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CROP PRODUCTION

Plot size and replications number for triticale experiments

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ABSTRACT: *The hybridization between wheat and rye crops resulted in the triticale crop, which presents rusticity, versatility in animal and human food and possibility of use as a cover plant. The objective of this research was to determine the optimal plot size and the replications number to evaluate the fresh weight of triticale in two evaluation moments. An experiment was carried out with the triticale cultivar IPR111. The experimental area was divided into 48 uniformity trials, each containing 36 basic experimental units of 0.51 m². The fresh weight was evaluated in 24 uniformity trials at 99 days after sowing (DAS) and in 24 uniformity trials at 127 DAS. The optimal plot size was determined by the method of the maximum curvature of the coefficient of variation and the replications number was determined in scenarios of treatments number and differences between means to be detected as significant by Tukey test. To determine the fresh weight of triticale, the optimal plot size is 3.12 m², with coefficient of variation of 13.69%. Six replications are sufficient to identify as significant, differences between treatment means of 25% for experiments with up to seven treatments and of 30% for experiments with up to 28 treatments, regardless of the experimental design.*
Key words: × *Triticosecale Wittmack, uniformity trials, experiment planning.*

Tamanho de parcela e número de repetições para experimentos de triticale

RESUMO: *O cruzamento das culturas de trigo e centeio resultou na obtenção da cultura do triticale, que apresenta rusticidade, versatilidade na alimentação animal e humana e possibilidade de uso como planta de cobertura. O objetivo deste trabalho foi determinar o tamanho ótimo de parcela e o número de repetições para avaliar a massa verde de triticale em dois momentos de avaliação. Foi conduzido um experimento com a cultivar de triticale IPR111, sendo a área experimental dividida em 48 ensaios de uniformidade, cada ensaio contendo 36 unidades experimentais básicas de 0,51 m². A massa verde foi avaliada em 24 ensaios aos 99 dias após a semeadura (DAS) e em 24 ensaios aos 127 DAS. O tamanho ótimo de parcela foi determinado pelo método da máxima curvatura do coeficiente de variação e o número de repetições foi determinado em combinações de número de tratamentos e de diferenças entre médias a serem detectadas como significativas pelo teste de Tukey. Para determinar a massa verde de triticale, o tamanho ótimo de parcela é de 3,12 m² com coeficiente de variação de 13,69%. Seis repetições são suficientes para identificar como significativas, diferenças entre médias de tratamentos de 25% para experimentos com até sete tratamentos e de 30% para experimentos com até 28 tratamentos, independentemente do delineamento experimental.*
Palavras-chave: × *Triticosecale Wittmack, ensaios de uniformidade, planejamento experimental.*

The hybridization between wheat (*Triticum* sp.) and rye (*Secale* sp.), aiming to combine the growth ability under unfavorable conditions of rye with the food use versatility of wheat, resulted in the origin of the triticale (× *Triticosecale* Wittmack - ZHU, 2018). Due to high protein concentration, the triticale has potential for use in animal feed, as fodder, silage and grain and in human diets as a complementary cereal in several foods and beverages (SUCU & ÇIFCI, 2016; ZHU, 2018). Furthermore, according to ALBRECHT et al. (2018), triticale is one of the most used species of cover crops in the western region of Parana State.

In experiments with triticale, several combinations between plot sizes and replications

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number were used, for example, plots of 6 m^2 to 16.8 m^2, evaluation areas in plots from 0.25 m^2 to 1.2 m^2 and three to four repetitions (SUCU & ÇIFCI, 2016; ALBRECHT et al., 2018). The lack of experimental protocols indicating the optimal plot size and number of replications, may result in experiments with low reliability or requiring excessive use of human, financial and time resources. Therefore, the objective of this research was to determine the optimal plot size and the replications number to evaluate the fresh weight of triticale in two evaluation moments.

An experiment was carried out with triticale cultivar IPR111 in the experimental area situated in latitude 29º 09' 25" S, longitude 56º 33' 16" W and altitude of 74 m, with Cfa climate and soil classified as "Haplic Plinthosol" (SANTOS et al., 2013). The seeding procedure was performed on June 3, 2016, with spacing of 0.17 m between rows and final plant population in the harvest of 1294117 plants ha^{-1}. Basic fertilization was carried out with 20 kg ha^{-1} of N, 80 kg ha^{-1} of P_2O_5 and 80 kg ha^{-1} of K_2O and two topdressing fertilizations were performed with 40 kg ha^{-1} of N in each one.

The useful area of the experiment was divided into 48 uniformity trials. Each uniformity trial of size 6 m × 3.06 m (18.36 m^2), was divided into 36 basic experimental units (BEU) of 1.0 m × 0.51 m (0.51 m^2 - one meter × three rows), forming a matrix of six rows and six columns. The fresh weight was evaluated in each BEU of 24 uniformity trials at 99 days after sowing (DAS) and in each BEU of 24 uniformity trials at 127 DAS, respectively, in anthesis completed and soft dough stages, as described by ZADOKS et al. (1974). Plants were cut near the soil surface and the fresh weight determined in grams per 0.51 m^2.

For each uniformity trial with fresh weight of 36 BEU, were determined the first-order spatial autocorrelation coefficient (obtained in the rows direction), variance, mean, variation coefficient of the trial, optimal plot size and the variation coefficient in the optimal plot size, using the equations described by PARANAÍBA et al. (2009) and applied by CARGNELUTTI FILHO et al. (2014). Next, the number of replications was determined in scenarios formed by combinations of i treatments (i = 3, 4, ..., 100) and d minimum differences among means of treatments to be detected as significant at 5% probability by Tukey test, expressed as a percentage of experiment mean (d = 10%, 15%, ..., 40%), by iterative process until convergence, as detailed and applied by CARGNELUTTI FILHO et al. (2014). Statistical analyzes were performed with Microsoft Office Excel® and software R (R DEVELOPMENT CORE TEAM, 2020).

There were no significant differences between the two evaluation moments (99 and 127 DAS) for the variables first-order spatial autocorrelation coefficient and mean (Table 1). For the variation coefficient of the trial, the optimal plot size and the variation coefficient in the optimal plot size, higher values were observed in the first evaluation moments, indicating greater variability between BEU in anthesis completed stage in relation to the soft dough stage. Among the 48 uniformity trials, the fresh weight ranged from 684.73 g to 1178.21 g per BEU (13426 kg ha^{-1} to 23102 kg ha^{-1}), with a overall mean of 904.93 g per BEU (17744 kg ha^{-1}). In the evaluation of forage yields at the dough stage in triticale lines, SUCU & ÇIFCI (2016) obtained forage production of up to 44.28 t ha^{-1} of fresh weight. Several genetic and management factors may explain the lower values of fresh weight in the present study, but the main one is related to the condition of lowland soils (Haplic Plinthosol), susceptible to waterlogging in which the experiment was conducted. Conversely, using the same cultivar as the present study, ALBRECHT et al. (2018) obtained lower fresh weight values in two-years experiments (5655 kg ha^{-1} and 7145 kg ha^{-1}), perhaps by the use of larger spacing.

The variation coefficient of the trial ranged from 13.56% to 34.21% (Table 1), with a mean value of 21.30% and the optimal plot size ranged from 1.61 m^2 to 3.12 m^2, with a mean value of 2.15 m^2 (Table 1). The variation coefficient in the optimal plot size ranged from 7.04% to 13.69% among the 48 uniformity trials, with a mean value of 9.44%, considered low by PIMENTEL-GOMES (2009). In order to ensure greater reliability in the experimental design, the highest optimal plot size among the 48 uniformity trials (3.12 m^2) was recommended for use in the evaluation of the fresh weight of triticale in anthesis completed and soft dough stages. The correspondent variation coefficient in the optimal plot size (13.69%), was used to calculate the number of replications for experiments in the completely randomized design and in the complete randomized block design.

The replications number varied between 2.7 and 69.5 (Figures 1A, B), with similar behavior for completely randomized and complete randomized block designs. The replications number increases with increasing number of treatments and of desired precision, i.e., with the lowest level of d (d = 10%), as expected and described in the literature (CARGNELUTTI FILHO et al., 2014). The number

Table 1 - First order spatial autocorrelation coefficient (ρ), variance (s^2), mean (m, in grams per basic experimental unit of 0.51 m^2), trial coefficient of variation (CV, in %), optimal plot size (Xo, in BEU of 0.51 m^2 and in m^2) and the coefficient of variation in the optimal plot size (CV_{Xo}, in %) for the fresh weight of triticale cultivar IPR111 in two evaluation moments (99 and 127 days after sowing).

DAS	Trial[1]	ρ	s^2	m (g)	CV (%)	Xo (BEU)	Xo (m^2)	CV_{Xo}(%)
99	1	0.43	112042.53	1048.17	31.93	5.50	2.81	12.30
99	2	-0.02	62451.56	859.91	29.06	5.53	2.82	12.36
99	3	0.00	53732.22	991.92	23.37	4.78	2.44	10.69
99	4	0.19	40932.75	864.54	23.40	4.73	2.41	10.57
99	5	0.26	39842.81	893.89	22.33	4.53	2.31	10.13
99	6	0.27	37274.89	863.12	22.37	4.52	2.31	10.11
99	7	0.11	28562.71	784.96	21.53	4.51	2.30	10.08
99	8	0.22	22868.68	891.59	16.96	3.80	1.94	8.50
99	9	0.63	33974.87	781.39	23.59	4.06	2.07	9.07
99	10	0.49	83221.06	925.56	31.17	5.29	2.70	11.83
99	11	0.48	46361.95	897.78	23.98	4.45	2.27	9.96
99	12	0.14	90843.89	880.91	34.21	6.12	3.12	13.69
99	13	0.28	69719.30	787.31	33.54	5.92	3.02	13.23
99	14	0.47	39477.58	814.58	24.39	4.53	2.31	10.14
99	15	0.48	36362.59	851.83	22.39	4.25	2.17	9.50
99	16	0.57	46891.67	689.58	31.40	5.09	2.60	11.39
99	17	0.08	37603.26	787.13	24.64	4.94	2.52	11.05
99	18	0.27	28483.60	684.73	24.65	4.83	2.46	10.79
99	19	0.40	50603.57	716.96	31.38	5.48	2.80	12.25
99	20	0.22	40985.31	1066.05	18.99	4.09	2.09	9.16
99	21	0.50	31180.30	915.73	19.28	3.82	1.95	8.53
99	22	-0.13	28463.63	1178.21	14.32	3.43	1.75	7.67
99	23	0.72	82776.31	928.20	31.00	4.52	2.30	10.10
99	24	0.32	34497.41	994.01	18.69	3.98	2.03	8.89
Mean – 99DAS	-	0.31a[2]	49131.44a	879.09a	24.94a	4.70a	2.39a	10.50a
127	1	0.40	23454.60	955.44	16.03	3.51	1.79	7.85
127	2	0.15	18318.79	910.05	14.87	3.51	1.79	7.85
127	3	0.56	22577.12	904.50	16.61	3.36	1.71	7.51
127	4	-0.01	22646.84	989.98	15.20	3.59	1.83	8.02
127	5	0.39	19484.21	1029.68	13.56	3.15	1.61	7.04
127	6	0.62	32430.10	897.52	20.06	3.67	1.87	8.21
127	7	0.44	60728.30	963.81	25.57	4.72	2.41	10.56
127	8	0.46	53637.44	1157.45	20.01	3.98	2.03	8.90
127	9	0.50	28626.97	1107.80	15.27	3.28	1.67	7.33
127	10	0.36	21798.58	943.72	15.64	3.49	1.78	7.81
127	11	0.44	20988.26	962.19	15.06	3.31	1.69	7.41
127	12	-0.07	27793.21	870.82	19.14	4.18	2.13	9.34
127	13	0.19	16580.49	877.05	14.68	3.46	1.77	7.75
127	14	0.56	34671.78	887.39	20.98	3.92	2.00	8.76
127	15	0.23	23939.28	756.72	20.45	4.29	2.19	9.60
127	16	0.29	33785.18	931.22	19.74	4.15	2.11	9.27
127	17	0.45	24691.31	985.84	15.94	3.43	1.75	7.68
127	18	0.23	23233.13	1028.53	14.82	3.46	1.77	7.74
127	19	0.52	42573.39	1008.68	20.46	3.94	2.01	8.80
127	20	0.16	33289.96	789.34	23.11	4.70	2.40	10.51
127	21	0.35	23316.17	836.07	18.26	3.89	1.98	8.69
127	22	0.13	24218.87	865.83	17.97	3.99	2.03	8.92
127	23	0.33	14526.17	768.15	15.69	3.53	1.80	7.89
127	24	0.32	18453.17	910.71	14.92	3.42	1.74	7.65
Mean – 127DAS	-	0.33a	27740.14b	930.77a	17.67b	3.75b	1.91b	8.38b
Overall Mean	-	0.32	38435.79	904.93	21.30	4.22	2.15	9.44

[1] Each uniformity trial of 6 m × 3.06 m (18.36 m^2), was divided into 36 basic experimental units (BEU) of 1.0 m × 0.51 m (0.51 m^2 - one meter × three rows), forming a matrix of six rows and six columns. [2] For each statistic (ρ, s^2, m, CV, Xo and CV_{Xo}), the means not followed by the same letter in the column (comparison of means between the evaluation days) differ at 5% significance by Student's t-test (bilateral) for independent samples.

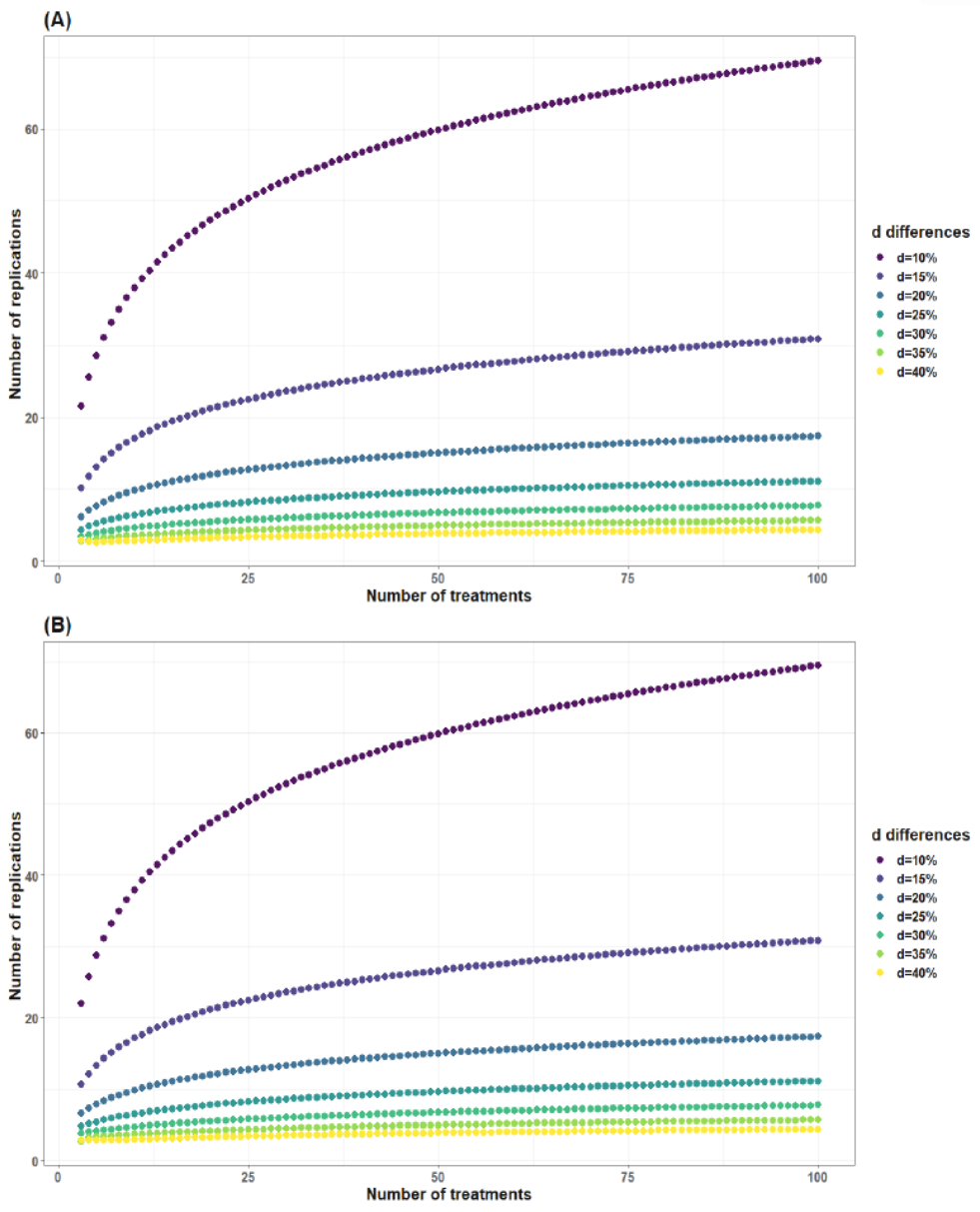

Figure 1 - Number of replications for experiments in the completely randomized design (A) and complete randomized block design (B), in scenarios formed by combinations of i treatments (i = 3, 4, ..., 100) and d least differences between treatment means to be detected as significant at 5% probability by Tukey test, expressed in percentage of the overall experimental mean (d = 10, 15, ..., 40%), to evaluate the fresh weight of triticale from optimal plot size (Xo = 3.12 m^2) and coefficient of variation in optimal plot size (CV_{Xo} = 13.69%).

of replications to the triticale fresh weight evaluation was presented in 1372 conditions (combination of 98 possible numbers of treatments × seven levels of d minimum differences among means × two experimental designs), allowing the reader/researcher to identify the number of replications recommend for each case. For example, if the researcher uses six replications, he can identify as significant, differences between treatment means of 25% for experiments with up to seven treatments, of 30% for experiments with

up to 28 treatments and of 35% for experiments with up to 100 treatments, regardless of the experimental design used. In black oats, CARGNELUTTI FILHO et al. (2014) recommended plots of 4.14 m^2 and four replications to identify as significant differences between treatment means of 26.7%.

In an experiment of triticale conducted by SUCU & ÇIFCI (2016) in Bursa, Turkey, plots of 6 m^2 and three replications were used in a complete randomized block design. However, for the evaluation of forage yields at the dough stage, forage samples were harvested in an area of 1.2 m^2. For experiments with triticale and six other species of cover crops, ALBRECHT et al. (2018) used a complete randomized block design with four replications and plot of 16.8 m^2 . However, for the evaluation of fresh and dry matter, they used a sample are of 0.25 m^2. In conclusion, to determine the fresh weight of triticale, the optimal plot size is 3.12 m^2, with coefficient of variation of 13.69%. Six replications are sufficient to identify as significant, differences between treatment means of 25% for experiments with up to seven treatments and of 30% for experiments with up to 28 treatments, regardless of the experimental design used.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

MT designed and supervised the experiment. ACM, RRS, FSS, LSS and AS performed the experiments and data collection. ACF performed the statistical analyses. MT prepared the draft of the manuscript. All authors critically revised the manuscript and approved of the final version.

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