Sample size determination for maize plants and cob traits under straw management at sowing

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Abstract

Certain types of management in maize crop experiments (*Zea mays* L) can determine sample size. This study aimed to estimate the sample size needed to determine traits of maize plants and cobs under various straw management and sowing methods in large plots. The experiment was performed in the experimental area of the Federal University of Technology in Paraná (Universidade Tecnológica Federal do Paraná - UTFPR), Pato Branco, Paraná. Different managements methods of oat straw were subjected to four sowing methods using plots with five 20 m long rows spaced at 0.8 m. A total of five traits were evaluated on 10 plants and four traits on eight cobs. The 95% confidence interval was estimated using 5,000 bootstrap simulations. For an error equal to 10% of the mean, the sample size for plant and cob insertion heights and stem diameter is less than six, and for cob length, diameter, number of rows and number of kernels per row, the sample size is less than five. The type of straw management and the method of sowing did not affect the sample size for traits of plants and cobs.

Keywords: *Zea mays* L, sampling, error estimation, bootstrap

Introduction

Experiments evaluating different types of straw management and sowing methods of maize (*Zea mays* L) generally require experimental units much larger than necessary, and sampling becomes necessary. It is known that sample size is directly related to the variability of the data and the desired reliability of the estimate and is inversely related to the estimation error established by the researcher (Barbetta et al, 2004; Confalonieri et al, 2006).

Studies evaluating fertilization management and sowing in maize (Lopes and Storck, 1995), sample size estimates for cob traits of different maize hybrids (Storck et al, 2007), and the sample sizes of plots with different maize genotypes (Martin et al, 2005a) have been reported. Palomino et al (2000) investigated the sample sizes of maize half-sib families. Moreover, to evaluate the pre-harvest traits of popcorn maize, samples of 5 to 25 plants per plot can be used without affecting the experimental accuracy (Catapatti et al, 2008).

Lúcio and Storck (1999) observed that the management of maize experiments affects experimental accuracy and that standardizing competition experiments with maize cultivars using management practices that reduce experimental error can increase accuracy. Additionally, maize thinning is a procedure that reduces experimental error, and insect control after the appearance of a pest in maize should be avoided (Lúcio and Storck, 1999). However, no information was found on the sample size of maize plants and cobs related to the type of crop residue management and mechanized sowing methods. Thus, this study aimed to estimate the sample size for traits of maize plants and cobs under different methods of straw management and sowing in large plots.

Materials and Methods

The experiment was performed in an experimental area at the Course of Agronomy, UTFPR - Campus Pato Branco, at the coordinates 26°10’36” south latitude and 52°41’20” west longitude and 765 m of altitude.

The different black oat straw management methods used were disked (leveler grid of double action), rolled (knife roll) and ground straw (straw crusher, adjusted to cut the straw to 0.07 m tall), which were performed seven days before sowing, and chemical management of the cover (dried straw) was performed 15 days prior to maize sowing.

The experimental units that were subjected to mechanical treatments were also subjected to chemical treatment, using 2.5 l ha^{-1} of the herbicide glyphosate athanor, of the straw to observe the effects of straw architecture and fractionation.

The different treatments were used on black oat (*Avena strigosa* Schreb) cover crop with an average dry weight of 7,759 kg ha^{-1} during the treatment period, which coincided with full flowering.

After the straw managements (28 November 2010), the hybrid DKB 240 YG was sown, using two furrow openers mechanisms (furrow openers with double disc and type shanks) in two operating speeds

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(4.5 and 7.0 km h^{-1}). The plots consisted of five rows, 20.0 m in length, spaced 0.8 m between rows. The 16 treatments (four straw managements x four sowing methods) were evaluated in a randomized block design with four replicates.

The initial plant height (IPH, cm) was randomly obtained from 10 plants per plot and assessed on 12 June 2010. IPH was defined as the distance from the ground level to the flag leaf insertion point. The initial stem diameter (ISD, cm) was randomly obtained from 10 plants per plot and assessed on 12 August 2010 using a caliper at the ground level. On 22 February 2011, the final plant height (FPH, cm), the final stem diameter (FSD, cm) at 20 cm from the ground level and the cob insertion height (CIH, cm) were assessed in other 10 plants per plot.

At harvest, eight cobs per plot were sampled to determine the cob length (CL, cm), cob diameter (CD, mm), number of kernel rows per ear (NK) and number of kernels per row (NKR).

For each of the nine traits (IPH, ISD, FPH, FSD, CIH, CL, CD, NK, and NKR), analysis of variance of the factorial experiments (four straw management x four sowing methods) was performed and F test using with $p < 0.05$.

For each of the 64 plots, the mean (m) and variance (s^2) for each trait was estimated using the measurements of all plants and cobs evaluated in each plot. The sample size (η) was estimated using the expression $\eta = t^2_{\alpha/2} s^2 / SA^2$, where SA is the semi-amplitude of the confidence interval, and $t_{\alpha/2}$ is the critical value of Student's t distribution, the right area of which is equal to $\alpha/2$ with $P(t>t_{\alpha/2}) = \alpha/2$, (n-1) degrees of freedom and α = 5% error probability (Barbetta et al, 2004). It was shown that SA is equal to 5 and 10% of the mean (m) or, SA = 0.05 m and SA = 0.10 m. Reversing the expression $\eta = t^2_{\alpha/2} s^2 / SA^2$, η was defined as the total number of plants (10) or cobs (8) per plot for calculating the estimation error (SA) as a percentage of the estimate of the mean (m) for each of the traits of each plot using the expression $SA = 100 t_{\alpha/2} s / m\sqrt{\eta}$, where s is the estimated sample standard deviation.

For each of the nine traits, the observed estimation error and the error of semi-amplitude was equal to 5% and to 10% of the mean (SA = 5% and SA = 10%). The Lilliefors test at 5% probability error was performed to verify the normality of the 64 values. Additionally, the Kruskal-Wallis nonparametric analysis of variance test was performed considering the 16 treatments in four replicates. The SAEG software was used for these analyses (SAEG, 2007).

The following statistics were obtained for the 64 values for each trait: minimum, mean, maximum and the lower and upper limit of the 95% bootstrap confidence interval, which was obtained with 5,000 simulations in the BioEstat 5.0 software (Ayres et al, 2007). The mean sample size of the different traits were compared two by two using a t test with 5,000 bootstrap simulations at 5% error probability using the BioEstat 5.0 software (Ayres et al, 2007).

Results and Discussion

Among the nine traits assessed in maize plants and cobs, the treatment effect was significant ($p<0.05$) only for IPH (Table 1). Therefore, the treatment effect was not considered in the management and method main effect or in the management x method interaction effect.

For the nine traits assessed, the variance component estimates among plots ($\hat{\sigma}^2$) was lower than the variance component estimates within plots ($\hat{\sigma}^2_\varepsilon$). Thus, for seven of the traits (IPH, ISD, FPH, FSD, CIH, CL and CD), the variance among the plots was significantly ($p < 0.05$) greater than zero. In these cases, the experimental plan, which uses the same number of plants and cobs per treatment, should provide a greater sample size, due to the number of repetitions required to compare treatment means with greater accuracy (Barbin, 2003). For six of the nine traits (the four cob traits and FPH and CIH), the variation coefficient among and within the plots was classified as low (approximately 10%). The correlation between the coefficient of variation (CV) and plot size is known (Martin et al, 2005b) in maize and other crops and is low for large plots. Because the present study was performed in large plots, it was inferred that the sample size taken from the plot was sufficient, as the accuracy was considered high.

In experiments involving the use of seeding ma-

Table 1 - Mean square treatment (MSt), mean square error between plots (MSeb), mean square error within plots (MSew), degrees of freedom (DF), mean, coefficient of variation between plots (CVb) and within plots (CVw), estimation of the variation between plots ($\hat{\sigma}^2$) and within plots ($\hat{\sigma}^2_\varepsilon$), and mean sample size (MSS) for an estimation error equal to 10% of the mean, for maize initial plant height (IPH, cm), initial stem diameter (ISD, mm), final plant height (FPH, cm), final stem diameter (FSD, mm) and cob insertion height (CIH, cm).

Trait	MSt (DF=15)	MSeb (DF=45)	MSew (DF=576)	Mean	CVb (%)	CVw (%)	$\hat{\sigma}^2$	$\hat{\sigma}^2_\varepsilon$	MSS
IPH	321.77*	91.88*	16.40	46.1	20.8	8.8	7.55	16.40	4.0
ISD	21.15ns	19.29*	5.12	22.4	19.6	10.1	1.42	5.12	5.2
FPH	681.2ns	615.1*	165.6	295.3	8.4	4.4	44.95	165.60	1.0
FSD	16.2ns	15.3*	5.42	24.4	16.0	9.5	0.99	5.42	4.6
CIH	260.8ns	144.7*	92.2	116.1	10.4	8.3	5.25	92.20	3.5

* significant by F test (p-value < 0.05); ns non-significant effect.

Table 2 - Mean square treatment (MSt), mean square error between plots (MSeb), mean square error within plots (MSew), degrees of freedom (DF), mean, coefficient of variation between plots (CVb) and within plots (CVw), estimation of the variation between plots ($\hat{\sigma}^2$) and within plots ($\hat{\sigma}_{\varepsilon}^2$), and mean sample size (MSS) for an estimation error equal to 10% of the mean, for cob length (CL, cm), cob diameter (CD, mm), number of rows (NR) and number of kernels per row (NKR).

Trait	MSt (DF=15)	MSeb (DF=45)	MSew (DF=576)	Mean	CVb (%)	CVw (%)	$\hat{\sigma}^2$	$\hat{\sigma}_{\varepsilon}^2$	MSS
CL	1.01^{ns}	1.55*	0.66	16.7	7.5	4.9	0.11	0.66	1.3
CD	3.65^{ns}	4.91*	3.01	46.5	4.8	3.7	0.24	3.01	0.8
NR	0.48^{ns}	1.11^{ns}	1.34	13.0	8.1	8.9	0.00	1.34	4.4
NKR	38.6^{ns}	31.97^{ns}	23.23	77.0	7.3	6.3	1.09	23.23	2.2

* significant by F test (p-value < 0.05); ns non-significant effect.

chines coupled to a tractor, the space required to standardize the operation for a given sowing treatment was greater than in genotype comparison experiments. In these experiments, evaluating traits in all of the plants grown in these large experimental units is a laborious and unnecessary process. Thus, sampling or random removal of part of the plants from each experimental unit reduces the required time and financial and human resources while maintaining the experimental accuracy at high levels to estimate the mean of each trait.

Considering that there are no differences between the 16 treatments for eight out of the nine traits assessed, the 64 plots (16 treatments and four replicates) constitute similar (homogeneous) subpopulations to be sampled. Thus, the mean sample size (MSS, Table 1 and 2) replaces the s^2 estimate for MSew in the expression and maintains the degrees of freedom (DF) for the sample size within each plot (DF = 9 for plant traits and DF = 7 for cob traits). For an estimation error equal to 10% of the mean (SA = 10%), fewer than five plants or cobs per plot could be sampled. Thus, taking a sample of five plants per plot to assess pre-harvest popcorn maize traits in the four replicate experiment does not affect the experimental accuracy (Catapatti et al, 2008).

The fit to a normal distribution was rejected (p = 0.05) for the 64 sample size values obtained for the two semi-amplitudes (SA = 5% and SA = 10%) with the estimation error and sample size from the plot using the Lilliefors' test for all traits. Additionally, using the Kruskal-Wallis test, it was observed that the treatment effects (straw management and sowing methods) on the sample size and estimation error were not significant (p > 0.05) for the plant and cob traits. Thus, both management methods that were assessed did not affect the sample size, which contradicts the conclusions of Lúcio and Storck (1999) that the experimental accuracy is related to management. Thus, these results indicate that the 64 plots are similar subpopulations with regard to the mean traits observed and the sample size.

The minimum, maximum, mean and the estimate per interval are described in Table 3 for the semi-amplitudes of 5 and 10% of the mean and for the estimation error as a percentage of the mean, which was obtained using the N observations of each plot.

The mean sample size (Table 3, mean of the 64 plots) is not necessarily equal to the mean sample size (Table 1 and 2, using the mean variance within the plot); however, in this case, they are similar for SA = 10%. The sample size per plot among the 64 plots allowed the authors to estimate the sample size interval and to compare the sample size among the different traits using the bootstrap resampling procedure (Ferreira, 2009), which does not require knowledge of the probability distribution. Based on the minimum and maximum values, a wide range of values for all traits was observed. However, estimates using the bootstrap interval (p-value of confidence = 0.95) show upper limits (UL) smaller than six plants or five cobs for a semi-amplitude equal to 10% of the mean. For a semi-amplitude equal to 5% of the mean (low estimation error), the UL of the confidence interval for the sample size is approximately 24 plant traits or 20 cob traits. The magnitude of sampling error (5 or 10% of the mean) is decided by the researcher, and the maximum estimation error in the present study (UL of the bootstrap confidence interval) was 7.4% (ICD) and 7.6% (NK). It is difficult to plan data collection using a different number of plants or cobs for the different traits to be assessed; however, it may be necessary, depending on the researcher's goals. In this case, using the t test with the bootstrap (p < 0.05), differ sample sizes among the traits were observed. Wide variability in the sample size of maize cob traits was also described by Storck et al (2007), who reasoned that the different traits measured and the different maize hybrids (single, triple and double-cross) used in the study were responsible. Interference from genetic and environmental sources affected the magnitude of the sample size estimates for maize cobs, as reported by Martin et al (2005a). These authors found sample size values for several maize cob traits that were superior (24 cobs) for the same estimation error of 10% of the mean. The introduction of human variation in the experiment to homogenize the management led to the conclusion that a more homogeneous method of distributing the fertilizer significantly reduced the experimental error (Lopes and Storck, 1995). It could be inferred that the sample size could be smaller due to reduced variability among the rep-

Table 3 - Minimum, mean, and maximum values, as well as lower limit (LL) and upper limit (UL) of the 95% bootstrap confidence interval for the sample size with semi-amplitudes (SA) equal to 5 and 10% of the mean and estimation error given the sample size used (N) for different trait: corn plant height (IPH, cm), initial stem diameter (ISD, mm), final plant height (FPH, cm), final stem diameter (FSD, mm), cob insertion height (CIH, cm), cob length (CL, cm), cob diameter (CD, mm), number of rows (NR) and number of kernels per row (NKR).

Trait	N	Minimum	Mean	Maximum	LL	UL
	Semi-amplitude = 5% of the mean					
IPH		2.3	16.1 bc*	212.6	11.4	22.1
ISD		3.7	21.1 a	67.6	18.5	23.6
FPH		0.4	3.9 f	23.7	2.9	4.8
FSD		2.2	18.7 ab	63.0	15.8	21.0
CIH		3.2	13.8 c	48.5	12.0	15.4
CL		0.7	5.4 e	18.1	4.4	6.1
CD		0.4	3.1 f	7.8	2.8	3.4
NR		0.0	17.7 b	42.8	16.1	19.1
NKR		1.0	8.9 d	35.6	7.4	10.4
	Semi-amplitude = 10% of the mean					
IPH		0.6	4.0 bc	53.2	3.0	6.0
ISD		0.9	5.3 a	16.9	4.6	6.0
FPH		0.1	1.0 f	5.9	0.7	1.2
FSD		0.5	4.7 ab	15.8	4.0	5.3
CIH		0.8	3.5 c	12.1	3.0	3.8
CL		0.2	1.3 e	4.5	1.1	1.5
CD		0.1	0.8 f	1.9	0.6	0.8
NR		0.0	4.4 b	10.7	4.0	4.8
NKR		0.3	2.2 d	8.9	1.8	2.6
	Estimation error, with N observations, as % of mean					
IPH	10	2.4	5.7 c	23.1	5.2	6.4
ISD	10	3.0	7.0 ab	13.0	6.4	7.4
FPH	10	1.0	2.8 f	7.7	2.6	3.1
FSD	10	2.3	6.6 b	12.6	6.1	7.0
CIH	10	2.8	5.7 c	11.0	5.3	6.0
CL	8	1.5	3.9 e	7.5	3.6	4.1
CD	8	1.1	3.0 f	4.9	2.8	3.1
NR	8	0.0	7.3 a	11.6	6.7	7.6
NKR	8	1.8	4.9 d	10.5	4.4	5.2

*Means of traits not connected by the same letter differ by t test 5,000 bootstrap simulations ($p = 0.05$)

licates. In experiments comparing maize hybrids, it was not advantageous to replace the use of borders for a proportionally greater number of replicates (Oliveira et al, 2005), which suggested that the plots should be small (without borders) for larger numbers of replicates. By fixing the number of plants per half-sib family, Palomino et al (2000) concluded that the expected gain with the selection decreases with the number of plants per plot because it reduces the number of replicates. These researchers also found that the collection of plants distributed in two or three rows provides greater experimental accuracy, which must be attributed to the principle of randomness (representativeness of the plot). For the present situation, larger plots must be used for technical reasons (the use of agricultural machinery for sowing) with no boundary required, the sampling of plants (or cobs) in small quantities does not mean that the number of replicates can be ignored for a suitable experimental accuracy. However, the effects of management treatment and sowing method did not affect the magnitude of the traits assessed or their variability because the sample size did not differ between treatments.

However, the fertilization hides soil N content variability with the consequence that larger sample sizes are required for unfertilized plots compared to fertilized plots with rice. Also, for row-seeded rice, the number of plants instead of linear centimeters as the sampling unit led to lower sample sizes. These results highlight the influence of experimental factors on within-plot variability and the importance of preliminary sampling for sample size determination (Confalonieri et al, 2006). Further investigations are required to examine the influence of the sample size and the structure of the population on the power of detecting (Reif et al, 2004).

For the smaller estimation error (SA = 5%), the differences in sample size for the different traits was more obvious (larger magnitude), suggesting that it may be important for researchers to use sample sizes that are specific to different traits.

Conclusions

The type of straw management and sowing method did not affect the sample size in plant and cob traits.

For an estimation error equal to 10% of the mean, the sample size for plants and insertion of cob height and stem diameter was less than six, and the sample size for length, diameter, number of rows and of kernels per row cob traits was less than five.

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