Sample sizes to estimate mean values for tassel traits in maize genotypes

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ABSTRACT. Tassel traits are important in maize breeding programs aiming to reduce the size and number of branches and maintain satisfactory pollen production in order to increase grain yield. The objectives of this study were to determine the sample size (number of tassels) required to estimate the mean values for tassel traits in maize genotypes and to verify the variability of sample size among genotypes. Twenty maize genotypes were evaluated in an experiment carried out in a randomized block design with three replicates. Twenty tassels were randomly collected in each plot, for a total of 1200 tassels. In each tassel, the following traits were measured: peduncle dry matter, branching space dry matter, central spike dry matter, tassel dry matter, peduncle length, branching space length, central spike length, tassel length, tassel dry matter to tassel length ratio, number of primary branches, number of secondary branches, and tassel branch number. Measures of central tendency and variability were calculated, analysis of variance and mean comparison tests were performed, normality was

verified, and the sample size was determined. In order to estimate the means with the same precision, the sample size for weight traits was greater than that for length traits. For tassel traits, 11, 20, and 43 tassels are sufficient to estimate the mean with a precision of 40, 30, and 20%, respectively, of the estimated mean at a 95% confidence level. These data show that there is sample size variability among maize genotypes.

Key words: *Zea mays* L.; Sampling; Experimental precision; Calculating study sample size

INTRODUCTION

Maize (*Zea mays* L.) is one of the most cultivated cereals in the world because of its use in a wide variety of industries, such as feed and foods industries. The estimated world maize production for the 2016/2017 agricultural year is 1013.87 million tons in an area of 178.62 million hectares (FAO, 2016). According to the Food and Agriculture Organization, the United States is the world's largest maize grower, followed by China and Brazil, with an estimated production of 355, 224, and 83 million tons, respectively, in the 2016/2017 agricultural year. The increased grain yield of modern maize cultivars is the result of agronomic practices and genetic gains derived through maize breeding programs (Lauer et al., 2012).

The morphology of staminate and pistillate inflorescences in maize and their separation through the plant favor the study and development of inbred lines and hybrid seed, along with accentuated heterotic responses in the F1 generation (Allard, 1999). For heterosis to occur the genitors should be divergent (Hallauer et al., 2010). In this sense, most maize traits contribute to grain yield, with tassel weight contributing to heterosis in grain yield in diallel crosses (Ribeiro et al., 2014).

Morphological tassel traits are of importance in maize breeding programs, in which inbred lines are developed with the aim of reducing the size and number of branches and maintaining satisfactory pollen production (Duvick, 2005; Fischer and Edmeades, 2010). Larger tassels act as a drain for photoassimilates, which could be directed toward grain production, and restrict the passage of solar radiation through the canopy (Edwards, 2011). In addition, smaller tassels produce lower levels of auxins and decrease apical dominance, which has an inhibitory effect on ear development (Sangoi et al., 2006). In addition to the environment, it permits the production of one or more ear per plant.

Thus, studies evaluating tassel traits related to grain yield have been carried out in half-sib families of an ESALQ-PB1 population (Andrade and Miranda Filho, 2008), in parental lines of Pioneer-brand maize hybrids (Lauer et al., 2012), in recombinant inbred lines in temperate and tropical climates (Brewbaker, 2015), in inbred lines of two heterotic groups (Nardino et al., 2016a), and in F1 hybrids (Nardino et al., 2016b). In general, those studies have confirmed the relationship between tassel traits and grain yield.

In order to generate reliable results from breeding programs involving maize tassels or other agricultural crops, it is important to accurately determine the sample size (number of tassels and/or plants) to be used. As reported by Storck et al. (2016), sampling should be undertaken when it is not possible to evaluate the entire experimental unit. An appropriate sample size enables the population mean to be effectively estimated, reducing the sampling error within the plot and subsequently, the experimental error. Furthermore, Bussab and

Morettin (2011) stated that the sample size is directly related to data variability and the desired reliability, and that it is inversely related to the level of error previously set by the researcher. Consistent with the above observations, larger sample sizes reduce the experimental error, but increase the demand on the size of the experimental area, manpower, financial resources, and time required for sampling. However, smaller sample sizes increase the experimental error (Cargnelutti Filho et al., 2012; Storck et al., 2016).

In maize, the sample size has been studied to estimate mean values for morphological and productive traits of ears (Martin et al., 2005; Storck et al., 2007), and morphological and productive traits of plants and ears in different soil tillage systems and straw (Modolo et al., 2013). Furthermore, Cargnelutti Filho et al. (2010) established the sample dimension to measure Pearson correlation coefficients among pairs of traits in maize hybrids. Also, the sample size was determined to estimate the coefficient of variation of the mean (Toebe et al., 2014) and Pearson correlation coefficients for different maize hybrids (Toebe et al., 2015).

The above studies presented significant results for the experimental design in maize crops. However, to our knowledge, no studies have investigated sample sizes for the estimation of mean values for tassel traits in maize genotypes, and it is assumed that the sample sizes differ among genotypes. The objectives of the present study were to determine the sample size (number of tassels) required to estimate mean values for tassel traits in maize genotypes and to verify sample size variability among genotypes.

MATERIAL AND METHODS

An experiment was carried out on maize during the 2015/2016 agricultural year in an experimental area located at 29°42'S, 53°49'W, and 95 m in altitude. Based on the Köppen climate classification updated by Peel et al. (2007), the climate of the region is Cfa, humid subtropical, with hot summers and without a dry season (Heldwein et al., 2009). The soil is classified as sandy loam typic Paleudalf (Santos et al., 2013).

Sowing was performed on October 21, 2015. The experimental design was a randomized block with 20 genotypes and three replicates, for a total of 60 plots. The 20 genotypes included 18 single-cross hybrids (30A68, 30F53, AG 8780, AG 9025, AM 9724, AS 1666, AS 1677, BM 3066, Celeron, DKB 230, DKB 290, MS 2010, MS 2013, P1630, P2530, SHS 7915, Status VIP, and SX 7331) and two three-way cross hybrids (20A55 and MS 3022). These 20 genotypes were used because they belong to a network of maize cultivars used in evaluation trials to identify genotypes adapted for the State of Rio Grande do Sul, in southern Brazil.

Each plot consisted of two rows, each 5-m long, with spacing of 0.80 m between rows and 0.20 m between plants. The plant density was adjusted by manually thinning to five plants per meter of each row, and a final population of 62,500 plants per hectare. Thus, each plot consisted of 50 plants, totaling 3000 plants in the experiment (20 genotypes x three plots per genotype x 50 plants per plot). Basic fertilizer was applied on the day of sowing, using the commercial NPK formulation at a 5-20-20 proportion, for a total of 37.5 kg/ha N, 150 kg/ha P_2O_5, and 150 kg/ha K_2O. Posteriorly, topdressing fertilization with 121.5 kg/ha N was divided between three applications, when the plants presented four, six, and eight expanded leaves (November 7 and 23, and December 10, 2015). Cultural practices regarding pest and weed control were followed to maintain competition-free conditions for the crop.

Twenty tassels were randomly collected from each plot and stored in paper packaging 104 days after sowing, when the plants were in the reproductive stage. The packages were

identified and dried in an oven at 55°C until the samples reached constant weight. The following traits were measured in each tassel: peduncle dry matter (PDM, considering the region between the flag leaf collar and the first branch), in grams per tassel; branching space dry matter (BSDM), in grams per tassel; central spike dry matter (CSDM), in grams per tassel; tassel dry matter (TDM = PDM + BSDM + CSDM), in grams per tassel; peduncle length (PL, considering the distance between the collar of the flag leaf and the first branch), in centimeters; branching space length (BSL), in centimeters; central spike length (CSL), in centimeters; tassel length (TL = PL + BSL + CSL), in centimeters; number of primary branches (NPB); number of secondary branches (NSB); and tassel branch number (TBN = NPB + NSB) (Figure 1). Weight traits were measured using a digital scale with precision of 0.01 g. Furthermore, the TDM to tassel length ratio (TDMTL) was calculated in grams per centimeter.

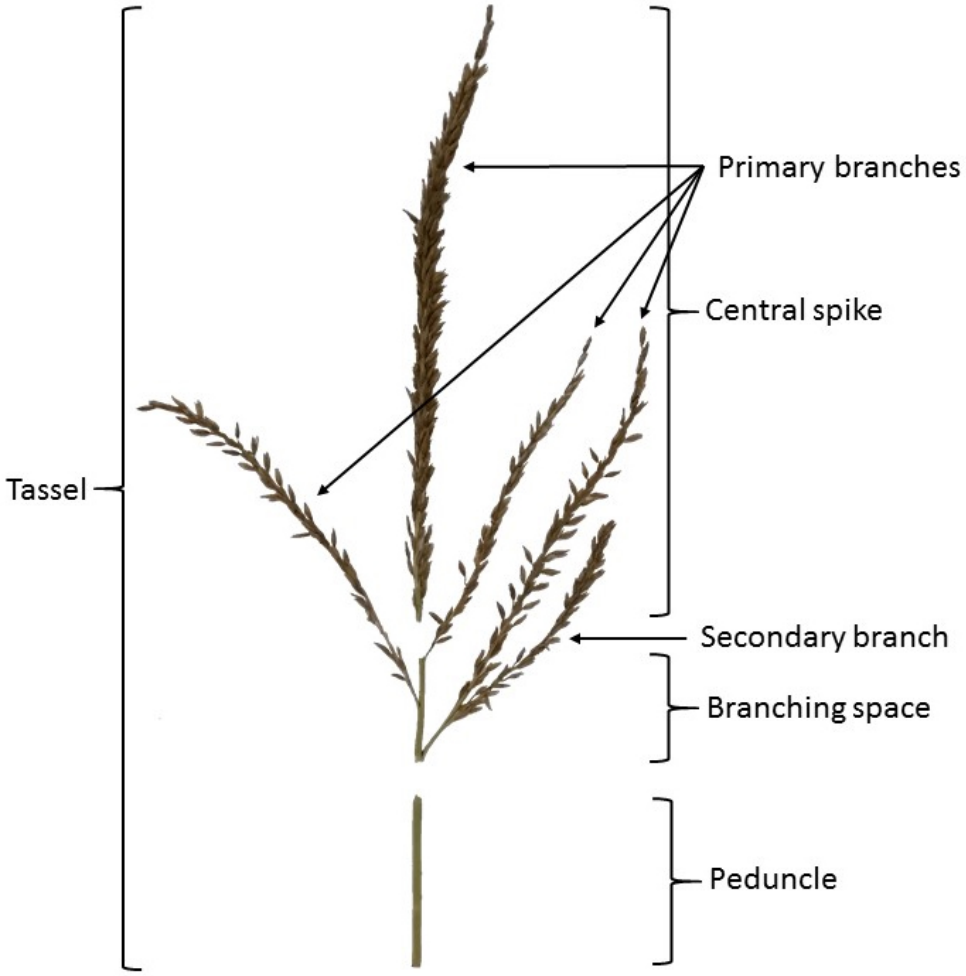

Figure 1. Representation of traits evaluated in a maize tassel from the 30F53 genotype, composed of one central spike, four primary branches, one secondary branch, a branching space, and peduncle, based on the methods described by Upadyayula et al. (2006).

The data set obtained from the 12 traits was subjected to analysis of variance and F test at 5% significance and mean values for genotypes were clustered by the Scott-Knott test (Scott and Knott, 1974) at a 5% significance level. For analysis of variance, a mathematical model of block design with sampling within plots was used, as defined by Storck et al. (2016). In order to evaluate experimental precision, selective accuracy (SA) (Resende and Duarte, 2007) was determined by the following: $SA = (1 - 1 / Fc)^{0.5}$, where Fc is the value derived from the F test for the genotype. According to the class limits established by Resende and Duarte (2007), the experimental precision was ranked as very high ($SA \geq 0.90$), high ($0.70 \leq SA < 0.90$), moderate ($0.50 \leq SA < 0.70$), and low ($SA < 0.50$).

Thereafter, normality of the data was verified by the Kolmogorov-Smirnov test (Siegel and Castellan Júnior, 2006) for the traits PDM, BSDM, CSDM, TDM, PL, BSL, CSL, TL, TDMTL, NPB, NSB, and TBN, of 20 tassels from each of the 60 plots, totaling 720 tests (20 genotypes x three plots per genotype x 12 traits). Normality was investigated in order to verify the suitability of the data set for the study of sample size based on the Student *t* distribution.

Based on data from 20 tassels sampled from each experimental unit (plot) of each genotype, the sample size (n) for the traits PDM, BSDM, CSDM, TDM, PL, BSL, CSL, TL, TDMTL, NPB, NSB, and TBN was determined using the following equation:

$$n = t^2_{\alpha/2} CV^2 / D^2$$

where CV is the coefficient of variation between 20 tassels (%); D is the semi-amplitude of the confidence interval for the mean (%) (established as D = 5, 10, 20, 30, and 40%); and *t* is the critical value of the Student's *t* distribution at the 5% significance level. Thus, 60 variables (sample size) were obtained by the combination of 12 traits (PDM, BSDM, CSDM, TDM, PL, BSL, CSL, TL, TDMTL, NPB, NSB, and TBN) at precision levels of 5% (D5), 10% (D10), 20% (D20), 30% (D30), and 40% (D40) of the estimated mean in the experimental unit.

In order to investigate variability in sample size among genotypes, the data set from these 60 variables (sample size) was subjected to analysis of variance using the mathematical model of randomized block design, as described by Storck et al. (2016). Genotype means were clustered using the Scott-Knott test (Scott and Knott, 1974) at a 5% significance level. Statistical analyzes were performed using the GENES software (Cruz, 2013) and Microsoft Office Excel.

RESULTS AND DISCUSSION

The mean TDM was 3.11 g/tassel, tassel length was 47.50 cm, and tassel branch number was 14.00 (Table 1). Similar results were obtained, respectively, by Upadyayula et al. (2006), Lauer et al. (2012), and Brewbaker (2015), proving that there was adequate plant development in the present experiment.

There was a significant effect of genotypes in relation to the 12 traits studied (PDM, BSDM, CSDM, TDM, PL, BSL, CSL, TL, TDMTL, NPB, NSB, and TBN), demonstrating the existence of genetic variability (Table 1). This variability permitted the separation of genotypes into groups by the Scott-Knott test (Table 2). The block effect was not significant, showing that the blocks were not heterogeneous. High SA values ($SA \geq 0.956$) ensured very high experimental precision in the evaluation of these 12 traits, as stated by Resende and Duarte (2007).

Table 1. Degrees of freedom (DF) and mean squares of the causes of variation (block, genotype, experimental error, and sampling error), mean, coefficient of experimental variation (CVe), coefficient of sampling variation (CVs), and selective accuracy for tassel traits of 20 maize genotypes.

Causes of variation	d.f.	Mean square					
		PDM	BSDM	CSDM	TDM	PL	BSL
Block	2	0.0053^{ns}	0.4019^{ns}	0.0352^{ns}	0.6155^{ns}	5.3038^{ns}	17.3976^{ns}
Genotype	19	0.4382*	39.7248*	1.1918*	47.5530*	207.8687*	380.5366*
Experimental error	38	0.0195*	1.6080*	0.0500*	2.0325*	17.8210*	16.6530*
Sampling error	1140	0.0034	0.3885	0.0176	0.5360	2.9877	3.0543
Mean	-	0.2592	2.1716	0.6746	3.1053	8.7803	12.1131
CVe (%)	-	53.92	58.39	33.14	45.91	48.08	33.69
CVs (%)	-	22.49	28.70	19.68	23.58	19.69	14.43
Selective accuracy	-	0.977	0.980	0.979	0.978	0.956	0.978
		CSL	TL	TDMTL	NPB	NSB	TBN
Block	2	6.7342^{ns}	68.9991^{ns}	0.0001^{ns}	72.7358^{ns}	3.4358^{ns}	106.6975^{ns}
Genotype	19	819.5201*	488.5188*	0.0214*	1054.1886*	100.1796*	1700.2900*
Experimental error	38	12.5195*	29.6708*	0.0009*	28.2288*	6.3543*	52.5615*
Sampling error	1140	6.8588	11.5412	0.0002	4.4512	1.1279	6.3673
Mean	-	26.6031	47.4964	0.0652	11.4092	2.5933	14.0025
CVe (%)	-	13.30	11.47	44.85	46.57	97.20	51.78
CVs (%)	-	9.84	7.15	20.52	18.49	40.95	18.02
Selective accuracy	-	0.992	0.969	0.980	0.987	0.968	0.984

PDM = peduncle dry matter, in grams per tassel; BSDM = branching space dry matter, in grams per tassel; CSDM = central spike dry matter, in grams per tassel; TDM = tassel dry matter, in grams per tassel; PL = peduncle length, in centimeters; BSL = branching space length, in centimeters; CSL = central spike length, in centimeters; TL = tassel length, in centimeters; TDMTL = tassel dry matter:tassel length ratio, in grams per centimeter; NPB = number of primary branches, in units; NSB = number of secondary branches, in units; TBN = tassel branch number, in units. *Significant effect as determined by the F test at a 5% significance level. ^{ns}Not significant.

The experimental error (variation among plots) was significant in relation to the traits PDM, BSDM, CSDM, TDM, PL, BSL, CSL, TL, TDMTL, NPB, NSB, and TBN (Table 1). Thus, it can be inferred that the coefficients of experimental variation (CVe) were superior to the coefficients of sampling variation (CVs) for all traits. Moreover, these coefficients presented high magnitude, i.e., $11.47\% \leq CVe \leq 97.20\%$, and $7.15\% \leq CVs \leq 40.95\%$. These results show there was greater variability of plants among experimental units than within experimental units. Therefore, increasing the number of replicates is appropriate to improve experimental precision (Barbin, 2003). As reported by Barbin (2003), this method is effective at reducing the estimate of variance of the estimated mean. The high values of CVe and CVs indicate that it is important to adjust the number of replicates and the sample size in order to improve the experimental precision. In this context, Storck et al. (2007) evaluated maize ears and suggested increasing the number of replicates and decreasing the number of ears in the plot, fixing the total number of ears assessed by genotype.

The mean P value (minimum level of significance) of the Kolmogorov-Smirnov test (Siegel and Castellan Júnior, 2006) relative to the data of 20 tassels in the 720 cases analyzed (20 genotypes x three plots per genotype x 12 traits) was 0.73. Data of the PDM, BSDM, CSDM, TDM, PL, BSL, CSL, TL, TDMTL, NPB, NSB, and TBN fully adhered to the normal distribution ($P > 0.20$) in 650 cases (90.3%). Considering a minor adjustment, i.e., $P > 0.05$, 695 cases (96.5%) had an adjusted normal distribution. Therefore, these results indicate that this database is suitable for the study of sample size determination based on the Student *t* distribution.

Table 2. Mean values for tassel traits evaluated in 20 maize genotypes.

Genotype	PDM	BSDM	CSDM	TDM	PL	BSL
20A55	0.36[a]	3.33[a]	0.88[a]	4.56[a]	10.25[a]	14.83[a]
30A68	0.33[b]	2.08[c]	0.77[b]	3.19[c]	10.03[a]	13.2[b]
30F53	0.2[d]	1.46[d]	0.86[a]	2.53[d]	6.98[c]	9.39[d]
AG8780	0.36[a]	2.25[c]	0.48[c]	3.09[c]	11.34[a]	14.92[a]
AG9025	0.13[e]	1.73[d]	0.69[b]	2.56[d]	5.74[c]	10.74[c]
AM9724	0.17[e]	2.4[c]	0.72[b]	3.29[c]	6.79[c]	12.61[b]
AS1666	0.26[c]	1.36[d]	0.69[b]	2.32[d]	10.2[a]	9.09[d]
AS1677	0.12[e]	1.5[d]	0.57[c]	2.19[d]	5.55[c]	11.37[c]
BM3066	0.26[c]	3.15[a]	0.67[b]	4.07[a]	7.76[b]	13.47[b]
Celeron	0.31[b]	1.82[d]	0.56[c]	2.69[d]	11.09[a]	10.68[c]
DKB230	0.12[e]	1.19[e]	0.33[d]	1.65[e]	6.3[c]	12.7[b]
DKB290	0.4[a]	2.82[b]	0.54[c]	3.76[b]	11.4[a]	15.78[a]
MS2010	0.27[c]	2.66[b]	0.69[b]	3.62[b]	10.49[a]	12.42[b]
MS2013	0.24[c]	3.41[a]	0.86[a]	4.5[a]	8.25[b]	13.15[b]
MS3022	0.3[b]	2.78[b]	0.73[b]	3.8[b]	8.89[b]	10.8[c]
P1630	0.2[d]	0.72[e]	0.75[b]	1.67[e]	8.7[b]	8.32[d]
P2530	0.22[d]	0.85[e]	0.82[a]	1.88[e]	8.06[b]	7.12[e]
SHS7915	0.24[c]	2.23[c]	0.76[b]	3.23[c]	8.43[b]	10.71[c]
StatusVIP	0.32[b]	2.87[b]	0.55[c]	3.74[b]	8.75[b]	15.79[a]
SX7331	0.38[a]	2.82[b]	0.58[c]	3.78[b]	10.63[a]	15.19[a]
	CSL	TL	TDMTL	NPB	NSB	TBN
20A55	24.55[e]	49.63[c]	0.09[a]	13.47[b]	2.72[b]	16.18[b]
30A68	31.74[a]	54.96[a]	0.06[c]	9.88[c]	2.05[c]	11.93[c]
30F53	30.89[b]	47.26[c]	0.05[c]	6.77[d]	0.92[d]	7.68[d]
AG8780	22.94[f]	49.19[c]	0.06[c]	11.82[c]	3.75[a]	15.57[b]
AG9025	29.94[b]	46.42[d]	0.05[c]	8.95[c]	1.43[c]	10.38[c]
AM9724	27.34[d]	46.73[d]	0.07[b]	10.45[c]	3.83[a]	14.28[b]
AS1666	28.81[c]	48.11[c]	0.05[c]	6.73[d]	1.65[c]	8.38[d]
AS1677	28.16[c]	45.09[e]	0.05[c]	8.05[d]	1.8[c]	9.85[c]
BM3066	21.35[g]	42.58[e]	0.09[a]	18.63[a]	3.72[a]	22.35[a]
Celeron	26.97[d]	48.74[c]	0.05[c]	11.07[c]	2.05[c]	13.12[c]
DKB230	24.78[e]	43.78[e]	0.04[d]	10.1[c]	2.5[b]	12.6[c]
DKB290	21.48[g]	48.66[c]	0.08[b]	12.7[b]	3.9[a]	16.6[b]
MS2010	28.71[c]	51.62[b]	0.07[b]	14.93[b]	3.77[a]	18.7[b]
MS2013	26.6[d]	47.99[c]	0.09[a]	14.18[b]	4[a]	18.18[b]
MS3022	24.87[e]	44.57[e]	0.08[a]	13.4[b]	3.23[b]	16.63[b]
P1630	30.71[b]	47.73[c]	0.04[d]	6.4[d]	0.27[d]	6.67[d]
P2530	32.23[a]	47.41[c]	0.04[d]	4.55[e]	0[d]	4.55[e]
SHS7915	27.41[d]	46.54[d]	0.07[b]	8.85[c]	2.57[b]	11.42[c]
StatusVIP	19.31[h]	43.84[e]	0.08[a]	19.82[a]	4.35[a]	24.17[a]
SX7331	23.29[f]	49.11[c]	0.08[b]	17.43[a]	3.37[a]	20.8[a]

PDM = peduncle dry matter, in grams per tassel; BSDM = branching space dry matter, in grams per tassel; CSDM = central spike dry matter, in grams per tassel; TDM = tassel dry matter, in grams per tassel; PL = peduncle length, in centimeters; BSL = branching space length, in centimeters; CSL = central spike length, in centimeters; TL = tassel length, in centimeters; TDMTL = tassel dry matter:tassel length ratio, in grams per centimeter; NPB = number of primary branches, in units; NSB = number of secondary branches, in units; TBN = tassel branch number, in units. Means not followed by the same superscript letter differ by the Scott-Knott test at a 5% significance level.

The results of analysis of variance of the sample size, and those of the Scott-Knott test, in relation to the 12 studied traits (PDM, BSDM, CSDM, TDM, PL, BSL, CSL, TL, TDMTL, NPB, NSB, and TBN) are shown in Tables 3, 4, 5, and 6. Regarding the analysis of variance of the sample size, the effect of genotypes was not found to be significant for the traits BSDM and TDMTL (Table 3). Consequently, as expected, no statistical differences were detected by the Scott-Knott test in terms of the sample sizes of those traits. Therefore, the average size for those traits is representative of all genotypes. Thus, 32 tassels per experimental unit for BSDM

(Table 4) and 17 tassels per experimental unit for TDMTL (Table 6) are sufficient to obtain estimates of the genotype mean with a precision of 10% (D10).

Table 3. Causes of variation (block and genotype) and respective degrees of freedom (DF), F test value for genotype (Fc), and coefficient of variation (CV) of sample sizes (number of tassels) for tassel traits in 20 maize genotypes.

Causes of variation	PDM	BSDM	CSDM	TDM	PL	BSL
Block (d.f. = 2)	ns	ns	ns	ns	ns	ns
Genotype (d.f. = 19)	*	ns	*	*	*	*
Fc	7.13	1.67	3.41	2.69	6.21	4.29
CV(%)	35.19	41.47	30.54	41.41	48.39	39.83
	CSL	TL	TDMTL	NPB	NSB	TBN
Block (d.f. = 2)	ns	ns	ns	ns	ns	ns
Genotype (d.f. = 19)	*	*	ns	*	*	*
Fc	2.45	3.76	1.62	2.75	6.70	3.10
CV(%)	40.72	38.25	50.82	40.56	138.63	43.91

PDM = peduncle dry matter, in grams per tassel; BSDM = branching space dry matter, in grams per tassel; CSDM = central spike dry matter, in grams per tassel; TDM = tassel dry matter, in grams per tassel; PL = peduncle length, in centimeters; BSL = branching space length, in centimeters; CSL = central spike length, in centimeters; TL = tassel length, in centimeters; TDMTL = tassel dry matter:tassel length ratio, in grams per centimeter; NPB = number of primary branches, in units; NSB = number of secondary branches, in units; TBN = tassel branch number, in units. *Significant effect as determined by the F test at a 5% significance level. nsNot significant.

For the other 10 traits, the effect of genotypes was significant, which demonstrates distinct sample sizes among genotypes (Table 3). Thus, four sample size groups were formed for PDM, three groups for CSDM and PL, and two for TDM, BSL, CSL, TL, NPB, NSB, and TBN by the Scott-Knott test, confirming that different sample sizes among genotypes are required to estimate the mean of these traits with the same precision (Tables 4, 5, and 6). Therefore, it can be inferred that there is sample size variability among genotypes, as verified in maize (Martin et al., 2005; Storck et al., 2007; Toebe et al., 2015). In this context, Martin et al. (2005) suggested sampling each genotype with its respective sample size or using the largest sample size determined in order to cover all genotypes.

The sample size used to estimate the mean of each trait, with semi-amplitude of the confidence interval equal to 5% of the mean estimate (greater precision, in this study), and a 95% confidence level, ranged from four tassels for TL of the 30A68 genotype to 6251 tassels for NSB of the P1630 genotype (Tables 4, 5, and 6). These results confirm the presence of sample size variability among traits of maize genotypes, as verified among traits (Storck et al., 2007; Toebe et al., 2014) and between pairs of traits in maize (Cargnelutti Filho et al., 2010; Toebe et al., 2015). In line with this, Storck et al. (2007) suggested using the sample size determined for the most important trait for the experiment. Another possibility proposed by those authors is to take an average sample size per group of traits, which includes the largest number of traits.

If a researcher utilizes the same sample size to evaluate these traits in an experiment, greater precision in estimates will be obtained in relation to TL, decreasing gradually in the following order: CSL, BSL, TBN, NPB, TDMTL, CSDM, PL, TDM, PDM, BSDM, and NSB. Under the general conditions of the present experiment, the data set demonstrated that to estimate the mean with the same precision, the sample size for weight traits is greater than that for length traits. Cargnelutti Filho et al. (2012) also observed the necessity of a larger sample size to evaluate weight traits in relation to other traits (length and diameter) in jack bean and velvet bean seeds.

Table 4. Sample size (number of tassels) of 20 maize genotypes for semi-amplitudes of the interval with 95% confidence equals to 5% (D5), 10% (D10), 20% (D20), 30% (D30), and 40% (D40) of the mean in relation to the traits peduncle dry matter, branching space dry matter, central spike dry matter, and tassel dry matter.

Genotype	Peduncle dry matter					Branching space dry matter				
	D5	D10	D20	D30	D40	D5	D10	D20	D30	D40
20A55	95[c]	24	6	3	2	142	36	9	4	3
30A68	85[c]	22	6	3	2	148	37	10	5	3
30F53	46[d]	12	3	2	1	85	22	6	3	2
AG8780	54[d]	14	4	2	1	98	25	7	3	2
AG9025	233[a]	59	15	7	4	150	38	10	5	3
AM9724	135[b]	34	9	4	3	169	43	11	5	3
AS1666	113[c]	29	8	4	2	76	19	5	3	2
AS1677	213[a]	54	14	6	4	112	28	7	4	2
BM3066	162[b]	41	11	5	3	143	36	9	4	3
Celeron	61[d]	16	4	2	1	108	27	7	3	2
DKB230	171[b]	43	11	5	3	106	27	7	3	2
DKB290	36[d]	9	3	1	1	103	26	7	3	2
MS2010	76[c]	19	5	3	2	201	51	13	6	4
MS2013	90[c]	23	6	3	2	105	27	7	3	2
MS3022	118[c]	30	8	4	2	126	32	8	4	2
P1630	144[b]	36	9	4	3	144	36	9	4	3
P2530	87[c]	22	6	3	2	87	22	6	3	2
SHS7915	138[b]	35	9	4	3	220	55	14	7	4
StatusVIP	39[d]	10	3	2	1	108	27	7	3	2
SX7331	37[d]	10	3	2	1	86	22	6	3	2
Mean	107	28	8	4	3	126	32	9	4	3
	Central spike dry matter					Tassel dry matter				
	D5	D10	D20	D30	D40	D5	D10	D20	D30	D40
20A55	72[c]	18	5	2	2	103[a]	26	7	3	2
30A68	50[c]	13	4	2	1	84[b]	21	6	3	2
30F53	43[c]	11	3	2	1	47[b]	12	3	2	1
AG8780	77[b]	20	5	3	2	76[b]	19	5	3	2
AG9025	47[c]	12	3	2	1	84[b]	21	6	3	2
AM9724	67[c]	17	5	2	2	122[a]	31	8	4	2
AS1666	39[c]	10	3	2	1	46[b]	12	3	2	1
AS1677	61[c]	16	4	2	1	73[b]	19	5	3	2
BM3066	77[b]	20	5	3	2	115[a]	29	8	4	2
Celeron	59[c]	15	4	2	1	70[b]	18	5	2	2
DKB230	56[c]	14	4	2	1	69[b]	18	5	2	2
DKB290	57[c]	15	4	2	1	73[b]	19	5	3	2
MS2010	130[a]	33	9	4	3	153[a]	39	10	5	3
MS2013	85[b]	22	6	3	2	83[b]	21	6	3	2
MS3022	67[c]	17	5	2	2	94[b]	24	6	3	2
P1630	81[b]	21	6	3	2	50[b]	13	4	2	1
P2530	37[c]	10	3	2	1	36[b]	9	3	1	1
SHS7915	92[b]	23	6	3	2	158[a]	40	10	5	3
StatusVIP	58[c]	15	4	2	1	82[b]	21	6	3	2
SX7331	60[c]	15	4	2	1	64[b]	16	4	2	1
Mean	66	17	5	3	2	85	22	6	3	2

Means not followed by the same letter differ by the Scott-Knott test at a 5% significance level. In columns referring to D10, D20, D30, and D40, the superscript letters are the same as in the column referring to D5, and therefore were not placed.

Table 5. Sample size (number of tassels) of 20 maize genotypes for semi-amplitudes of the interval with 95% confidence equals to 5% (D5), 10% (D10), 20% (D20), 30% (D30), and 40% (D40) of the mean in relation to the traits peduncle length, branching space length, central spike length, and tassel length.

Genotype	Peduncle length					Branching space length				
	D5	D10	D20	D30	D40	D5	D10	D20	D30	D40
20A55	85[c]	22	6	3	2	42[b]	11	3	2	1
30A68	52[c]	13	4	2	1	40[b]	10	3	2	1
30F53	54[c]	14	4	2	1	40[b]	10	3	2	1
AG8780	22[c]	6	2	1	1	24[b]	6	2	1	1
AG9025	172[a]	43	11	5	3	66[a]	17	5	2	2
AM9724	89[c]	23	6	3	2	36[b]	9	3	1	1
AS1666	53[c]	14	4	2	1	32[b]	8	2	1	1
AS1677	188[a]	47	12	6	3	53[b]	14	4	2	1
BM3066	114[b]	29	8	4	2	20[b]	5	2	1	1
Celeron	26[c]	7	2	1	1	32[b]	8	2	1	1
DKB230	208[a]	52	13	6	4	33[b]	9	3	1	1
DKB290	29[c]	8	2	1	1	25[b]	7	2	1	1
MS2010	31[c]	8	2	1	1	53[b]	14	4	2	1
MS2013	111[b]	28	7	4	2	44[b]	11	3	2	1
MS3022	85[c]	22	6	3	2	48[b]	12	3	2	1
P1630	152[a]	38	10	5	3	80[a]	20	5	3	2
P2530	64[c]	16	4	2	1	89[a]	23	6	3	2
SHS7915	72[c]	18	5	2	2	42[b]	11	3	2	1
StatusVIP	31[c]	8	2	1	1	16[b]	4	1	1	1
SX7331	25[c]	7	2	1	1	16[b]	4	1	1	1
Mean	84	22	6	3	2	42	11	3	2	2
	Central spike length					Tassel length				
	D5	D10	D20	D30	D40	D5	D10	D20	D30	D40
20A55	20[a]	5	2	1	1	12[a]	3	1	1	1
30A68	11[b]	3	1	1	1	4[b]	1	1	1	1
30F53	12[b]	3	1	1	1	5[b]	2	1	1	1
AG8780	25[a]	7	2	1	1	9[b]	3	1	1	1
AG9025	7[b]	2	1	1	1	7[b]	2	1	1	1
AM9724	18[b]	5	2	1	1	9[b]	3	1	1	1
AS1666	8[b]	2	1	1	1	7[b]	2	1	1	1
AS1677	17[b]	5	2	1	1	7[b]	2	1	1	1
BM3066	18[b]	5	2	1	1	10[b]	3	1	1	1
Celeron	14[b]	4	1	1	1	6[b]	2	1	1	1
DKB230	14[b]	4	1	1	1	13[a]	4	1	1	1
DKB290	21[a]	6	2	1	1	10[b]	3	1	1	1
MS2010	29[a]	8	2	1	1	17[a]	5	2	1	1
MS2013	19[a]	5	2	1	1	9[b]	3	1	1	1
MS3022	27[a]	7	2	1	1	16[a]	4	1	1	1
P1630	22[a]	6	2	1	1	11[b]	3	1	1	1
P2530	11[b]	3	1	1	1	10[b]	3	1	1	1
SHS7915	28[a]	7	2	1	1	17[a]	5	2	1	1
StatusVIP	20[a]	5	2	1	1	7[b]	2	1	1	1
SX7331	16[b]	4	1	1	1	5[b]	2	1	1	1
Mean	18	5	2	1	1	10	3	2	1	1

Means not followed by the same superscript letter differ by the Scott-Knott test at 5% significance level. In columns referring to D10, D20, D30, and D40, the letters are the same as in the column referring to D5, and therefore, are not shown.

Table 6. Sample size (number of tassels) of 20 maize genotypes for semi-amplitudes of the interval with 95% confidence equals to 5% (D5), 10% (D10), 20% (D20), 30% (D30), and 40% (D40) of the mean in relation to the traits tassel dry matter by tassel length ratio, number of primary branches, number of secondary branches, and tassel branch number.

Genotype	Tassel dry matter: tassel length ratio					Number of primary branches				
	D5	D10	D20	D30	D40	D5	D10	D20	D30	D40
20A55	82	21	6	3	2	102[a]	26	7	3	2
30A68	70	18	5	2	2	77[a]	20	5	3	2
30F53	39	10	3	2	1	91[a]	23	6	3	2
AG8780	51	13	4	2	1	38[b]	10	3	2	1
AG9025	70	18	5	2	2	97[a]	25	7	3	2
AM9724	113	29	8	4	2	56[b]	14	4	2	1
AS1666	36	9	3	1	1	47[b]	12	3	2	1
AS1677	65	17	5	2	2	77[a]	20	5	3	2
BM3066	84	21	6	3	2	50[b]	13	4	2	1
Celeron	57	15	4	2	1	37[b]	10	3	2	1
DKB230	44	11	3	2	1	55[b]	14	4	2	1
DKB290	61	16	4	2	1	18[b]	5	2	1	1
MS2010	110	28	7	4	2	65[a]	17	5	2	2
MS2013	62	16	4	2	1	45[b]	12	3	2	1
MS3022	59	15	4	2	1	69[a]	18	5	2	2
P1630	43	11	3	2	1	95[a]	24	6	3	2
P2530	33	9	3	1	1	76[a]	19	5	3	2
SHS7915	108	27	7	3	2	57[b]	15	4	2	1
StatusVIP	58	15	4	2	1	37[b]	10	3	2	1
SX7331	48	12	3	2	1	37[b]	10	3	2	1
Mean	65	17	5	3	2	62	16	5	3	2
	Number of secondary branches					Tassel branch number				
	D5	D10	D20	D30	D40	D5	D10	D20	D30	D40
20A55	234[b]	59	15	7	4	89[a]	23	6	3	1
30A68	368[b]	92	23	11	6	68[a]	17	5	2	1
30F53	860[b]	215	54	24	14	89[a]	23	6	3	1
AG8780	168[b]	42	11	5	3	28[b]	7	2	1	1
AG9025	1547[b]	387	97	43	25	115[a]	29	8	4	1
AM9724	246[b]	62	16	7	4	61[b]	16	4	2	1
AS1666	314[b]	79	20	9	5	46[b]	12	3	2	1
AS1677	478[b]	120	30	14	8	79[a]	20	5	3	1
BM3066	226[b]	57	15	7	4	46[b]	12	3	2	1
Celeron	214[b]	54	14	6	4	39[b]	10	3	2	1
DKB230	253[b]	64	16	8	4	40[b]	10	3	2	1
DKB290	167[b]	42	11	5	3	23[b]	6	2	1	2
MS2010	380[b]	95	24	11	6	74[a]	19	5	3	2
MS2013	254[b]	64	16	8	4	44[b]	11	3	2	2
MS3022	230[b]	58	15	7	4	67[a]	17	5	2	2
P1630	6251[a]	1563	391	174	98	107[a]	27	7	3	2
P2530	-	-	-	-	-	76[a]	19	5	3	2
SHS7915	236[b]	59	15	7	4	45[b]	12	3	2	2
StatusVIP	161[b]	41	11	5	3	36[b]	9	3	1	2
SX7331	172[b]	43	11	5	3	31[b]	8	2	1	2
Mean	672	169	43	20	11	61	16	5	3	2

Means not followed by the same superscript letter differ by the Scott-Knott test at a 5% significance level. In columns referring to D10, D20, D30, and D40, the superscript letters are the same as in the column referring to D5, and therefore, are not shown.

If the researcher selects the largest average number of sample size (NSB trait), 11, 20, and 43 tassels are sufficient to estimate the mean values for tassel traits with a precision of 40% (D40), 30% (D30), and 20% (D20), respectively, of the mean estimate and a 95% confidence level. Taking the average sample size for genotypes in groups of traits for a precision of 10% (D10) of the estimated mean, weight traits (PDM, BSDM, CSDM, and TDM) can be sampled with 32 tassels (Table 3), length traits (PL, BSL, CSL, and TL) with 22 tassels (Table 4), and branching traits (NPB, NSB, and TBN) with 169 tassels. Furthermore, assuming that the sample size is set at 43 tassels (20% precision) to estimate the mean values of treatments in an experiment with three replicates, 15 tassels can be sampled by repetition.

In conclusion, for tassel traits, 11, 20, and 43 tassels are sufficient to estimate the mean with a precision of 40, 30, and 20%, respectively, of the estimated mean at a 95% confidence level.

There is sample size variability among maize genotypes for peduncle dry matter, central spike dry matter, tassel dry matter, peduncle length, branching space length, central spike length, tassel length, number of primary branches, number of secondary branches, and tassel branch number.

Conflicts of interest

The authors declare no conflict of interest.

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