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Plot size and number of replicates in times of sowing and cuts of millet

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Key words:
Pennisetum glaucum (L.) R. Brown
maximum curvature of the coefficientof variation model
experiment planning

ABSTRACT
The objective of this study was to determine the optimum plot size (Xo) and number of replicates to evaluate millet shoot fresh matter in times of sowing and cuts. Uniformity trials of 6 × 4 m (24 m^2) were carried out in three sowing times, in the agricultural year of 2013-2014. Each uniformity trial was divided into 24 basic experimental units (BEU) of 1 × 1 m (1 m^2) and the shoot fresh matter of plants in each BEU was weighed. The Xo was determined by the method of maximum curvature of the coefficient of variation model. The number of replicates for experiments in completely randomized and randomized block design, in scenarios of combinations of i treatments (i = 3, 4, ..., 50) and d minimal differences between treatment means, to be detected as significant at 0.05 probability level by Tukey test, expressed in percentage of the experiment mean (d = 10, 12, ..., 30%), was determined by iterative process until convergence. The optimum plot size to evaluate millet shoot fresh matter is 4.97 m^2, for the three times of sowing and cuts. For the evaluation of up to 50 treatments, in completely randomized and randomized block design, five replicates are sufficient to identify as significant, at 0.05 probability level by Tukey test, differences between treatment means of 28.66% of the mean of the experiment.

Palavras-chave:
Pennisetum glaucum (L.) R. Brown
curvatura máxima do modelo do coeficiente de variação
planejamento experimental

Tamanho de parcela e número de repetições em milheto em épocas de semeadura e cortes

RESUMO
O objetivo deste trabalho foi determinar o tamanho ótimo de parcela (Xo) e o número de repetições para avaliar a massa verde de parte aérea de milheto em épocas de semeadura e cortes. Foram conduzidos ensaios de uniformidade de 6 × 4 m (24 m^2) em três épocas de semeadura, no ano agrícola 2013-2014. Cada ensaio de uniformidade foi dividido em 24 unidades experimentais básicas (UEB) de 1 × 1 m (1 m^2) e pesada a massa verde da parte aérea das plantas de cada UEB. O Xo foi determinado por meio do método da curvatura máxima do modelo do coeficiente de variação. O número de repetições para experimentos nos delineamentos inteiramente casualizados e blocos ao acaso, em cenários formados pelas combinações de i tratamentos (i = 3, 4, ..., 50) e diferenças mínimas entre médias de tratamentos a serem detectadas como significativas a 0,05 de probabilidade, pelo teste de Tukey, expressas em percentagem da média do experimento (d = 10, 12, ..., 30%) foi realizado por processo iterativo até a convergência. O tamanho ótimo de parcela para avaliar a massa verde da parte aérea de milheto é de 4,97 m^2, para as três épocas de semeadura e cortes. Para avaliar até 50 tratamentos nos delineamentos inteiramente casualizados e blocos ao acaso, cinco repetições são suficientes para identificar, como significativas, pelo teste de Tukey, a 0,05 de probabilidade, diferenças entre médias de tratamentos de 28,66% da média do experimento.

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Introduction

Millet (*Pennisetum glaucum* (L.) R. Brown) is an annual species of tropical climate, from the Poaceae family, which adapts to various conditions of climate and soil. This crop stands out for the high forage production potential for both silage and pasture, due to its nutritional quality and regrowth capacity (Kollet et al., 2006; Costa et al., 2011; Pinho et al., 2013), intensifying the livestock production in the state of Rio Grande do Sul. In addition, it shows differences in performance with respect to its development depending on the times of sowing, and its phytomass production is influenced by the cut regimes (Lemos et al., 2003; Coimbra & Nakagawa, 2006).

Studies conducted with millet in the form of pasture showed satisfactory performance in animal feeding (Roman et al., 2008; Jochims et al., 2010; Pacheco et al., 2014). In these studies, the fresh matter was used for the determination of the mass of pasture. According to Montagner et al. (2011), the voluntary consumption of pasture by the animals and, consequently, their development, are influenced by the amount of pasture and, more precisely, by the mass of green leaves.

Millet, due to its high potential for biomass production and its various forms of use, is an interesting alternative that can integrate agriculture and livestock farming (Priesnitz et al., 2011). In field experiments with millet, it is important to dimension plot size and number of replicates correctly. Adequate determination guarantees more precision and validates the extrapolation of the results because, regardless of the objectives of the experiments, the purpose is to detect significant differences between the evaluated treatments (Donato et al., 2008).

One way to contribute to the improvement in the quality of the experiments is to apply adequate methods, based on practical rules that minimize experimental error and maximize the amount of information that can be obtained from an experiment (Brito et al., 2012). Thus, it is essential the utilization of adequate methods that allow the determination of the optimum plot size and number of replicates. The method of maximum curvature of the coefficient of variation model is indicated to obtain the optimum size of experimental plots (Paranaíba et al., 2009) and has already been used in the estimate of the optimum plot size for grasses, such as corn (Cargnelutti Filho et al., 2011) and black oat (Cargnelutti Filho et al., 2014).

Experiments using millet, in which fresh matter was evaluated, used variable plot sizes and number of replicates, such as plots of 6 m^2 (1.5 m wide and 4.0 m long), with five replicates (Pinho et al., 2013), plots of 12 m^2 (2.5 m wide and 4.8 m long) and four replicates (Priesnitz et al., 2011) and plots of 15 m^2, with six replicates (Moreira et al., 2003), all of them arranged in a randomized block design. On the other hand, experiments using the fresh matter for the determination of mass of forage in millet pasture employed larger plot sizes, 1,350 m^2, with two replicates (Jochims et al., 2010) and 13,000 m^2, with five replicates (Pacheco et al., 2014) in a completely randomized experimental design.

Given the expressive importance of the millet crop for animal feeding, studies on experimental planning aiming to estimate plot size and number of replicates to evaluate its shoot fresh matter are essential and still unknown. Thus, this study aimed to determine the optimum plot size (Xo) and number of replicates for the shoot fresh matter of millet (*Pennisetum glaucum* (L.) R. Brown) in times of sowing and cut.

Material and Methods

Uniformity trials were conducted using the millet crop (*Pennisetum glaucum* (L.) R. Brown) at the experimental area of the Department of Plant Science of the Federal University of Santa Maria, in Santa Maria-RS, Brazil (29° 42' S; 53° 49' W; 95 m) in the agricultural year of 2013-2014. Uniformity trials, also referred to as blank experiments, are conducted without treatments and with homogeneous cultural practices over the entire area (Storck et al., 2011).

The millet cultivar 'BRS 1501' was sown broadcast in three sowing times (October 18, 2013, November 26, 2013, and December 3, 2013) at density of 50 kg ha^{-1}, in experimental areas of 500, 800 and 750 m^2, respectively. Basal fertilization used 40 kg ha^{-1} of N, 160 kg ha^{-1} of P_2O_5 and 160 kg ha^{-1} of K_2O. As top-dressing, 100 kg ha^{-1} of N were applied in the entire area, in each sowing time.

Each uniformity trial with size of 6 × 4 m (24 m^2) was divided into 24 basic experimental units (BEU) of 1 × 1 m (1 m^2) forming a matrix of six rows and four columns. In each sowing time, shoot fresh matter was collected in three trials, in each evaluation period, and plants were cut at 10 cm from the soil surface in order to evaluate the regrowth.

In the first sowing time, the first cut of fresh matter was performed in three trials at 39 days after sowing (DAS), in three trials at 46 DAS, in three trials at 54 DAS and in three trials at 62 DAS. The second fresh matter cut was performed at 54, 62 and 80 DAS, respectively, in the trials with the first cut performed at 39, 46 and 54 DAS. The third fresh matter cut was performed at 69 and 82 DAS, respectively, in the trials with second cut at 54 and 62 DAS. In the second sowing time, only one cut was performed in each trial, in three trials, at 43 DAS, in three trials at 51 DAS, in three trials at 58 DAS and in three trials at 64 DAS. In the third sowing time, the first fresh matter cut was performed in three trials at 36 DAS, in three trials at 44 DAS, in three trials at 51 DAS and in three trials at 57 DAS. The second fresh matter cut was performed at 57 and 65 DAS, respectively, in the trials with first cut at 36 and 44 DAS. The third fresh matter cut was performed at 99 DAS, both in trials with second cut at 57 DAS and in trials with second cut at 65 DAS.

For each uniformity trial with the data of fresh matter of the 24 BEU, the following variables were determined: first-order spatial autocorrelation coefficient (ρ) in the direction of the rows, variance (s^2), mean (m) and the coefficient of variation of the trial (CV) in percentage. The estimation of ρ started from the BEU in the row 1, column 1, until the row 1, column 4, returning from row 2, column 4, until the row 2, column 1, and so on, until the end, in the BEU in row 6, column 1.

Subsequently, for each sowing time, in each period of evaluation of the trials and cuts, the optimum plot size (Xo) was determined by the method of maximum curvature of the coefficient of variation model, according to the equation:

$$Xo = \frac{10\sqrt[3]{2\left(1-\rho^2\right)s^2 m}}{m} \qquad (1)$$

where:

ρ - first-order spatial autocorrelation coefficient;

s^2 - variance; and

m - mean.

The coefficient of variation in the optimum plot size (CV_{Xo}), in percentage, was determined using the equation described by Paranaíba et al. (2009):

$$CV_{Xo} = \frac{\sqrt{\frac{(1-\rho^2)s^2}{m^2}}}{\sqrt{Xo}} \times 100 \qquad (2)$$

For the estimation of the statistics ρ, s^2, m, CV, Xo and CV_{Xo}, the following parameters were calculated: mean, standard deviation, coefficient of variation and p-value of the Kolmogorov-Smirnov normality test. The means of the statistics (ρ, s^2, m, CV, Xo and CV_{Xo}) were compared between cuts in the same sowing time and between sowing times, by the Student's t-test for independent samples, at 0.05 probability level. These comparisons of means were performed two by two and the results were represented by letters on the side of the means.

The number of replicates was calculated based on the least significant difference (d) of the Tukey test, expressed in percentage of the mean of the experiment, estimated by the equation:

$$d = \frac{q_{\alpha(i;DFE)}\sqrt{\frac{MSE}{r}}}{m} \times 100 \qquad (3)$$

where:

$q_{\alpha(i;DFE)}$ - critical value of the Tukey test at level α of probability of error (α = 0.05);

i - number of treatments;

DFE - degrees of freedom of the error, i(r-1) for the completely randomized design and (i-1)(r-1) for the randomized block design;

MSE - mean square of the error;

r - number of replicates; and

m - mean of the experiment.

Substituting the equation of the experimental coefficient of variation:

$$CV = \frac{\sqrt{MSE}}{m} \times 100 \qquad (4)$$

in percentage, in Eq. 4 and isolating r, the following expression is obtained:

$$r = \left(\frac{q_{\alpha(i;DFE)}CV}{d}\right)^2 \qquad (5)$$

The CV, in percentage, corresponds to the CV_{Xo} (Cargnelutti Filho et al., 2014).

Then, based on the mean of the highest CV_{Xo} values, between the sowing times, the number of replicates (r) was determined by an iterative process until convergence, for experiments in completely randomized design (CRD) and randomized block (RBD) in scenarios formed by the combinations of i (i = 3, 4, ..., 50) and d (d = 10, 12, ..., 30%). The statistical analyses were performed using the application Microsoft Office Excel®.

Results and Discussion

A scenario with three sowing times, 21 evaluations, three cuts of millet fresh matter per sowing time (except for the second sowing time, which had only one cut) in 63 trials and 1,512 basic experimental units (63 trials x 24 basic experimental units), provides an adequate database for the proposed study.

The set of results shows wide variability of the estimates of all the evaluated statistics: first-order spatial autocorrelation coefficient (ρ), variance (s^2), mean (m), coefficient of variation of the trial (CV), optimum plot size (Xo) and coefficient of variation in the optimum plot size (CV_{Xo}), obtained from the shoot fresh matter of millet in the trials of the first sowing time (6.68 ≤ CV ≤ 85.30%), second sowing time (12.21 ≤ CV ≤ 48.99%) and third sowing time (8.40 ≤ CV ≤ 70.53%) (Tables 1 and 2). The variability of the statistics between the trials was also reported by Cargnelutti Filho et al. (2014), and is considered as important, since it reflects actual conditions of the crop at field and provides consistency to the study on plot size and number of replicates, along with the large database.

According to the normality of the data, verified through the Kolmogorov-Smirnov test, all the estimated statistics (ρ, s^2, m, CV, Xo and CV_{Xo}) have good adherence to normal distribution (P ≥ 0.35) (Tables 1 and 2), evidencing that the inferences based on the means of the trials are adequate for the study on plot size and also provide reliability to the other inferences.

The total fresh matter productions were 5,764.19, 3,726.19 and 5,840.23 g m^{-2} for the first, second and third sowing times, respectively (Tables 1 and 2). The mean fresh matters obtained in the first, second and third sowing times were 1,999.35, 3,726.19 and 2,058.89 g m^{-2}, respectively (Table 3). In general, there were decreases in millet shoot fresh matter with the increment in cuts, for each sowing time (Tables 1 and 2). However, aiming the maximization of millet production for animal feeding in different periods, there was an increase in total fresh matter production when there were cuts. These results also confirm that phytomass production influences the sowing time and number of cuts, regardless of the vegetative stage of the plants (Coimbra & Nakagawa, 2006).

The cuts performed in the first and third sowing time aimed to evaluate the regrowth capacity, because millet has great importance in animal feeding. Guimarães Júnior et al. (2009) showed the capacity of regrowth of the crop when correctly managed. The values of fresh matter of the performed cuts ranged from 1,545.22 to 2,395.35 g m^{-2} or 15.45 to 23.95 t ha^{-1}, respectively (Tables 1 and 2). For the same cultivar ('BRS-1501'), lower values of approximately 12 t ha^{-1} in cuts were observed by Pinho et al. (2013) and values ranging from 6.28 to 28.98 t ha^{-1} by Guimarães Júnior et al. (2009) in five periods of cut. The satisfactory performance of the crop and the good development of the regrowth are also related to cut height (10 cm from soil surface). Thus, Kollet et al. (2006) recommend cut between 6 and

Table 1. First-order spatial autocorrelation coefficient (ρ), variance (s^2), mean (m), coefficient of variation of the trial (CV, in %), optimum plot size (Xo, in m^2) and coefficient of variation in the optimum plot size (CV_{Xo}, in %) for millet shoot fresh matter (g m^{-2}) evaluated in three uniformity trials per evaluation period, in the first cut in four evaluation periods at 39, 46, 54 and 62 days after sowing (DAS), in the second cut in three evaluation periods at 54, 62 and 80 DAS and in the third cut in two evaluation periods at 69 and 82 DAS, of the first sowing time (October 18, 2013)

Sowing time	Cut	Trial(1)	ρ	s^2	m	CV (%)	Xo (m^2)	CV_{Xo} (%)
First evaluation period = 39 DAS								
1	1	1	0.37	112,463.69	963.04	34.82	5.94	13.28
1	1	2	0.24	57,002.56	896.29	26.64	5.12	11.44
1	1	3	0.47	119,451.85	989.13	34.94	5.75	12.86
Second evaluation period = 46 DAS								
1	1	1	0.20	197,996.95	1,051.79	42.31	7.00	15.66
1	1	2	0.09	179,330.98	1,406.13	30.12	5.65	12.63
1	1	3	0.41	91,123.04	1,137.92	26.53	4.89	10.94
Third evaluation period = 54 DAS								
1	1	1	0.24	271,771.85	2,155.75	24.18	4.79	10.72
1	1	2	0.51	639,425.13	3,125.00	25.59	4.59	10.27
1	1	3	0.24	455,754.00	3,212.50	21.01	4.37	9.77
Fourth evaluation period = 62 DAS								
1	1	1	0.05	397,867.64	3,824.58	16.49	3.79	8.46
1	1	2	-0.03	321,399.68	4,112.88	13.78	3.36	7.51
1	1	3	-0.07	449,605.82	4,086.92	16.41	3.77	8.43
Mean(2)			0.23 b	274,432.77 a	2,246.83 a	26.07 a	4.92 a	11.00 a
Standard deviation			0.19	180,969.55	1,331.02	8.53	1.05	2.35
CV (%)			84.69	65.94	59.24	32.74	21.33	21.33
p-value(3)			0.99	0.90	0.51	0.97	1.00	1.00
First evaluation period = 54 DAS								
1	2	1	0.50	172,092.26	1,303.21	31.83	5.34	11.94
1	2	2	0.68	276,295.28	1,714.67	30.66	4.66	10.42
1	2	3	0.65	386,906.17	1,624.50	38.29	5.53	12.37
Second evaluation period = 62 DAS								
1	2	1	0.19	160,552.16	2,021.38	19.82	4.23	9.47
1	2	2	0.25	165,480.22	2,123.04	19.16	4.10	9.16
1	2	3	0.35	172,480.74	1,848.96	22.46	4.46	9.97
Third evaluation period = 80 DAS								
1	2	1	0.45	952,677.99	2,794.58	34.93	5.79	12.94
1	2	2	0.62	1,006,039.71	2,356.67	42.56	6.08	13.59
1	2	3	0.60	281,861.24	1,962.25	27.06	4.56	10.19
Mean(2)			0.48 a	397,153.97 a	1,972,14 a	29.64 a	4.97 a	11.12 a
Standard deviation			0.18	338,772.97	432.92	8.22	0.72	1.62
CV (%)			37.66	85.30	21.95	27.72	14.54	14.54
p-value(3)			0.88	0.39	0.99	0.99	0.77	0.76
First evaluation period = 69 DAS								
1	3	1	0.35	168,165.12	1,197.42	34.25	5.90	13.19
1	3	2	0.53	216,364.61	1,327.46	35.04	5.61	12.55
1	3	3	0.54	182,304.37	1,147.25	37.22	5.80	12.97
Second evaluation period = 82 DAS								
1	3	1	0.47	363,268.68	1,834.38	32.86	5.52	12.33
1	3	2	0.54	483,324.03	2,304.88	30.16	5.04	11.27
1	3	3	0.33	154,360.95	1,459.92	26.91	5.06	11.31
Mean(2)			0.46 a	261,297.96 a	1,545.22 a	32.74 a	5.49 a	12.27 a
Standard deviation			0.10	132,711.60	446.03	3.69	0.37	0.82
CV (%)			21.24	50.79	28.87	11.28	6.68	6.68
p-value(3)			0.79	0.66	0.87	0.99	0.95	0.95

(1) Each uniformity trial with size of 6 × 4 m (24 m^2) was divided into 24 basic experimental units of 1 × 1 m (1 m^2), forming a matrix with six rows and four columns. (2) For each statistic (ρ, s^2, m, CV, Xo and CV_{Xo}), means not followed by the same letter in the column (comparison of means between cuts) differ at 0.05 probability level by Student's t-test for independent samples. (3) Normality test of Kolmogorov-Smirnov

10 cm for a good regrowth and higher fresh matter production by plants.

The mean values of the coefficient of variation (CV) of the trials oscillated from 21.49 to 32.74% (Tables 1 and 2), increasing along the cuts. Coefficients of variation of such magnitude are below the value reported in a study evaluating millet fresh matter conducted by Pinho et al. (2013), who observed CV values of 51.95 and 52.62%, in cuts 1 and 2, respectively. In study performed by Priesnitz et al. (2011), evaluating the fresh biomass of two millet cultivars, CV values of 17.67% were observed; on the other hand, Guimarães Júnior et al. (2009) obtained CV values below 10.62% for shoot fresh matter of three millet genotypes, in five periods of cut. For studies on plot size using the method of maximum curvature of the CV model, similar CV values of 19.99 and 21.66% were observed for black oat in two evaluation periods (Cargnelutti Filho et al., 2014).

The optimum plot sizes (Xo) and the coefficient of variation in the optimum plot size showed no significant difference for cuts performed in the same sowing time (Tables 1 and 2). On the other hand, between sowing times, there was significant

Table 2. First-order spatial autocorrelation coefficient (ρ), variance (s^2), mean (m), coefficient of variation of the trial (CV, in %), optimum plot size (Xo, in m^2) and coefficient of variation in the optimum plot size (CV_{Xo}, in %) for millet shoot fresh matter (g m^{-2}) evaluated in three uniformity trials per evaluation period, in the first cut in four evaluation periods at 43, 51, 58 and 64 days after sowing (DAS) of the second sowing time (November 26, 2013) and in the first cut in four evaluation periods at 36, 44, 51 and 57 DAS, in the second cut in two evaluation periods at 57 and 65 DAS and in the third cut in two evaluation periods at 99 DAS, of the third sowing time (December 3, 2013)

Sowing time	Cut	Trial[(1)]	ρ	s^2	m	CV (%)	Xo (m^2)	CV_{Xo} (%)
First evaluation period = 43 DAS								
2	1	1	0.31	219,564.17	3,030.46	15.46	3.51	7.84
2	1	2	0.28	305,290.35	2,963.54	18.64	4.00	8.94
2	1	3	0.55	704,711.65	2,801.46	29.97	5.01	11.21
Second evaluation period = 51 DAS								
2	1	1	0.38	625,623.22	3,772.46	20.97	4.22	9.44
2	1	2	0.34	1,070,665.17	4,474.04	23.13	4.56	10.20
2	1	3	0.55	460,732.17	3,478.50	19.51	3.76	8.40
Third evaluation period = 58 DAS								
2	1	1	0.49	479,470.95	3,547.79	19.52	3.87	8.66
2	1	2	0.32	1,388,023.10	4,213.17	27.96	5.20	11.63
2	1	3	0.36	479,476.61	4,064.79	17.04	3.70	8.27
Fourth evaluation period = 64 DAS								
2	1	1	0.41	667,299.30	3,901.54	20.94	4.18	9.36
2	1	2	0.60	898,060.42	4,043.38	23.44	4.14	9.25
2	1	3	0.28	884,907.01	4,423.17	21.27	4.37	9.76
Mean			0.40	681,985.34	3,726.19	21.49	4.21	9.41
Standard deviation			0.11	334,126.15	567.64	4.19	0.51	1.15
CV (%)			27.76	48.99	15.23	19.49	12.21	12.21
p-value[(3)]			0.78	0.96	0.97	0.79	0.92	0.93
First evaluation period = 36 DAS								
3	1	1	0.49	165,611.30	1,740.21	23.39	4.36	9.75
3	1	2	0.19	138,373.30	1,495.42	24.88	4.92	11.01
3	1	3	0.31	169,753.77	1,837.88	22.42	4.50	10.06
Second evaluation period = 44 DAS								
3	1	1	0.42	184,350.09	2,432.54	17.65	3.72	8.31
3	1	2	0.37	184,472.61	2,337.46	18.37	3.87	8.66
3	1	3	0.48	237,636.25	2,022.58	24.10	4.48	10.01
Third evaluation period = 51 DAS								
3	1	1	-0.21	663,666.75	3,338.67	24.40	4.85	10.84
3	1	2	0.32	413,758.38	2,716.88	23.68	4.65	10.40
3	1	3	0.40	261,641.48	2,470.00	20.71	4.17	9.31
Fourth evaluation period = 57 DAS								
3	1	1	-0.01	309,106.25	3,040.42	18.29	4.06	9.08
3	1	2	0.39	735,727.19	2,801.17	30.62	5.41	12.10
3	1	3	0.41	411,330.82	2,510.96	25.54	4.77	10.67
Mean[(2)]			0.30 b	322,952.35 a	2,395.35 a	22.84 a	4.48 a	10.02 a
Standard deviation			0.21	198,731.91	546.60	3.68	0.48	1.08
CV (%)			70.53	61.54	22.82	16.13	10.76	10.76
p-value[(3)]			0.35	0.70	0.99	0.96	1.00	1.00
First evaluation period = 57 DAS								
3	2	1	0.39	229,707.28	1,675.33	28.61	5.18	11.59
3	2	2	0.57	296,203.64	2,058.42	26.44	4.55	10.18
3	2	3	0.50	276,700.89	2,006.25	26.22	4.70	10.51
Second evaluation period = 65 DAS								
3	2	1	0.62	207,273.69	1,143.29	39.82	5.82	13.01
3	2	2	0.56	163,861.74	1,390.79	29.11	4.88	10.92
3	2	3	0.33	207,586.59	1,439.38	31.65	5.63	12.59
Mean[(2)]			0.49 a	230,222.31 a	1,618.91 a	30.31 a	5.13 a	11.47 a
Standard deviation			0.11	48.901.54	362.45	5.07	0.51	1.14
CV (%)			22.68	21.24	22.39	16.72	9.97	9.97
p-value[(3)]			0.90	0.99	0.98	0.81	0.99	0.99
First evaluation period = 99 DAS								
3	3	1	0.50	473,930.43	1,922.21	35.81	5.78	12.92
3	3	2	0.45	490,688.41	2,469.83	28.36	5.04	11.27
3	3	3	0.28	440,912.90	2,002.88	33.15	5.87	13.13
Second evaluation period = 99 DAS								
3	3	1	0.30	166,267.80	1,302.67	31.30	5.63	12.60
3	3	2	0.31	179,637.16	1,740.88	24.35	4.75	10.62
3	3	3	0.27	171,891.90	1,517.38	27.32	5.17	11.57
Mean[(2)]			0.35 b	320,554.76 a	1,825.97 a	30.05 a	5.37 a	12.02 a
Standard deviation			0.10	162,922.39	407.78	4.18	0.45	1.01
CV (%)			27.99	50.83	22.33	13.90	8.40	8.40
p-value[(3)]			0.56	0.63	1.00	1.00	0.94	0.94

(1) Each uniformity trial with size of 6 × 4 m (24 m^2) was divided into 24 basic experimental units of 1 × 1 m (1 m^2), forming a matrix with six rows and four columns. (2) In the third sowing time, for each statistic (ρ, s^2, m, CV, Xo and CV_{Xo}), means not followed by the same letter in the column (comparison of means between cuts) differ at 0.05 probability level by Student's t-test for independent samples. (3) Normality test of Kolmogorov-Smirnov

Table 3. Mean of the first-order spatial autocorrelation coefficient (ρ), variance (s^2), mean (m), coefficient of variation of the trial (CV, in %), optimum plot size (Xo, in m^2) and coefficient of variation in the optimum plot size (CV_{Xo}, in %) of millet shoot fresh matter (g m^{-2}), evaluated in three sowing times, in the agricultural year of 2013-2014

	Sowing time 1 (October18, 2013)	Sowing time 2 (November 26, 2013)	Sowing time 3 (December 3, 2013)
ρ	0.36 a	0.40 a	0.36 a
s^2	312,420.99 b	681,985.34 a	299,170.44 b
m	1,999.35 b	3,726.19 a	2,058.89 b
CV (%)	28.74 a	21.49 b	26.51 a
Xo (m^2)	5.06 a	4.21 b	4.87 a
CV_{Xo} (%)	11.32 a	9.41 b	10.88 a

(1) For each statistic (ρ, s^2, m, CV, Xo and CV_{Xo}), means not followed by the same letter in the row (comparison of means between sowing times) differ at 0.05 probability level by Student's t-test for independent samples

Table 4. Number of replicates for experiments in completely randomized design in scenarios formed by the combinations of i treatments (i = 3, 4, ..., 50) and d minimal differences between treatment means to be detected as significant at 0.05 probability level by Tukey test, expressed in percentage of the experiment mean (d = 10, 12, ..., 30%), to evaluate millet fresh matter based on optimum plot size (Xo = 4.97 m^2) and coefficient of variation in the optimum plot size (CV_{Xo} = 11.10%)

i	%										
	10	12	14	16	18	20	22	24	26	28	30
3	14.58	10.46	7.99	6.39	5.30	4.53	3.97	3.53	3.23	2.99	2.79
4	17.14	12.18	9.20	7.27	5.96	5.03	4.34	3.83	3.42	3.12	2.88
5	19.09	13.50	10.14	7.96	6.47	5.42	4.64	4.06	3.61	3.26	2.98
6	20.68	14.58	10.90	8.52	6.90	5.74	4.89	4.25	3.75	3.36	3.06
7	22.02	15.49	11.55	9.00	7.26	6.02	5.10	4.41	3.88	3.46	3.13
8	23.19	16.28	12.12	9.42	7.57	6.26	5.29	4.56	4.00	3.55	3.20
9	24.21	16.98	12.62	9.79	7.86	6.48	5.46	4.70	4.10	3.64	3.26
10	25.13	17.60	13.07	10.13	8.11	6.68	5.62	4.82	4.20	3.71	3.33
11	25.97	18.17	13.47	10.43	8.35	6.86	5.76	4.93	4.29	3.79	3.38
12	26.73	18.69	13.85	10.71	8.56	7.03	5.90	5.04	4.38	3.85	3.44
13	27.43	19.17	14.19	10.97	8.76	7.18	6.02	5.14	4.45	3.92	3.49
14	28.08	19.62	14.52	11.21	8.94	7.33	6.13	5.23	4.53	3.98	3.54
15	28.69	20.03	14.82	11.43	9.12	7.46	6.24	5.32	4.60	4.03	3.58
16	29.26	20.42	15.10	11.64	9.28	7.59	6.34	5.40	4.67	4.09	3.63
17	29.79	20.79	15.36	11.84	9.43	7.71	6.44	5.48	4.73	4.14	3.67
18	30.29	21.13	15.61	12.03	9.58	7.83	6.53	5.55	4.79	4.19	3.71
19	30.77	21.46	15.85	12.21	9.72	7.94	6.62	5.62	4.85	4.24	3.75
20	31.23	21.77	16.08	12.38	9.85	8.04	6.70	5.69	4.90	4.28	3.79
21	31.66	22.07	16.29	12.54	9.97	8.14	6.78	5.76	4.96	4.33	3.82
22	32.07	22.35	16.50	12.70	10.09	8.23	6.86	5.82	5.01	4.37	3.86
23	32.47	22.63	16.69	12.85	10.21	8.33	6.93	5.88	5.06	4.41	3.89
24	32.84	22.89	16.88	12.99	10.32	8.41	7.00	5.94	5.11	4.45	3.92
25	33.21	23.13	17.06	13.12	10.43	8.50	7.07	5.99	5.15	4.49	3.95
26	33.56	23.37	17.24	13.26	10.53	8.58	7.14	6.04	5.20	4.52	3.99
27	33.89	23.61	17.41	13.38	10.63	8.66	7.20	6.10	5.24	4.56	4.01
28	34.22	23.83	17.57	13.51	10.72	8.73	7.26	6.15	5.28	4.59	4.04
29	34.53	24.04	17.72	13.62	10.81	8.81	7.32	6.20	5.32	4.63	4.07
30	34.83	24.25	17.88	13.74	10.90	8.88	7.38	6.24	5.36	4.66	4.10
31	35.12	24.45	18.02	13.85	10.99	8.95	7.44	6.29	5.40	4.69	4.13
32	35.41	24.65	18.16	13.96	11.07	9.01	7.49	6.33	5.43	4.72	4.15
33	35.68	24.84	18.30	14.06	11.16	9.08	7.54	6.38	5.47	4.75	4.18
34	35.95	25.02	18.44	14.16	11.23	9.14	7.59	6.42	5.51	4.78	4.20
35	36.21	25.20	18.57	14.26	11.31	9.20	7.64	6.46	5.54	4.81	4.23
36	36.46	25.38	18.69	14.36	11.39	9.26	7.69	6.50	5.57	4.84	4.25
37	36.71	25.55	18.82	14.45	11.46	9.32	7.74	6.54	5.60	4.87	4.27
38	36.95	25.71	18.94	14.54	11.53	9.38	7.78	6.58	5.64	4.89	4.29
39	37.18	25.87	19.05	14.63	11.60	9.43	7.83	6.61	5.67	4.92	4.32
40	37.41	26.03	19.17	14.72	11.67	9.49	7.87	6.65	5.70	4.94	4.34
41	37.63	26.18	19.28	14.80	11.73	9.54	7.92	6.68	5.73	4.97	4.36
42	37.84	26.33	19.39	14.88	11.80	9.59	7.96	6.72	5.76	4.99	4.38
43	38.06	26.48	19.49	14.96	11.86	9.64	8.00	6.75	5.78	5.02	4.40
44	38.26	26.62	19.60	15.04	11.92	9.69	8.04	6.79	5.81	5.04	4.42
45	38.46	26.76	19.70	15.12	11.98	9.74	8.08	6.82	5.84	5.06	4.44
46	38.66	26.89	19.80	15.20	12.04	9.79	8.12	6.85	5.87	5.09	4.46
47	38.86	27.03	19.90	15.27	12.10	9.83	8.16	6.88	5.89	5.11	4.48
48	39.04	27.16	19.99	15.34	12.16	9.88	8.19	6.91	5.92	5.13	4.49
49	39.23	27.29	20.09	15.41	12.21	9.92	8.23	6.94	5.94	5.15	4.51
50	39.41	27.41	20.18	15.48	12.27	9.97	8.26	6.97	5.97	5.17	4.53

Table 5. Number of replicates for experiments in randomized block design, in scenarios formed by the combination of i treatments (i = 3, 4, ..., 50) and d minimal differences between treatment means to be detected as significant at 0.05 probability level by Tukey test, expressed in percentage of the experiment mean (d = 10, 12, ..., 30%), to evaluate millet fresh matter based on optimum plot size (Xo = 4.97 m^2) and coefficient of variation in the optimum plot size (CV_{Xo} = 11.10%)

i	%										
	10	12	14	16	18	20	22	24	26	28	30
3	15.08	10.95	8.48	6.88	5.79	5.00	4.42	3.92	3.40	2.96	2.29
4	17.42	12.46	9.49	7.56	6.24	5.31	4.63	4.10	3.70	3.37	3.10
5	19.28	13.69	10.32	8.15	6.66	5.60	4.82	4.24	3.79	3.43	3.14
6	20.81	14.71	11.03	8.66	7.03	5.87	5.02	4.38	3.88	3.50	3.18
7	22.12	15.59	11.65	9.10	7.36	6.12	5.20	4.51	3.98	3.56	3.23
8	23.26	16.35	12.19	9.50	7.65	6.34	5.37	4.64	4.08	3.63	3.28
9	24.27	17.04	12.68	9.85	7.92	6.54	5.53	4.76	4.17	3.70	3.33
10	25.18	17.65	13.12	10.18	8.17	6.73	5.67	4.87	4.25	3.77	3.38
11	26.01	18.21	13.52	10.47	8.39	6.90	5.81	4.98	4.34	3.83	3.43
12	26.76	18.73	13.89	10.75	8.60	7.07	5.93	5.08	4.42	3.89	3.48
13	27.46	19.20	14.23	11.00	8.79	7.22	6.05	5.17	4.49	3.95	3.52
14	28.11	19.64	14.54	11.24	8.97	7.36	6.16	5.26	4.56	4.01	3.57
15	28.71	20.06	14.84	11.46	9.14	7.49	6.27	5.34	4.63	4.06	3.61
16	29.28	20.44	15.12	11.67	9.30	7.61	6.37	5.42	4.69	4.11	3.65
17	29.81	20.81	15.38	11.86	9.45	7.73	6.46	5.50	4.75	4.16	3.69
18	30.31	21.15	15.63	12.05	9.60	7.85	6.55	5.57	4.81	4.21	3.73
19	30.79	21.48	15.87	12.23	9.73	7.95	6.64	5.64	4.87	4.26	3.77
20	31.24	21.79	16.09	12.40	9.86	8.06	6.72	5.71	4.92	4.30	3.80
21	31.67	22.08	16.30	12.56	9.99	8.15	6.80	5.77	4.97	4.34	3.84
22	32.08	22.37	16.51	12.71	10.11	8.25	6.87	5.83	5.02	4.38	3.87
23	32.48	22.64	16.71	12.86	10.22	8.34	6.95	5.89	5.07	4.42	3.90
24	32.85	22.90	16.89	13.00	10.33	8.42	7.02	5.95	5.12	4.46	3.94
25	33.22	23.14	17.07	13.13	10.44	8.51	7.08	6.00	5.16	4.50	3.97
26	33.56	23.38	17.25	13.27	10.54	8.59	7.15	6.05	5.21	4.53	4.00
27	33.90	23.61	17.41	13.39	10.64	8.67	7.21	6.11	5.25	4.57	4.02
28	34.22	23.84	17.58	13.51	10.73	8.74	7.27	6.16	5.29	4.60	4.05
29	34.54	24.05	17.73	13.63	10.82	8.81	7.33	6.20	5.33	4.64	4.08
30	34.84	24.26	17.88	13.75	10.91	8.88	7.39	6.25	5.37	4.67	4.11
31	35.13	24.46	18.03	13.86	11.00	8.95	7.44	6.30	5.40	4.70	4.13
32	35.41	24.66	18.17	13.96	11.08	9.02	7.50	6.34	5.44	4.73	4.16
33	35.69	24.85	18.31	14.07	11.16	9.08	7.55	6.38	5.48	4.76	4.18
34	35.96	25.03	18.44	14.17	11.24	9.15	7.60	6.42	5.51	4.79	4.21
35	36.21	25.21	18.57	14.27	11.32	9.21	7.65	6.46	5.54	4.82	4.23
36	36.47	25.38	18.70	14.36	11.39	9.27	7.70	6.50	5.58	4.84	4.25
37	36.71	25.55	18.82	14.46	11.46	9.33	7.74	6.54	5.61	4.87	4.28
38	36.95	25.72	18.94	14.55	11.53	9.38	7.79	6.58	5.64	4.90	4.30
39	37.18	25.88	19.06	14.64	11.60	9.44	7.83	6.62	5.67	4.92	4.32
40	37.41	26.03	19.17	14.72	11.67	9.49	7.88	6.65	5.70	4.95	4.34
41	37.63	26.18	19.28	14.81	11.74	9.54	7.92	6.69	5.73	4.97	4.36
42	37.85	26.33	19.39	14.89	11.80	9.59	7.96	6.72	5.76	5.00	4.38
43	38.06	26.48	19.50	14.97	11.86	9.64	8.00	6.76	5.79	5.02	4.40
44	38.27	26.62	19.60	15.05	11.93	9.69	8.04	6.79	5.82	5.04	4.42
45	38.47	26.76	19.70	15.12	11.99	9.74	8.08	6.82	5.84	5.07	4.44
46	38.67	26.90	19.80	15.20	12.04	9.79	8.12	6.85	5.87	5.09	4.46
47	38.86	27.03	19.90	15.27	12.10	9.84	8.16	6.88	5.89	5.11	4.48
48	39.05	27.16	20.00	15.35	12.16	9.88	8.20	6.92	5.92	5.13	4.50
49	39.23	27.29	20.09	15.42	12.21	9.92	8.23	6.95	5.95	5.15	4.52
50	39.42	27.42	20.18	15.49	12.27	9.97	8.27	6.97	5.97	5.17	4.53

differences between plot sizes, showing a mean value of Xo of 4.97, basic experimental units of 1 m^2 (4.97 m^2) and this value was obtained by the mean of the two highest values that did not differ statistically (Table 3). In the studies conducted by Pinho et al. (2013), Priesnitz et al. (2011), Moreira et al. (2003), Jochims et al. (2010) and Pacheco et al. (2014), plots larger than 4.97 m^2 were used, which is the reason why these studies provide reliability to the information in the literature. In future experiments with the millet crop, using the optimum plot size, it is possible to increase experimental precision, reduce costs and labor, and have greater control of the experiment, since it can be conducted in area smaller than that in the previously mentioned studies.

Besides Xo, for the other statistics, except for the first-order spatial autocorrelation coefficient, there were also significant differences between sowing times (Table 3). Therefore, in general, the times influenced the variability of the statistics and experimental planning; as to Xo, it must take into consideration the sowing time.

The number of replicates to evaluate millet fresh matter ranged from 2.79 (three treatments and d = 30%) to 39.41 (50 treatments and d = 10%) (Table 4) in the completely randomized design (CRD) and from 2.29 (three treatments and d = 30%) to 39.42 (50 treatments and d = 10%) (Table 5) in the randomized block design (RBD) in scenarios formed by the combinations of treatments varying from three to 50 and minimal differences between treatments detected as significant at 0.05 probability level by Tukey test, expressed in percentage of the overall mean of the experiment (d = 10, 12, ..., 30%).

Not all the estimated numbers of replicates are operationally viable, due to the high cost for experiment conduction and the demand for time and labor. It should be considered that, the higher the desired precision, the more replicates are necessary. Thus, based on the information generated (Tables 4 and 5) using Xo = 4.97 m^2, the researcher can combine treatments, minimal differences between means of treatments and number of replicates considered as necessary in future experiments.

Since it is not possible to conduct field experiments with 4.53 replicates, it is recommended to use five replicates in CRD and RBD experiments. This number of replicates (r = 5) was also used in experiments in CRD by Pacheco et al. (2014) and RBD by Pinho et al. (2013). Using the equation of the least significant difference of the Tukey test, expressed in percentage of the experiment mean with 50 treatments, five replicates, α = 5% and CV_{Xo} = 11.10%, results in d = 28.65% for CRD and d = 28.66% for RBD; then, it is possible to infer that in order to evaluate millet shoot fresh matter in CRD and RBD with up to 50 treatments, five replicates are sufficient to identify significant differences between treatment means of 28.66% of the experiment mean by Tukey test at 0.05 probability level.

In the studies conducted by Moreira et al. (2003), Priesnitz et al. (2011) and Pinho et al. (2013), approximately 30% of the fresh matter is dry matter. Dry matter is also a parameter widely used and of great importance in animal feeding. Therefore, if this percentage is maintained in all the basic experimental units, it is possible to use the same plot size and number of replicates of the fresh matter for the dry matter.

Conclusions

1. The optimum plot size to evaluate millet shoot fresh matter is 4.97 basic experimental units of 1 m^2 (4.97 m^2) for the three times of sowing and cuts.

2. For the evaluation of up to 50 treatments in completely randomized and randomized block design, five replicates are sufficient to identify as significant, by Tukey test at 0.05 probability level, differences between treatment means of 28.66% of the experiment mean.

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