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Statistics/ Original Article

Sampling sufficiency for estimating the mean of wheat traits

Abstract – The objective of this work was to determine the sample size necessary for estimating the means of wheat (*Triticum aestivum*) traits, obtained through measurement, counting, and weighing. Seventeen uniformity trials were performed with 1,790 plants harvested randomly, whose following traits were evaluated: lengths of the main stem and main stem ear (measurement); number of leaves, stems, and ears (counting); and mass of fresh and dry matter of leaves, stems, and ears (weighing). The Bartlett and Kolmogorov-Smirnov tests and Welch's analysis of variance were performed. Skewness, central tendency, and variability were determined, and sample size was calculated to estimate the means of the 13 evaluated traits, considering estimation errors (semi-amplitudes of the 95% confidence interval) equal to 5, 10, 15, and 20% of the mean. There is a decrease in the sample size to estimate the means of wheat traits obtained through weighing, counting, and measuring, in this order. In an experiment to estimate the mean of wheat traits obtained by weighing, counting, and measuring with a maximum error of 10% of the mean at a 95% confidence interval, 117, 76, and 9 plants per treatment are needed, respectively.

Index terms: *Triticum aestivum*, experimental precision, uniformity trial.

Suficiência amostral para estimar a média de caracteres de trigo

Resumo – O objetivo deste trabalho foi determinar o tamanho de amostra necessário para estimar a média de caracteres de trigo (*Triticum aestivum*) obtidos por meio de medição, contagem e pesagem. Dezessete ensaios de uniformidade foram feitos com 1,790 plantas colhidas aleatoriamente, cujos seguintes caracteres foram avaliados: comprimentos do colmo principal e da espiga do colmo principal (medição); número de folhas, colmos e espigas (contagem); e massa de matéria fresca e seca de folhas, colmos e espigas (pesagem). Realizaram-se os testes de Bartlett e Kolmogorov-Smirnov e a análise de variância de Welch. Determinaram-se as medidas de assimetria, tendência central e variabilidade, e calculou-se o tamanho de amostra para a estimação da média dos 13 caracteres avaliados, tendo-se considerado erros de estimação (semiamplitudes do intervalo de confiança de 95%) iguais a 5, 10, 15 e 20% da média. Há um decréscimo do tamanho de amostra para estimar a média dos caracteres de trigo obtidos por meio de pesagem, contagem e medição, nesta ordem. Em um experimento para estimar a média dos caracteres de trigo obtidos por pesagem, contagem e medição com erro máximo de 10% da média e nível de confiança de 95%, são necessárias 117, 76 e 9 plantas por tratamento, respectivamente.

Termos para indexação: *Triticum aestivum*, precisão experimental, ensaio de uniformidade.

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Introduction

Wheat (*Triticum aestivum* L.) belongs to the Poaceae family and is one of the main food crops cultivated in various environments and geographical regions, with relevance in diet, due to the quantity and quality of its protein and diversity of derived products (Borém & Scheeren, 2015). Because of wheat importance, the experiments with this crop should be planned properly, prioritizing the obtaining of high experimental precision (low coefficient of variation) and, consequently, reliability in the inferences on the evaluated treatments. In experiments conducted in the field, there are several traits that can be evaluated by measuring, counting, and weighing. When evaluating a trait, it is common to observe variation between plants, even between those subjected to the same treatment. Therefore, it is essential to size the number of plants under evaluation, to obtain reliable information on the trait to be estimated (Storck et al., 2016).

Sample sizing can be performed from data obtained in uniformity trials (experiments without treatment). It is important to perform uniformity trials in various scenarios, such as the combination of agricultural years, sowing dates, and cultivars. These scenarios enable plants to develop under different environmental conditions, expanding their variability. With these databases, it is possible to size a representative sample size of scenarios with wide variability.

For wheat crop, studies on sampling sufficiency have been conducted which demonstrated variation in sample size between regions of adaptation and Brazilian states as to estimate the means of gluten strength (Castro et al., 2016), between characteristics of severity of yellow spot and the area under the disease progress curve (Sari et al., 2020) and between instruments used to determine the hectoliter mass (Martin et al., 2022).

For other species of the Poaceae family, such as sorghum (Silva et al., 2005), maize (Toebe et al., 2014; Wartha et al., 2016), black oat (Cargnelutti Filho et al., 2015), millet (Kleinpaul et al., 2017) and rye (Bandeira et al., 2018a, 2018b, 2019), sample sizing studies were also conducted to estimate the mean of traits. For these species, the authors showed that, to estimate the mean with the same precision, there was variation of sample size between traits. In addition, for the same trait, there was variation in sample size between genotypes (Toebe et al., 2014; Wartha et al., 2016; Bandeira et al., 2018a, 2018b, 2019), sowing times (Bandeira et al., 2018a, 2018b, 2019), evaluation times (Cargnelutti Filho et al., 2015; Kleinpaul et al., 2017; Bandeira et al., 2018b), and agricultural years (Toebe et al., 2014).

The inclusion of sample sizing studies is assumed to aggregate important information to support the planning of experiments with better precision and, consequently, with greater reliability in the results.

The objective of this work was to determine the sample size necessary for estimating the means of wheat traits, obtained through measurement, counting, and weighing.

Materials and Methods

Seventeen uniformity trials (experiments without treatments) with wheat were conducted in an experimental area (29º42'S, 53º49'W, at 95 m altitude). In this site, the climate is Cfa – humid subtropical, according to the Köppen-Geiger's classification; and the soil is Argissolo Vermelho distrófico arênico (Ultisol) (Santos et al., 2018).

The trials were formed by the combination of agricultural years, sowing dates, and cultivars (Table 1). In all uniformity trials, which measured 20×8 m (160 m^2), the mechanized sowing of wheat was carried out in rows spaced at 0.20 m apart, using 420 seed m^{-2}. Basal fertilization consisted of 9 kg ha^{-1} N, 36 kg ha^{-1} P_2O_5, and 36 kg ha^{-1} K_2O; subsequently, two top-dressing fertilizations with 41 kg ha^{-1} N were carried out in the development stages V_3 (three expanded leaves) and V_6 (six expanded leaves). Other cultural management practices were performed evenly throughout the experimental area.

The evaluations were performed when the crop was at the dough grain development stage (reproductive stage). For that, samples of 100 and 110 plants were established in each trial conducted in 2018 and 2019 crop season, respectively. These 1,790 plants were randomly harvested and separated into three parts (leaf, stem, and ear). The plants were cut near the soil surface and, immediately after cutting, fresh matter was determined with a digital scale to obtain the mass value (grams per plant).

In each plant, traits were evaluated as follows: measuring (cm) of the main stem length (SL, obtained by the distance between the base of the plant and the flag leaf insertion node) and of the main stem ear length

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(EL); counting of the number of leaves (NL), number of stems (NS), and number of ears (NE); and weighing (g per plant) of the fresh matter of leaves (FML), fresh matter of stems (FMS), fresh matter of ears (FME), fresh matter of shoots (FMSH = FML+FMS+FME), dry matter of leaves (DML), dry matter of stems (DMS), dry matter of ears (DME) and dry matter of shoots (DMSH = DML+DMS+DME).

The following statistics were calculated: p-value by the Kolmogorov-Smirnov's normality test, skewness, mean, median, minimum and maximum values, variance, and coefficient of variation. The Bartlett's test was performed to check the homogeneity of variances between the uniformity trials, and the Welch's analysis of variance was employed to check whether the means of traits differed between the uniformity trials. In case of occurences of heterogeneity of variances, the Welch's analysis of variance is an appropriate procedure to check whether the means of traits differ between uniformity trials.

For each trial and trait, based on the pilot sample, the sample size (n) was determined for the estimation of errors (e) fixed at 5, 10, 15, and 20% of the mean (m), that is, 0.05×m (higher precision), 0.10×m, 0.15×m, and 0.20×m (lower precision), with a confidence level (1-α) of 95%. The estimation error corresponds to the semi-amplitude of the confidence interval. The 'n' was determined by the expression $n = [(t_{\alpha/2}\ s)/e]^2$ (Bussab & Morettin, 2017), in which $t_{\alpha/2}$ is the critical value of the Student's t-distribution, whose area on the right is equal to α/2, that is, the value of t, such that $P(t>t_{\alpha/2}) = \alpha/2$, with α=5% probability of error and n-1 degrees of freedom (n = 100, and 110 plants in the 2018 and 2019 trials, respectively, in the present study), and 's' is the standard deviation estimate.

Statistical analyses were performed by the applications of Microsoft Office Excel, Genes (Cruz, 2016), and R software (R Core Team, 2022).

Table 1. Composition of uniformity trials with wheat (*Triticum aestivum*) cultivars in the 2018 and 2019 crop season.

Trial	Sowing (month/day)	Cultivar	Evaluation (month/day)	DAS[(1)]	No. of plats evaluated
			2018		
1	05/28	TBIO Energia I	09/21	116	100
2	05/28	TBIO Energia II	09/11	106	100
3	06/20	TBIO Energia I	10/04	106	100
4	06/20	TBIO Energia II	09/28	100	100
5	08/03	TBIO Energia I	10/23	81	100
6	08/03	TBIO Energia II	10/19	77	100
7	08/03	TBIO Sossego	10/24	82	100
8	08/03	TBIO Toruk	10/21	79	100
			2019		
9	06/07	TBIO Energia I	10/04	119	110
10	06/07	TBIO Energia II	09/13	98	110
11	06/07	TBIO Audaz	10/03	118	110
12	06/27	TBIO Energia I	10/10	105	110
13	06/27	TBIO Energia II	10/09	104	110
14	06/27	TBIO Audaz	10/22	117	110
15	07/18	TBIO Energia I	10/23	97	110
16	07/18	TBIO Energia II	10/23	97	110
17	07/18	TBIO Audaz	10/24	98	110

[(1)]DAS, number of days after sowing on the evaluation date.

Results and Discussion

The chi-square values of the Bartlett's test were the following: SL, 110.5; EL, 146.9; NL, 1,007.6; NS, 796.3; NE, 706.7; FML, 2,064.4; FMS, 1,578.9; FME, 728.5; FMSH, 1,498.5; DML, 1,371.3; DMS, 1,025.6; DME, 496.7; and DMSH, 876.3. The 13 traits showed heterogeneous variances (p-value < 0.0001) among the 17 uniformity trials (combination of agricultural years, sowing dates, and cultivars), which indicates different sample size between the trials.

The F-test values of the Welch's analysis of variance were the following: SL, 140.2; EL, 128.2; NL, 61.5; NS, 44.3; NE, 37.1; FML, 99.8; FMS, 67.1; FME, 38.6; FMSH, 48.8; DML, 76.5; DMS, 44.1; DME, 50.1; and DMSH, 30.0. These results show that there are differences between the means of the uniformity trials (p-value < 0.0001) for the 13 traits.

The Kolmogorov-Smirnov's test for the 221 cases (17 trials × 13 traits per trial) shows p-values between <0.001 and 0.996, with 0.313 average (Table 2). The higher is the p-value, the greater is the adherence of the data to the normal distribution curve. Thus, assuming 5% significance level, the normality was met in 71% of the cases. A lower adherence to the normal distribution was observed for the traits obtained by counting (NL, NS, and NE). Skewness coefficients close to zero (-0.56 ≤ skewness ≤ 1.75) and the proximity of the mean to the median (Table 3) indicate that the data showed good fit or slight distances from the normal distribution curve. Therefore, this data set is suitable for the study of sample sizing based on the Student's t-distribution.

A wide variation was found between plants within the uniformity trials and between uniformity trials for all traits, on the basis of the minimum and maximum values (Table 4) and of the variance and the coefficient of variation (Table 5). Such wide variation – promoted by 17 trials involving six sowing dates, five cultivars, and evaluations performed between 77 and 119 days after sowing (Table 1) – is important for the studies on sample sizing, as it contemplates plants of different sizes (small, medium, and large), which are common in field experiments.

The coefficient of variation (CV) of the 13 traits ranged between 7.85%, for EL in trial 7, and 54.37% for DME in trial 1, with 31.08% average (Table 5). In the average of the 17 uniformity trials, the coefficients of variation of traits FML, FMS, FME, FMSH, DML,

Table 2. P-value of the Kolmogorov-Smirnov's normality test and skewness coefficient of the traits evaluated in wheat plants (*Triticum aestivum*) cultivated in 17 uniformity trials[(1)].

Trial	Traits												
	SL	EL	NL	NS	NE	FML	FMS	FME	FMSH	DML	DMS	DME	DMSH
	p-value of the Kolmogorov-Smirnov's normality test												
1	0.707	0.970	0.007	0.000	0.001	0.245	0.196	0.137	0.164	0.251	0.131	0.380	0.256
2	0.256	0.295	0.236	0.005	0.001	0.393	0.483	0.653	0.772	0.593	0.363	0.543	0.421
3	0.931	0.894	0.029	0.000	0.000	0.007	0.037	0.053	0.027	0.008	0.194	0.020	0.104
4	0.930	0.288	0.027	0.004	0.000	0.015	0.067	0.036	0.103	0.038	0.278	0.108	0.178
5	0.993	0.504	0.005	0.000	0.000	0.075	0.127	0.224	0.157	0.040	0.327	0.180	0.172
6	0.449	0.422	0.009	0.000	0.000	0.132	0.348	0.468	0.450	0.218	0.763	0.429	0.455
7	0.831	0.493	0.015	0.000	0.000	0.208	0.847	0.906	0.343	0.360	0.942	0.808	0.935
8	0.746	0.299	0.005	0.000	0.000	0.093	0.077	0.028	0.034	0.201	0.012	0.012	0.019
9	0.664	0.274	0.000	0.000	0.000	0.481	0.302	0.875	0.649	0.595	0.521	0.957	0.710
10	0.797	0.666	0.022	0.000	0.000	0.222	0.163	0.090	0.078	0.096	0.134	0.162	0.146
11	0.910	0.051	0.000	0.000	0.000	0.331	0.916	0.433	0.818	0.446	0.958	0.175	0.621
12	0.407	0.000	0.000	0.000	0.000	0.470	0.157	0.980	0.837	0.380	0.864	0.940	0.996
13	0.930	0.042	0.000	0.000	0.000	0.265	0.469	0.422	0.303	0.472	0.717	0.860	0.584
14	0.857	0.189	0.000	0.000	0.000	0.069	0.194	0.554	0.323	0.168	0.342	0.611	0.664
15	0.922	0.239	0.034	0.000	0.000	0.424	0.364	0.248	0.431	0.262	0.334	0.414	0.375
16	0.892	0.439	0.001	0.000	0.000	0.292	0.567	0.220	0.199	0.276	0.222	0.532	0.131
17	0.814	0.356	0.063	0.000	0.000	0.572	0.271	0.232	0.284	0.673	0.144	0.243	0.285
	Skewness												
1	0.22	-0.03	0.66	0.79	0.60	0.83	0.69	0.86	0.74	0.70	0.64	0.81	0.64
2	0.07	-0.45	0.67	0.57	0.28	0.72	0.63	0.52	0.64	0.76	0.79	0.59	0.75
3	0.36	-0.07	1.19	0.43	0.50	1.69	1.27	1.32	1.36	1.75	0.84	1.08	1.10
4	-0.28	0.30	0.82	1.08	1.41	1.03	0.79	0.91	0.88	0.89	0.87	0.72	0.84
5	-0.04	0.14	1.40	1.58	1.28	1.63	1.72	1.35	1.73	1.61	1.48	1.29	1.63
6	0.19	0.00	0.89	0.48	0.65	0.83	0.54	0.85	0.61	0.60	0.42	0.90	0.54
7	0.36	-0.33	0.26	0.06	0.34	0.49	0.49	0.09	0.43	0.41	0.25	0.05	0.20
8	0.26	0.67	0.79	1.11	1.20	1.02	1.46	1.37	1.34	1.05	1.50	1.35	1.36
9	-0.09	0.05	0.55	0.84	0.84	0.58	0.53	0.06	0.41	0.55	0.52	-0.07	0.28
10	-0.08	-0.30	0.33	0.97	0.54	0.80	0.74	0.82	0.81	0.83	0.76	0.72	0.81
11	-0.18	-0.31	0.60	0.00	-0.24	0.77	0.14	0.54	0.26	0.51	0.26	0.77	0.23
12	0.13	0.34	0.74	0.84	0.52	0.45	0.34	0.36	0.42	0.24	0.26	0.26	0.21
13	0.34	-0.22	0.66	0.57	-0.04	0.69	0.79	0.61	0.70	0.59	0.75	0.54	0.63
14	-0.27	-0.45	0.81	0.43	0.38	0.79	0.45	0.44	0.52	0.67	0.54	0.39	0.40
15	-0.01	0.18	0.30	0.42	0.36	0.74	0.40	0.57	0.55	0.70	0.47	0.49	0.48
16	0.12	-0.56	0.58	0.29	0.16	0.61	0.47	0.36	0.48	0.60	0.57	0.33	0.46
17	0.33	-0.17	0.36	0.21	0.47	0.73	0.94	1.10	0.93	0.71	0.75	1.16	0.87

[(1)]Uniformity trials defined in Table 1. SL, main stem length (cm); EL, main stem ear length (cm); NL, number of leaves; NS, number of stems; NE, number of ears; FML, fresh matter of leaves (g per plant); FMS, fresh matter of stems (g per plant); FME, fresh matter of ears (g per plant); FMSH, fresh matter of shoots (FML+FMS+FME) (g per plant); DML, dry matter of leaves (g per plant); DMS, dry matter of stems (g per plant); DME, dry matter of ears (g per plant); and DMSH, dry matter of shoots (DML+DMS+DME) (g per plant).

DMS, DME, and DMSH (34.01 ≤ CV ≤ 40.76%, with 36.30% average) were higher than those obtained for the traits NL, NS and NE (29.25 ≤ CV ≤ 32.78%, with 30.78% average) and the traits SL and EL (10.39 ≤ CV ≤ 10.90%, with 10.64% average). Thus, for the same precision, a larger sample size is expected to estimate the means of the traits obtained by weighing (FML, FMS, FME, FMSH, DML, DMS, DME and DMSH) than those obtained by count (NL, NS and NE) and by measurement (SL and EL), in this order. However, means estimated from a single sample size would show precision increase for the traits obtained by weighing, counting, and measuring, in that order.

The sample sizes for estimating the mean, with estimation error (semi-amplitude of the 95% confidence interval) equals to 5% of the estimate of the mean (m), that is, 0.05×m (higher precision, in the present study), ranged between 10 plants for EL in trial 7 and 466

Table 3. Mean and median of the traits evaluated in wheat plants (*Triticum aestivum*) cultivated in 17 uniformity trials[(1)].

Trial	Traits												
	SL	EL	NL	NS	NE	FML	FMS	FME	FMSH	DML	DMS	DME	DMSH
							Mean						
1	50.16	8.77	8.65	3.60	3.13	5.34	9.91	2.57	17.82	1.43	2.90	0.77	5.10
2	60.81	9.97	14.46	3.97	3.10	8.45	12.22	3.19	23.86	1.90	2.74	0.83	5.47
3	48.27	9.41	6.52	2.97	2.89	3.08	6.69	3.24	13.01	0.95	1.92	1.05	3.92
4	50.30	10.89	14.21	5.47	5.02	11.26	17.56	6.53	35.35	2.45	4.16	1.76	8.37
5	41.48	7.67	7.35	2.73	2.61	2.61	4.76	1.87	9.24	0.85	1.45	0.62	2.93
6	41.94	9.08	6.65	3.38	3.13	3.14	6.62	2.59	12.36	0.86	1.83	0.77	3.46
7	44.14	8.71	6.61	2.54	2.47	3.39	5.95	2.09	11.43	0.95	1.66	0.68	3.29
8	34.22	8.01	6.74	2.99	2.74	2.78	5.10	2.42	10.31	0.88	1.28	0.77	2.93
9	55.68	7.59	4.58	2.31	2.31	1.67	4.71	2.60	8.98	0.52	1.68	0.98	3.19
10	56.70	8.91	5.55	2.39	2.27	3.33	5.58	2.30	11.21	0.91	1.68	0.68	3.26
11	51.61	8.18	6.22	2.00	1.89	2.47	4.92	2.51	9.90	0.70	1.75	0.95	3.39
12	50.60	7.60	6.76	2.31	2.14	2.22	4.38	2.44	9.04	0.67	1.35	0.83	2.85
13	46.58	8.37	4.83	2.48	2.35	2.19	4.26	3.14	9.59	0.66	1.23	1.12	3.02
14	48.33	8.81	5.20	2.75	2.71	1.51	3.76	3.94	9.21	0.55	1.26	1.79	3.60
15	46.72	7.03	5.72	2.78	2.73	1.90	3.65	2.17	7.72	0.66	1.24	0.75	2.65
16	48.01	9.18	4.50	3.12	3.01	2.30	4.65	3.68	10.63	0.73	1.48	1.41	3.62
17	43.79	7.83	8.05	3.23	3.02	2.43	3.66	2.55	8.65	0.87	1.30	0.86	3.04
							Median						
1	50.05	8.85	8.00	3.00	3.00	5.03	9.16	2.35	16.62	1.31	2.61	0.73	4.63
2	61.20	10.25	14.00	4.00	3.00	8.18	11.66	3.03	23.02	1.78	2.59	0.78	5.11
3	48.30	9.45	6.00	3.00	3.00	2.80	6.19	3.01	12.11	0.88	1.80	0.97	3.71
4	50.60	10.90	13.00	5.00	5.00	9.64	15.68	5.54	32.03	2.15	3.81	1.63	7.63
5	41.45	7.60	7.00	3.00	3.00	2.43	4.31	1.71	8.50	0.79	1.35	0.56	2.67
6	41.25	9.00	6.00	3.00	3.00	2.94	6.27	2.47	11.73	0.82	1.76	0.72	3.23
7	44.05	8.80	6.00	3.00	2.00	3.25	5.95	2.13	11.19	0.92	1.68	0.70	3.26
8	33.95	8.00	6.00	3.00	2.00	2.49	4.50	2.18	9.53	0.81	1.16	0.70	2.68
9	56.00	7.50	4.00	2.00	2.00	1.62	4.59	2.60	8.87	0.51	1.68	0.99	3.14
10	56.60	9.00	5.50	2.00	2.00	3.14	5.26	2.22	10.55	0.84	1.60	0.66	3.09
11	52.00	8.20	6.00	2.00	2.00	2.35	4.89	2.31	9.64	0.66	1.71	0.85	3.25
12	51.00	7.50	6.00	2.00	2.00	2.16	4.31	2.41	8.85	0.66	1.35	0.83	2.85
13	46.70	8.35	4.50	2.00	2.00	2.05	4.11	3.11	9.07	0.64	1.18	1.13	2.98
14	48.45	8.90	5.00	3.00	3.00	1.40	3.58	3.78	8.81	0.52	1.20	1.72	3.52
15	46.45	7.00	6.00	3.00	3.00	1.82	3.52	2.09	7.39	0.67	1.16	0.71	2.48
16	48.30	9.35	4.00	3.00	3.00	2.14	4.52	3.44	10.23	0.67	1.41	1.35	3.47
17	43.60	7.80	8.00	3.00	3.00	2.35	3.46	2.37	8.06	0.85	1.20	0.78	2.84

[(1)]Uniformity trials defined in Table 1. SL, main stem length (cm); EL, main stem ear length (cm); NL, number of leaves; NS, number of stems; NE, number of ears; FML, fresh matter of leaves (g per plant); FMS, fresh matter of stems (g per plant); FME, fresh matter of ears (g per plant); FMSH, fresh matter of shoots (FML+FMS+FME) (g per plant); DML, dry matter of leaves (g per plant); DMS, dry matter of stems (g per plant); DME, dry matter of ears (g per plant); and DMSH, dry matter of shoots (DML+DMS+DME) (g per plant).

plants for DME in trial 1 (Table 6). These sizes were calculated in Microsoft Office Excel, by the following expressions, respectively: =ARREDONDAR.PARA. CIMA((((INVT(0.05;99)*0.6836)/(0.05*8.7120))^2);0) = 10 plants and =ARREDONDAR.PARA. CIMA((((INVT(0.05;99)*0.4201)/(0.05*0.7727))^2);0) = 466 plants.

Therefore, in relation to ear length, it can be inferred that, with 95% confidence, the confidence interval of the mean (m) obtained with 10 plants is m±0.05m, that is, m±0.44 cm because the mean height of the 100 plants sampled was 8.71 cm (Table 3). At another extreme, the precision of m±0.05 m is obtained with 466 plants for the mass of dry matter of ears and, in this situation, the value would be m±0.04 g because the mean dry matter mass of ears of the 100 plants sampled was 0.77 g.

Table 4. Minimum and maximum value of traits evaluated in wheat plants (*Triticum aestivum*) cultivated in 17 uniformity trials[(1)].

Trial	Traits												
	SL	EL	NL	NS	NE	FML	FMS	FME	FMSH	DML	DMS	DME	DMSH
							Minimum value						
1	34.50	6.00	4.00	2.00	1.00	1.70	1.86	0.39	3.95	0.31	0.40	0.07	0.78
2	47.60	5.70	3.00	1.00	1.00	2.27	2.84	0.39	5.86	0.52	0.58	0.07	1.29
3	36.50	6.80	2.00	1.00	1.00	0.87	2.05	0.89	3.81	0.30	0.64	0.29	1.23
4	29.80	9.10	4.00	2.00	2.00	3.81	5.63	2.33	12.04	0.88	1.40	0.66	2.94
5	31.10	6.00	3.00	2.00	1.00	1.06	1.63	0.43	3.32	0.30	0.46	0.14	1.00
6	28.00	7.50	3.00	2.00	1.00	1.03	2.63	1.08	5.18	0.28	0.67	0.28	1.42
7	33.60	6.40	3.00	2.00	2.00	1.94	3.37	0.96	6.73	0.47	0.75	0.31	1.64
8	26.20	6.60	3.00	2.00	1.00	1.03	1.83	0.85	3.80	0.30	0.41	0.23	0.98
9	43.20	5.50	3.00	2.00	2.00	0.90	2.79	1.12	4.89	0.27	0.88	0.40	1.55
10	41.00	6.00	2.00	2.00	1.00	1.60	2.83	0.94	5.48	0.44	0.86	0.24	1.58
11	36.00	5.40	3.00	1.00	1.00	0.84	1.06	0.37	2.27	0.18	0.42	0.13	0.73
12	42.00	6.00	4.00	2.00	1.00	0.83	2.78	0.73	5.28	0.26	0.70	0.20	1.56
13	37.00	6.20	3.00	2.00	1.00	0.78	1.96	1.18	4.20	0.27	0.47	0.35	1.19
14	36.20	6.50	3.00	2.00	1.00	0.63	1.82	1.20	3.91	0.20	0.54	0.46	1.25
15	35.00	5.40	2.00	1.00	1.00	0.43	1.41	0.76	2.95	0.18	0.49	0.25	1.15
16	36.10	6.00	2.00	2.00	1.00	0.75	2.11	1.61	5.01	0.29	0.65	0.52	1.55
17	35.00	5.70	4.00	2.00	2.00	1.04	1.77	0.88	4.16	0.34	0.59	0.27	1.43
							Maximum value						
1	65.00	11.40	17.00	7.00	6.00	12.00	22.57	6.73	38.36	3.12	6.72	2.16	11.42
2	76.40	12.40	34.00	9.00	6.00	20.10	28.12	7.77	55.99	4.60	6.98	2.02	13.07
3	64.30	11.60	16.00	6.00	6.00	8.80	16.05	9.24	34.09	2.79	4.24	2.84	9.87
4	67.50	13.30	30.00	12.00	12.00	27.86	37.36	14.97	77.73	5.76	9.19	3.82	18.51
5	52.50	9.30	18.00	6.00	6.00	7.04	13.29	5.13	25.46	2.19	3.94	1.69	7.82
6	56.60	11.20	14.00	6.00	6.00	6.37	12.85	5.80	23.02	1.61	3.33	1.75	6.58
7	59.10	10.50	12.00	4.00	4.00	5.34	9.69	3.36	17.51	1.50	2.94	1.14	5.27
8	46.50	10.10	13.00	6.00	6.00	6.08	13.06	5.77	23.79	1.84	3.34	1.93	6.71
9	68.00	9.40	7.00	3.00	3.00	2.83	7.37	4.58	14.78	0.86	2.67	1.73	5.25
10	70.00	11.00	9.00	4.00	3.00	6.07	9.94	4.59	19.85	1.68	3.01	1.42	5.81
11	65.20	10.30	11.00	3.00	3.00	5.05	9.22	5.62	18.82	1.40	3.37	2.44	6.61
12	61.00	10.00	11.00	3.00	3.00	4.00	6.55	4.49	14.55	1.07	2.20	1.52	4.67
13	59.00	10.10	8.00	4.00	4.00	4.10	8.53	6.65	19.28	1.22	2.61	2.36	6.14
14	57.40	10.50	10.00	4.00	4.00	3.07	6.14	7.61	16.51	1.08	2.39	3.18	6.40
15	58.20	8.60	11.00	5.00	5.00	4.36	6.95	4.70	16.01	1.39	2.36	1.51	5.12
16	61.40	11.50	10.00	5.00	5.00	4.75	8.68	6.47	18.62	1.48	2.89	2.60	6.59
17	55.50	9.60	14.00	5.00	5.00	5.30	7.78	5.94	18.35	1.81	2.51	2.11	6.20

[(1)]Uniformity trials defined in Table 1. SL, main stem length (cm); EL, main stem ear length (cm); NL, number of leaves; NS, number of stems; NE, number of ears; FML, fresh matter of leaves (g per plant); FMS, fresh matter of stems (g per plant); FME, fresh matter of ears (g per plant); FMSH, fresh matter of shoots (FML+FMS+FME) (g per plant); DML, dry matter of leaves (g per plant); DMS, dry matter of stems (g per plant); DME, dry matter of ears (g per plant); and DMSH, dry matter of shoots (DML+DMS+DME) (g per plant).

These results show that, for the same precision, the sample sizes vary between traits, as found for wheat (Sari et al., 2020), sorghum (Silva et al., 2005), maize (Toebe et al., 2014; Wartha et al., 2016), black oat (Cargnelutti Filho et al., 2015), millet (Kleinpaul et al., 2017), and rye (Bandeira et al., 2018a, 2018b, 2019). It was also observed that, for the same trait, there is variation for the sample size between the uniformity trials (combination of agricultural years, sowing dates, and cultivars). The results of the present study corroborate those of other studies, which also showed that, for the same trait, there is variation in the sample size between genotypes (Toebe et al., 2014; Wartha et al., 2016; Bandeira et al., 2018a, 2018b, 2019), sowing times (Bandeira et al., 2018a, 2018b, 2019), evaluation times (Cargnelutti Filho et al., 2015; Kleinpaul et al., 2017; Bandeira et al., 2018b), and agricultural years (Toebe et al., 2014).

Table 5. Variance and coefficient of variation of the traits evaluated in wheat plants (*Triticum aestivum*) cultivated in 17 uniformity trials[(1)].

Trial	Traits												
	SL	EL	NL	NS	NE	FML	FMS	FME	FMSH	DML	DMS	DME	DMSH
							Variance						
1	26.99	1.27	10.63	1.66	1.77	4.59	19.99	1.79	58.34	0.34	1.90	0.18	5.23
2	51.79	1.82	39.58	2.43	1.65	14.64	28.89	2.34	107.11	0.72	1.48	0.17	5.70
3	28.77	1.27	6.17	1.00	0.93	2.03	8.02	2.01	30.55	0.17	0.56	0.21	2.46
4	43.13	0.79	38.77	5.73	4.85	33.67	63.09	10.74	276.10	1.47	3.61	0.69	14.51
5	21.50	0.51	7.12	0.76	0.68	1.14	3.72	0.62	13.28	0.12	0.36	0.07	1.34
6	36.28	0.53	6.05	1.19	1.23	1.48	5.53	0.99	19.13	0.10	0.43	0.10	1.49
7	27.14	0.47	3.07	0.27	0.27	0.62	2.04	0.32	6.42	0.05	0.21	0.04	0.63
8	20.64	0.49	5.49	1.26	1.08	1.21	4.13	1.10	16.05	0.12	0.29	0.12	1.39
9	26.23	0.57	1.03	0.22	0.22	0.15	0.88	0.38	3.35	0.01	0.12	0.06	0.44
10	40.19	1.11	2.21	0.30	0.24	1.03	2.83	0.58	10.59	0.07	0.26	0.06	0.94
11	39.69	0.96	2.78	0.26	0.24	0.78	2.51	1.27	10.68	0.06	0.31	0.24	1.31
12	18.00	0.73	2.81	0.22	0.21	0.42	0.88	0.47	4.04	0.03	0.11	0.07	0.46
13	22.08	0.73	1.17	0.31	0.36	0.49	2.16	1.08	9.29	0.04	0.20	0.17	1.00
14	17.49	0.74	2.62	0.52	0.54	0.33	0.92	1.70	6.97	0.04	0.15	0.41	1.22
15	21.37	0.47	4.52	0.67	0.70	0.67	1.80	0.68	7.75	0.07	0.21	0.08	0.86
16	24.13	1.14	2.84	0.82	0.74	0.75	2.17	1.44	10.27	0.07	0.24	0.27	1.23
17	13.58	0.59	6.17	0.76	0.68	0.68	1.60	1.38	9.34	0.09	0.21	0.18	1.21
							Coefficient of variation (%)						
1	10.36	12.85	37.70	35.75	42.51	40.17	45.11	52.09	42.88	40.57	47.51	54.37	44.81
2	11.83	13.54	43.51	39.29	41.39	45.28	43.97	47.96	43.37	44.68	44.44	49.55	43.65
3	11.11	11.97	38.10	33.65	33.32	46.19	42.35	43.75	42.47	43.81	38.96	43.65	40.06
4	13.06	8.18	43.82	43.75	43.86	51.53	45.22	50.20	47.00	49.49	45.68	47.00	45.51
5	11.18	9.28	36.30	32.03	31.70	40.97	40.53	41.98	39.44	40.01	41.44	42.81	39.55
6	14.36	8.03	36.98	32.24	35.37	38.77	35.50	38.29	35.40	35.92	35.68	39.95	35.29
7	11.80	7.85	26.50	20.50	21.11	23.29	23.99	27.03	22.17	22.86	27.29	28.78	24.15
8	13.28	8.73	34.76	37.58	37.98	39.58	39.83	43.37	38.87	39.43	42.14	45.39	40.26
9	9.20	9.99	22.20	20.10	20.10	23.17	19.97	23.74	20.38	22.40	20.42	25.54	20.87
10	11.18	11.86	26.83	22.73	21.41	30.55	30.17	33.10	29.03	29.21	30.55	36.44	29.79
11	12.21	12.00	26.80	25.34	26.17	35.85	32.15	44.94	33.01	35.02	31.86	51.66	33.74
12	8.38	11.24	24.77	20.10	21.48	29.19	21.36	28.04	22.23	27.01	24.08	32.18	23.80
13	10.09	10.18	22.42	22.33	25.46	32.09	34.50	33.01	31.77	31.47	36.21	36.35	33.23
14	8.65	9.78	31.13	26.31	27.09	38.10	25.54	33.10	28.66	35.86	30.35	35.81	30.74
15	9.89	9.70	37.16	29.37	30.58	43.03	36.74	37.88	36.05	40.38	36.70	37.03	34.90
16	10.23	11.62	37.45	29.05	28.65	37.52	31.65	32.63	30.14	35.77	33.05	36.97	30.68
17	8.41	9.85	30.88	27.09	27.29	33.95	34.57	46.01	35.34	33.66	35.48	49.38	36.24

[(1)]Uniformity trials defined in Table 1. SL, main stem length (cm); EL, main stem ear length (cm); NL, number of leaves; NS, number of stems; NE, number of ears; FML, fresh matter of leaves (g per plant); FMS, fresh matter of stems (g per plant); FME, fresh matter of ears (g per plant); FMSH, fresh matter of shoots (FML+FMS+FME) (g per plant); DML, dry matter of leaves (g per plant); DMS, dry matter of stems (g per plant); DME, dry matter of ears (g per plant); and DMSH, dry matter of shoots (DML+DMS+DME) (g per plant).

For the estimation error of 10%, a larger sample size was observed for the traits FML, FMS, FME, FMSH, DML, DMS, DME, and DMSH ($16 \leq n \leq 117$), with the average of 55 plants, than the sample size required for NL, NS, and NE ($16 \leq n \leq 76$), with an average of 40 plants, and sample size required for SL and EL ($3 \leq n \leq 9$), with an average of 5 plants (Table 6). As expected, these results are due to the higher coefficient of variation of the traits obtained by weighing (FML, FMS, FME, FMSH, DML, DMS, DME, and DMSH) than to those obtained by count (NL, NS, and NE) and by measurement (SL and EL), in this order. A similar order was observed in black oat, for which a larger sample size was necessary to estimate fresh matter

Table 6. Sample size (number of plants) for estimating the means of traits evaluated in wheat plants (*Triticum aestivum*) cultivated in 17 uniformity trials(1), for estimation errors (semi-amplitudes of the confidence interval) equal to 5% and 10% of the mean (m), that is, 0.05×m (higher precision) and 0.10×m (lower precision), with confidence level (1-α) of 95%.

Trial	Traits												
	SL	EL	NL	NS	NE	FML	FMS	FME	FMSH	DML	DMS	DME	DMSH
	Estimation error equal to 5% of the mean												
1	17	27	224	202	285	255	321	428	290	260	356	466	317
2	23	29	299	244	270	323	305	363	297	315	312	387	301
3	20	23	229	179	175	337	283	302	285	303	239	301	253
4	27	11	303	302	303	419	323	397	348	386	329	348	327
5	20	14	208	162	159	265	259	278	245	253	271	289	247
6	33	11	216	164	197	237	199	231	198	204	201	252	197
7	22	10	111	67	71	86	91	116	78	83	118	131	92
8	28	12	191	223	228	247	250	297	238	245	280	325	256
9	14	16	78	64	64	85	63	89	66	79	66	103	69
10	20	23	114	82	73	147	144	173	133	135	147	209	140
11	24	23	113	101	108	202	163	318	172	193	160	420	179
12	12	20	97	64	73	134	72	124	78	115	92	163	90
13	16	17	80	79	102	162	188	172	159	156	207	208	174
14	12	16	153	109	116	229	103	173	130	203	145	202	149
15	16	15	218	136	147	291	213	226	205	257	212	216	192
16	17	22	221	133	129	222	158	168	143	202	172	215	148
17	12	16	150	116	118	182	188	333	197	179	198	384	207
	Estimation error equal to 10% of the mean												
1	5	7	56	51	72	64	81	107	73	65	89	117	80
2	6	8	75	61	68	81	77	91	75	79	78	97	76
3	5	6	58	45	44	85	71	76	72	76	60	76	64
4	7	3	76	76	76	105	81	100	87	97	83	87	82
5	5	4	52	41	40	67	65	70	62	64	68	73	62
6	9	3	54	41	50	60	50	58	50	51	51	63	50
7	6	3	28	17	18	22	23	29	20	21	30	33	23
8	7	3	48	56	57	62	63	75	60	62	70	82	64
9	4	4	20	16	16	22	16	23	17	20	17	26	18
10	5	6	29	21	19	37	36	44	34	34	37	53	35
11	6	6	29	26	27	51	41	80	43	49	40	105	45
12	3	5	25	16	19	34	18	31	20	29	23	41	23
13	4	5	20	20	26	41	47	43	40	39	52	52	44
14	3	4	39	28	29	58	26	44	33	51	37	51	38
15	4	4	55	34	37	73	54	57	52	65	53	54	48
16	5	6	56	34	33	56	40	42	36	51	43	54	37
17	3	4	38	29	30	46	47	84	50	45	50	96	52

(1)Uniformity trials defined in Table 1. SL, main stem length (cm); EL, main stem ear length (cm); NL, number of leaves; NS, number of stems; NE, number of ears; FML, fresh matter of leaves (g per plant); FMS, fresh matter of stems (g per plant); FME, fresh matter of ears (g per plant); FMSH, fresh matter of shoots (FML+FMS+FME) (g per plant); DML, dry matter of leaves (g per plant); DMS, dry matter of stems (g per plant); DME, dry matter of ears (g per plant); and DMSH, dry matter of shoots (DML+DMS+DME) (g per plant).

and dry matter obtained by weighing, than sample sizes required to estimate the number of leaves and tillers obtained by count, and plant height obtained by measurement (Cargnelutti Filho et al., 2015). In maize, Toebe et al. (2014) also determined a larger sample size for traits obtained by weighing than those obtained by count and measurement, in this order.

If the researcher allows of 20% estimation error, that is, 0.20×m (lower precision, in the present study) and a 95% confidence level, the number of plants to be sampled is 1 to 30 plants (Table 7). It is evident that this low number of plants (≤ 30) would be easily evaluated in an experiment. However, it would lead to low precision in estimating the means of the traits;

Table 7. Sample size (number of plants) for estimating the means of traits evaluated in wheat plants (*Triticum aestivum*) cultivated in 17 uniformity trials[(1)], for estimation errors (semi-amplitudes of the confidence interval) equal to 15% and 20% of the mean (m), that is, 0.15×m (higher precision) and 0.20×m (lower precision), with confidence level (1-α) of 95%.

Trial	Traits												
	SL	EL	NL	NS	NE	FML	FMS	FME	FMSH	DML	DMS	DME	DMSH
	Estimation error equal to 15% of the mean												
1	2	3	25	23	32	29	36	48	33	29	40	52	36
2	3	4	34	28	30	36	34	41	33	35	35	43	34
3	3	3	26	20	20	38	32	34	32	34	27	34	29
4	3	2	34	34	34	47	36	45	39	43	37	39	37
5	3	2	24	18	18	30	29	31	28	29	31	33	28
6	4	2	24	19	22	27	23	26	22	23	23	28	22
7	3	2	13	8	8	10	11	13	9	10	14	15	11
8	4	2	22	25	26	28	28	33	27	28	32	37	29
9	2	2	9	8	8	10	7	10	8	9	8	12	8
10	3	3	13	10	9	17	16	20	15	15	17	24	16
11	3	3	13	12	12	23	19	36	20	22	18	47	20
12	2	3	11	8	9	15	8	14	9	13	11	19	10
13	2	2	9	9	12	18	21	20	18	18	23	24	20
14	2	2	17	13	13	26	12	20	15	23	17	23	17
15	2	2	25	16	17	33	24	26	23	29	24	24	22
16	2	3	25	15	15	25	18	19	16	23	20	24	17
17	2	2	17	13	14	21	21	37	22	20	22	43	23
	Estimation error equal to 20% of the mean												
1	2	2	14	13	18	16	21	27	19	17	23	30	20
2	2	2	19	16	17	21	20	23	19	20	20	25	19
3	2	2	15	12	11	22	18	19	18	19	15	19	16
4	2	1	19	19	19	27	21	25	22	25	21	22	21
5	2	1	13	11	10	17	17	18	16	16	17	19	16
6	3	1	14	11	13	15	13	15	13	13	13	16	13
7	2	1	7	5	5	6	6	8	5	6	8	9	6
8	2	1	12	14	15	16	16	19	15	16	18	21	16
9	1	1	5	4	4	6	4	6	5	5	5	7	5
10	2	2	8	6	5	10	9	11	9	9	10	14	9
11	2	2	8	7	7	13	11	20	11	13	10	27	12
12	1	2	7	4	5	9	5	8	5	8	6	11	6
13	1	2	5	5	7	11	12	11	10	10	13	13	11
14	1	1	10	7	8	15	7	11	9	13	10	13	10
15	1	1	14	9	10	19	14	15	13	17	14	14	12
16	2	2	14	9	9	14	10	11	9	13	11	14	10
17	1	1	10	8	8	12	12	21	13	12	13	24	13

[(1)]Uniformity trials defined in Table 1. SL, main stem length (cm); EL, main stem ear length (cm); NL, number of leaves; NS, number of stems; NE, number of ears; FML, fresh matter of leaves (g per plant); FMS, fresh matter of stems (g per plant); FME, fresh matter of ears (g per plant); FMSH, fresh matter of shoots (FML+FMS+FME) (g per plant); DML, dry matter of leaves (g per plant); DMS, dry matter of stems (g per plant); DME, dry matter of ears (g per plant); and DMSH, dry matter of shoots (DML+DMS+DME) (g per plant).

for instance, if the data from trial 1 were applied, the values of traits would be as follows: SL, m±10.03 cm; EL, m±1.75 cm; NL, m±1.73 leaves; NS, m±0.72 stems; NE, m±0.63 ears; FML, m±1.07 grams; FMS, m±1.98 grams; FME, m±0.51 grams; FMSH, m±3.56 grams; DML, m±0.29 grams; DMS, m±0.58 grams; DME, m±0.15 grams; DMSH- m±1.02 grams.

In practice, by the results of the present study, the researcher can choose the sample size to estimate the means of these traits with the desired precision. For instance, if the option is to allow of the maximum estimation error of 10%, that is, 0.10×m, 117 plants would be sufficient to estimate the means of these 13 traits, under the conditions of the 17 uniformity trials (Table 6). Thus, when planning an experiment to be conducted in the field, in a completely randomized experimental design to estimate the mean of each treatment with 10% precision, 117 plants per treatment should be evaluated. If the experiment is planned with four replicates per treatment, 30 plants per replicate (117/4 = 29.25) should be sampled, that is, 30 plants per plot. Furthermore, if five treatments were evaluated in the experiment, 600 plants (120 per treatment) should be sampled.

It is worth pointing out that, for the traits obtained by count (NL, NS, and NE) and by measurement (SL and EL), individual evaluations of the plants are needed, which requires more labor and time than for FML, FMS, FME, FMSH, DML, DMS, DME, and DMSH, since, for these traits, plants can be weighed together. Optionally, the researcher can guarantee the maximum estimation error of 10%, by sampling 117, 76, and 9 plants, for the traits obtained respectively by weighing, counting, and measuring.

Conclusions

1. The sample size for estimating the means of wheat (*Triticum aestivum*) traits, obtained by weighing, counting and measuring, decreases in this attainment order.

2. To estimate the means of wheat traits with 10% maximum error of the mean and a confidence level of 95%, the sufficient sample size is 117 plants – for fresh and dry matter of leaves, stems, ears, and shoots –, 76 plants for the number of leaves, stems, and ears; and nine plants for the length of main stem and length of main stem of ear.

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