMA, H. N.; MARQUES, F. A.; OLIVEIRA, J. B.; CUNHA, T. J. F. *Sistema brasileiro de classificação de solos*. Brasília, DF: Embrapa, 2025.

SILVA, E. S.; OLIVEIRA, R. F.; MEDEIROS, D. A.; DIAS, J. A.; MALTA, A. O.; SILVA, S. I. A. Adubação foliar nitrogenada e boratada no crescimento e produção do algodão colorido. *Agropecuária Científica no Semiárido*, v. 15, n. 2, p. 86-91, 2019.

SINGH, A. S.; MASUKU, M. B. Sampling techniques & determination of sample size in applied statistics research: an overview. *International Journal of Economics, Commerce and Management*, v. 2, n. 11, p. 1-22, 2014.

TOEBE, M.; BANDEIRA, C. T.; FORTES, S. K. G.; CARVALHO, J. O.; TARTAGLIA, F. L.; TAMBARA, A. L.; MELO, P. J. Dimensionamento amostral e associação linear entre caracteres de *Crotalaria spectabilis*. *Bragantia*, v. 76, n. 1, p. 45-53, 2017.

UNITED STATES DEPARTMENT OF AGRICULTURE (USDA). Soil Survey Staff. *Keys to soil taxonomy*. 13. ed. Washington, DC: USDA, 2022.

WICKHAM, H. *ggplot2*: elegant graphics for data analysis. New York: Springer-Verlag, 2016.

WU, B.; ZUO, W.; YANG, P.; ZHANG, W. Optimal water and nitrogen management increases cotton yield through improving leaf number and canopy light environment. *Field Crops Research*, v. 290, e108745, 2023.

ZAMAN, I.; ALI, M.; SHAHZAD, K.; TAHIR, M. S.; MATLOOB, A.; AHMAD, W.; ALAMRI, S.; KHURSHID, M. R.; QURESHI, M. M.; WASAYA, A.; BAIG, K. S.; SIDDIQUI, M. H.; FAHAD, S.; DATTA, R. Effect of plant spacings on growth, physiology, yield and fiber quality attributes of cotton genotypes under nitrogen fertilization. *Agronomy*, v. 11, e2589, 2021.