

Plot size and number of replicates for experiments with forage sorghum

Abstract – The objective of this work was to define the optimal plot size and number of replicates for the evaluation of the fresh weight of sorghum (*Sorghum bicolor*) hybrids. Thirty-two uniformity trials were carried out with two hybrids, in two sowing dates and four evaluation periods. Each trial was divided into 48 basic experimental units (BEUs) of 0.5 m², and fresh weight was determined for each BEU. The mean, variance, coefficient of variation, first-order spatial autocorrelation coefficient, optimal plot size, and coefficient of variation of the optimal plot size were calculated. The number of replicates was determined on the basis of the largest calculated plot size, through an iterative process, for the combinations of number of treatments and differences among means to be detected as significant by Tukey's test, at 5% probability. The optimal plot size ranged from 1.79 to 2.58 m², and the number of replicates from 2.6 (~3) to 49.2 (~50). The optimal plot size is 2.58 m², and five replicates are sufficient to identify as significant the differences between treatment means of 35%.

Index terms: *Sorghum bicolor*, experiment planning, uniformity trials.

Tamanho de parcela e número de repetições para experimentos com sorgo-forrageiro

Resumo – O objetivo deste trabalho foi definir o tamanho ótimo de parcela e o número de repetições para a avaliação da massa de matéria fresca de híbridos de sorgo (*Sorghum bicolor*). Trinta e dois ensaios de uniformidade foram realizados com dois híbridos, em duas datas de semeadura e quatro períodos de avaliação. Cada ensaio foi dividido em 48 unidades experimentais básicas (UEBs) de 0,5 m², e a massa de matéria fresca foi determinada para cada UEB. Calcularam-se a média, a variância, o coeficiente de variação, o coeficiente de autocorrelação espacial de primeira ordem, o tamanho ótimo de parcela e o coeficiente de variação do tamanho ótimo de parcela. O número de repetições foi determinado com base no maior tamanho de parcela, por meio de um processo iterativo, para as combinações de número de tratamentos e de diferenças entre médias a serem detectadas como significativas pelo teste de Tukey, a 5% de probabilidade. O tamanho ótimo de parcela oscilou entre 1,79 e 2,58 m², e o número de repetições entre 2,6 (~3) e 49,2 (~50). O tamanho ótimo de parcela é de 2,58 m², e cinco repetições são suficientes para identificar como significativas as diferenças entre médias de tratamentos de 35%.

Termos para indexação: *Sorghum bicolor*, planejamento experimental, ensaios de uniformidade.

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Introduction

Forage sorghum [*Sorghum bicolor* (L.) Moench] is a C₄ crop widely adapted to different environmental conditions, with high water use efficiency, forage yield, and a less demanding fertility than other agricultural crops (Híbrido BRS 655..., 2009; Borba et al., 2012; Cardoso et al., 2012; Qu et al., 2014; Barcelos et al., 2016; BRS 658..., 2016; Tolentino et al., 2016; Pino & Heinrichs 2017; Manarelli et al., 2019). Among the forage sorghum hybrids available in the market, 'BRS 655' and 'BRS 658' hybrids stand out for their productive stability, resistance to drought and high-quality forage, with high levels of protein and digestibility (Híbrido BRS 655..., 2009; BRS 658..., 2016).

Studies on forage sorghum have been carried out mostly under field conditions (Nascimento et al., 2014; Galvão et al., 2015; Frias et al., 2018; Lima et al., 2017; Guimarães et al., 2018); in these experiments, variations in the use of plot size (6 m² to 28.9 m²), number of replicates (three to five), and the useful evaluation area (three plants to 6.8 m²) are highlighted. These variations indicate a lack of experimental protocols for the crop. In grain sorghum, Lopes et al. (2005) determined the optimal plot size for measuring grain yield and recommended the use of 3.2 m² plots. These authors also indicated that the increase of population density did not contribute to the increase of grain yield, however, it increased the experimental precision.

The definition of experimental protocols for agricultural crops is important for experiments to be carried out with a reliable representation of population pattern, with a known level of error, and without the excessive use of labor and financial and time resources which does not result in large gains in precision. Paranaíba et al. (2009) propose the method of the maximum curvature of the coefficient of variation, to scale the plot size. Cargnelutti Filho et al. (2014) used an iterative process up to convergence, to determine the number of replicates, in scenarios formed by combinations of the number of treatments and the differences among means to be detected as significant by Tukey's test. Although Lopes et al. (2005) have defined the optimal plot size for grain yield in grain sorghum, no studies were found on the optimal plot size for the evaluation of fresh weight in new forage sorghum hybrids.

The objective of this work was to define the optimal plot size and number of replicates for the evaluation of the fresh weight of sorghum hybrids.

Materials and Methods

During the 2016/2017 agricultural harvest, 32 uniformity trials (experiments without treatments) were carried out with two forage sorghum hybrids [*Sorghum bicolor* (L.) Moench], in the experimental area of the Universidade Federal do Pampa, in the municipality of Itaqui (29°09'25"S, 56°33'16"W, at 74 m altitude), in the state of Rio Grande do Sul, Brazil. The climate of the region is a Cfa, according to Köppen-Geiger's classification, and the soil is a Plintossolo Háplico, according to the Brazilian soil classification system (Santos et al., 2018), which corresponds to the Ultisol classification (Soil Survey Staff, 1999). The sowings of the uniformity trials were carried out on November 12, 2016 and on November 14, 2016, using the hybrids 'BRS 655' and 'BRS 658', at 0.5 m spacing between rows and 0.16 m between plants.

Out of the 32 uniformity trials, 16 were sown with 'BRS 655' (eight trials in November 12, 2016, and eight in November 14, 2016), and the other 16 were sown with 'BRS 658' (eight trials in November 12, 2016, and eight in November 14, 2016). Each uniformity trial was composed of six sowing rows measuring 8 m long, at 0.5 m spacing between rows, totaling 24 m². To evaluate the fresh weight, the uniformity trials were divided into 48 basic experimental units (BEUs) of 0.5 m² (1.0 m long sowing row with six plants). Out of the eight uniformity trials for each hybrid and sowing date, two were separated for each cut date or evaluation period (75, 86, 96, and 140 days after sowing, for the first sowing date, and 74, 85, 95, and 144 days after sowing for the second sowing date). As forage sorghum can be managed at different times, the evaluation was performed for two trials at each evaluation time, to ensure that the experimental design was representative within the growth and development stages of the crop. The growth stages were considered between the final leaf visible in whorl (stage 4) and the physiological maturity (maximum dry matter accumulation – stage 9), according to Vanderlip & Reeves (1972). The experimental area was 768 m² (evaluations were performed for two hybrids × two

sowing dates $\times$ eight uniformity trials per hybrid and sowing date $\times$ 48 BEUs of 0.5 m²).

Sowing was carried out manually with two seeds per point and subsequent thinning, to guarantee the population of 125,000 plants ha⁻¹ during the evaluations, and a density close to that recommended for the two hybrids, which is 120,000 plants ha⁻¹ (Híbrido BRS 655..., 2009; BRS 658..., 2016). Basic fertilization was carried out uniformly with 200 kg ha⁻¹ N-P₂O₅-K₂O of the formulation 05-20-20, which is in accordance with the soil analysis and recommendations for the crop. In addition, two cover fertilizations were carried out, one with 90 kg ha⁻¹ N, applied when plants had from 4 to 6 expanded leaves, and the other with 100 kg ha⁻¹ N, applied when plants had from 6 to 9 expanded leaves.

To determine the fresh weight, plants in each BEU were cut close to the soil surface and weighed immediately. For each uniformity trial of the fresh weight of 48 BEUs, the mean (represented by m , in g), variance (s^2), first-order spatial autocorrelation coefficient (ρ , obtained in the rows direction), coefficient of variation of the trial (CV_{trial} , %), optimal plot size (X_o , in BEU and m²), and the coefficient of variation in the optimal plot size (CV_{X_o} , %) were determined, using the equations described by Paranaíba et al. (2009) and applied by Cargnelutti Filho et al. (2014), as follows:

$$X_o = \left(10 \sqrt[3]{2(1-\rho^2)s^2m} \right) / m, \text{ and} \\ CV_{X_o} = \left(\sqrt{(1-\rho^2)s^2 / m^2} \right) / \sqrt{X_o} \times 100.$$

Next, based on the largest calculated plot size, the number of replicates was determined (in scenarios formed by the combinations of i treatments ($i = 3, 4, \dots, 100$) and d minimum differences among the treatment means to be detected as significant at 5% probability, by Tukey's test, expressed as a percentage of the experiment mean ($d = 10\%, 15\%, \dots, 35\%$), through an iterative process up to convergence, as detailed by Cargnelutti Filho et al. (2014). Thus, the number of replicates was estimated through the following equation:

$$D = \left(q_{\alpha(i; DFE)} \sqrt{EMS / r} \right) / m \times 100,$$

in which: $q_{\alpha(i; DFE)}$ is the critical value of Tukey's test, at level α probability ($\alpha = 0.05$, in this study); i is the number of treatments; DFE is the number of degrees of freedom of the error, which is $[i(r-1)]$ for the completely randomized design, and $[(i-1)(r-1)]$

for the randomized complete block design; EMS is the error mean square; r is the number of replicates; and m is the mean of the experiment. By replacing the expression of the experimental coefficient of variation ($CV = \sqrt{EMS} / m \times 100$), in percentage, in the expression for the calculation of d , and isolating r , the following expression is obtained:

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

In the present study, CV is expressed as a percentage and corresponds to the CV_{X_o} , since this is the CV expected for an experiment with the optimal plot size (X_o) determined. The number of replicates (r) was determined for experiments with completely randomized design and randomized complete block design, through an iterative process up to convergence, using the CV_{X_o} . Statistical analyses were performed with Microsoft Office Excel and R software (R Core Team, 2020).

Results and Discussion

There was no difference between the evaluated sorghum hybrids for any of the statistics used (Table 1). The mean of fresh weight per BEU was 2,864.59 g, and uniformity trials ranged from 1,934.16 g to 4,009.14 g per BEU. The 'BRS 655' hybrid produced a mean of 2,983.82 g per BEU and, according to Híbrido BRS 655... (2009), this hybrid produces 50.0 Mg ha⁻¹ mean fresh weight. 'BRS 658' produced mean of 2,745.37 g per BEU and, according to BRS 658... (2016), the mean this hybrid also produces 50.0 Mg ha⁻¹ fresh weight. Considering that the experimental area is located in a marginal region for the forage sorghum cultivation, under a "Plintossolo Háplico" (Ultisol) with low-capacity of water infiltration, it is possible to verify the quality in the conduction of the uniformity trials, to generate a higher fresh weight than the indicated by the developers of these hybrids (Híbrido BRS 655..., 2009; BRS 658..., 2016).

The CV was high, according to the classification by Pimentel-Gomes (2009), in most of the 32 trials, and it ranged from 15.22% to 27.53% with an average of 21.83%, that is, using plots of 0.5 m², the trials showed low precision, in most of the evaluated conditions (Table 1). This fact evidences the need to use larger plots. Additionally, the value of the first-

order spatial autocorrelation coefficient (ρ) oscillated between -0.05 and 0.61, with a mean value ($\rho = 0.24$) close to zero. According to Paranaíba et al. (2009) and Cargnelutti Filho et al. (2014), ρ values close to zero indicate less spatial autocorrelation and, consequently,

the need of a larger plot size. The optimal plot size (X_o) ranged from 1.79 to 2.58 m² (Table 1). The mean value of $X_o = 2.20$ m² was close to the values of the two hybrids, that is, $X_o = 2.25$ m² for 'BRS 655', and $X_o = 2.16$ m² for 'BRS658'. Using the optimal plot

Table 1. First order spatial autocorrelation coefficient (ρ), variance (s^2), mean (g/basic experimental unit of 0.50 m²), trial coefficient of variation (CV, %), optimal plot size (X_o , in basic experimental unit of 0.50 m² and in m²), and the coefficient of variation in the optimal plot size (CV_{X_o} , %) for the fresh weight of two forage sorghum (*Sorghum bicolor*) hybrids sown in two dates and evaluated in days after sowing (DAS).

Sowing date	Trial ⁽¹⁾	DAS	Growth stage ⁽²⁾	ρ	s^2	Mean (g)	CV (%)	X_o (BEU)	X_o (m ²)	CV_{X_o} (%)
Cultivar BRS 655										
11/12/2016	1	75	5	0.17	275278	2,801.68	18.73	4.08	2.04	9.13
11/12/2016	2	75	5	-0.05	548858	3,330.36	22.25	4.62	2.31	10.33
11/12/2016	3	86	5	-0.03	682281	4,009.14	20.60	4.39	2.20	9.82
11/12/2016	4	86	5	0.31	977640	3,591.57	27.53	5.15	2.58	11.52
11/12/2016	5	96	7	0.26	382017	3,586.94	17.23	3.81	1.90	8.52
11/12/2016	6	96	7	0.04	537707	3,828.04	19.16	4.18	2.09	9.36
11/12/2016	7	140	9	0.50	621121	3,219.45	24.48	4.47	2.24	10.00
11/12/2016	8	140	9	0.41	741013	3,239.06	26.58	4.90	2.45	10.95
11/14/2016	9	74	4	0.29	241857	1,934.16	25.43	4.91	2.46	10.99
11/14/2016	10	74	4	0.00	238053	2,251.42	21.67	4.55	2.27	10.16
11/14/2016	11	85	5	0.01	357969	2,928.51	20.43	4.37	2.19	9.77
11/14/2016	12	85	5	0.09	362521	2,907.86	20.71	4.40	2.20	9.83
11/14/2016	13	95	6	0.08	388594	2,598.28	23.99	4.85	2.43	10.85
11/14/2016	14	95	6	0.26	377757	2,521.73	24.37	4.80	2.40	10.74
11/14/2016	15	144	9	0.30	295103	2,430.64	22.35	4.49	2.25	10.04
11/14/2016	16	144	9	0.43	236261	2,562.23	18.97	3.89	1.94	8.69
Mean – BRS 655				0.19a ⁽³⁾	454002a	2,983.82a	22.15a	4.49a	2.25a	10.05a
Cultivar BRS 658										
11/12/2016	1	75	5	0.42	547928	2,819.65	26.25	4.84	2.42	10.82
11/12/2016	2	75	5	0.10	182731	2808.27	15.22	3.58	1.79	8.00
11/12/2016	3	86	5	0.16	405236	3,355.62	18.97	4.12	2.06	9.22
11/12/2016	4	86	5	0.26	642964	3,270.12	24.52	4.82	2.41	10.78
11/12/2016	5	96	7	0.37	561210	3,117.35	24.03	4.64	2.32	10.38
11/12/2016	6	96	7	0.37	424333	3,196.11	20.38	4.15	2.08	9.28
11/12/2016	7	140	9	0.38	286028	2,702.34	19.79	4.06	2.03	9.08
11/12/2016	8	140	9	0.48	414634	2,738.49	23.51	4.39	2.20	9.82
11/14/2016	9	74	4	0.61	244073	2,057.74	24.01	4.17	2.09	9.33
11/14/2016	10	74	4	0.19	283193	2,507.51	21.22	4.43	2.21	9.90
11/14/2016	11	85	5	0.06	338859	2,982.29	19.52	4.23	2.12	9.47
11/14/2016	12	85	5	0.09	358622	3,000.62	19.96	4.29	2.15	9.59
11/14/2016	13	95	6	0.43	473842	2,543.82	27.06	4.92	2.46	11.00
11/14/2016	14	95	6	0.10	153299	2,355.47	16.62	3.80	1.90	8.49
11/14/2016	15	144	9	0.21	129546	2,158.36	16.68	3.76	1.88	8.40
11/14/2016	16	144	9	0.38	367897	2,312.23	26.23	4.90	2.45	10.96
Mean – BRS 658				0.29a	363400a	2,745.37a	21.50a	4.32a	2.16a	9.66a
Overall Mean				0.24	408701	2,864.59	21.83	4.41	2.20	9.85

⁽¹⁾Each uniformity trial of 3 m × 8 m (24 m²) was divided into 48 basic experimental units (BEUs) of 0.5 m × 1 m (0.50 m² – 1 row × 1.0 m), forming a matrix of 6 rows and 8 columns. ⁽²⁾Growth stages defined by Vanderlip & Reeves (1972), as follows: 4, final leaf visible in whorl; 5, boot, head extended into flag leaf sheath; 6, half-bloom (half of the plants at some stage of bloom); 7, soft dough; and, 9, physiological maturity (maximum dry matter accumulation). ⁽³⁾For each statistics (ρ , s^2 , m, CV, X_o , and CV_{X_o}), the means followed by equal letters, in the columns (comparison of means between the forage sorghum hybrids), do not differ by Student's t-test (bilateral), at 5% probability, for independent samples.

size, the corresponding CV went down; and, through the classification by Pimentel-Gomes (2009), most uniformity trials showed an overall value of 9.85%, being 10.05% for 'BRS 655' and 9.66% for 'BRS 658'.

As there was no difference between hybrids for any of the statistics used, the overall optimal size (2.20 m²) and the corresponding CV (9.85%) could be used in the calculation of the number of replicates. However, the highest values of these statistics among the 32 uniformity trials were used, that is, $X_o = 2.58$ m² and CV_{X_o} of 11.52%, as in the trial 4 of the 'BRS 655' hybrid (Table 1). The use of the highest values of X_o and CV_{X_o} allows a safe recommendation for the optimal plot size and number of replicates – even in trials with less precision –, increasing the reliability of the results obtained in such trials.

The number of replicates ranged between 2.6 (~3) and 49.2 (~50), depending on the experimental design, number of treatments, and the d minimum differences among means of treatments to be detected as significant (Figure 1). In general, the number of replicates was similar between the two experimental designs (completely randomized design and randomized complete block design). The number of replicates augmented with the increase of the number of treatments and with the reduction of the d minimum differences, that is, with the increase of precision, as already described by Cargnelutti Filho et al. (2014). For the highest precision level ($d = 10\%$) and the largest number ($i = 100$) of treatments, $49.2 \approx 50$ replicates would be necessary. This is unfeasible from a practical point of view, since very large agricultural experiments (with many treatments and replicates) occupy large areas and, usually, they show greater variability that makes it difficult to detect significant differences between treatments. Therefore, such numbers of treatments and replicates should be avoided, in order to minimize experimental variability. However, at the lowest level of precision ($d = 35\%$) and the lowest number of treatments ($i = 3$), three replicates would be sufficient.

In total, 1176 scenarios were formed (combination of 2 experimental designs $\times$ 98 possible number of treatments $\times$ 6 levels of precision), and the corresponding number of replicates (Figure 1). Given these scenarios, it is up to the researcher to select the number of replicates that contemplates the chosen experimental design, the number of treatments,

and the desired precision for the research. If six replicates are used, experiments with up to 5, 25, and 100 treatments can be conducted for d minimum differences respectively of 20%, 25%, and 30%. If five replicates are used, experiments with up to 11, 57, and 100 treatments can be conducted, respectively, for d minimum differences of 25%, 30%, and 35%. When four replicates are chosen, experiments with up to 3, 18, and 86 treatments can be conducted for d minimum differences respectively of 25%, 30%, and

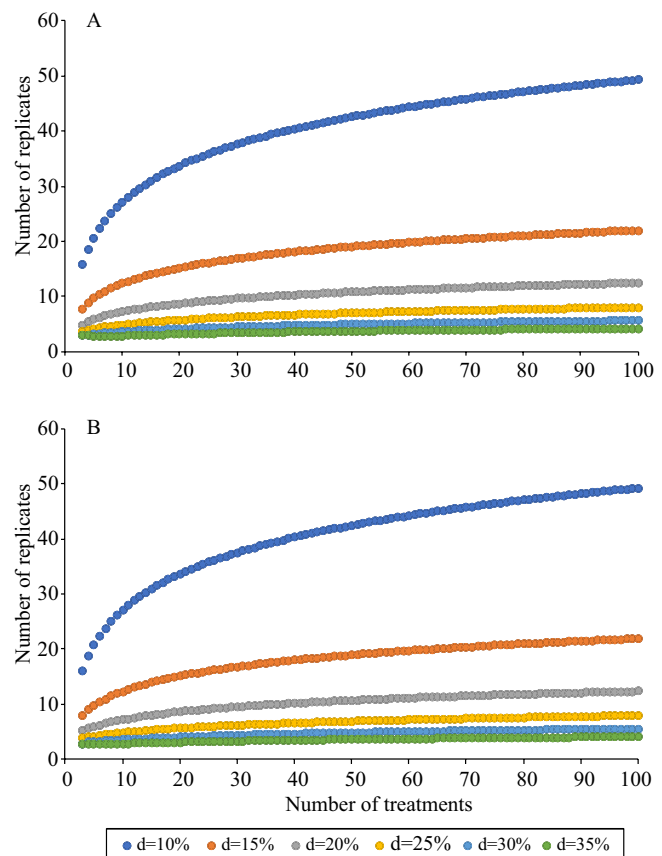


Figure 1. Number of replicates in the experiments to evaluate the fresh weight of forage sorghum (*Sorghum bicolor*) hybrids ('BRS 655' and 'BRS 658') from optimal plot size ($X_o = 2.58$ m²) and coefficient of variation in optimal plot size ($CV_{X_o} = 11.52\%$). The experiments were carried out in completely randomized design (A) and randomized complete block design (B), in scenarios formed by combinations of i treatments ($i = 3, 4, \dots, 100$) and d least differences among treatment means to be detected as significant at 5% probability, by Tukey's test, expressed in percentage of the overall experimental mean ($d = 10, 15, \dots, 35\%$).

35%. Finally, the use of only three replicates would be recommended for up to 3 and 15 treatments for a minimum differences respectively of 30% and 35%. However, it is important to highlight that the final number of plots (treatments $\times$ replicates) should be greater than 20, considering the interference of the number of degrees of freedom of the error with the estimate of the mean squared error, of the F-statistics, in the analysis of variance, and with the values of the least significant difference in mean-comparison tests.

Conclusion

The optimal plot size for the fresh weight evaluation of the 'BRS 655' and 'BRS 658' forage sorghum (*Sorghum bicolor*) hybrids is 2.58 m², and five replicates are sufficient to identify significant differences between treatment means of 35%.

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