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Estimate of the number of experiments and importance of morphoagronomic characters in lettuce

Estimativa do número de experimentos e importância de caracteres morfoagronômicos em alface

ABSTRACT: In scientific research, the repetition of the experiment and the selection of variables-response improve the precision of the results and make the conclusions more assertive, allowing generalizations from the joint analysis. However, there are no official rules about how many times experiment should be repeated, as well as which variables best discriminate the evaluated treatments. For this reason, this research aimed to estimate the number of repetitions of experiments and indicate the importance of morphoagronomic characters of lettuce cv. ‘Veneranda’. Data from four experiments were considered, in which four soil covers were evaluated (SoilTain DW[®] geosynthetic blanket, white and black polypropylene mulching and uncovered soil) in the completely randomized design, with five replications. The number of experiments was estimated by the repeatability coefficient and the importance of the characters, estimated by Singh criteria. In general, the repeatability coefficients were of medium magnitude, in which the number of measurements ranged from 1 to 9 experiments and, for yield, four experiments are enough to accurately indicate the best treatments. The most discriminant variables were the number of leaves (39.47%) and their length (20.79%) and plant height (17.06%); leaf width and root mass variables may be discarded in future studies (0% contribution). The results indicate that the execution of a single experiment, common in lettuce researches, does not allow accurate conclusions, and should be a criterion to be considered in the experimental planning.

RESUMO: Na pesquisa científica, a repetição do experimento e a seleção das variáveis-resposta melhoram a precisão dos resultados e tornam mais assertiva as conclusões, possibilitando generalizações a partir da análise conjunta. Porém, não há informações quanto ao número de vezes que o experimento deverá ser conduzido, nem quais as variáveis que melhor discriminam os tratamentos avaliados. Por esta razão, nesta pesquisa, objetivou-se estimar o número de repetições de experimentos e indicar a importância de caracteres morfoagronômicos da alface cv. Veneranda. Foram considerados os dados de quatro experimentos, em que foram avaliadas quatro coberturas do solo (manta geossintética SoilTain DW[®], mulching de polipropileno branco e preto, e o solo descoberto) no delineamento inteiramente casualizado, com cinco repetições. O número de experimentos foi estimado pelo coeficiente de repetibilidade e a importância dos caracteres, pelo critério de Singh. Em geral, os coeficientes de repetibilidade foram de média magnitude, em que o número de experimentos variou de 1 a 9, sendo que, para a produtividade, quatro experimentos são suficientes para indicar, com acurácia, os melhores tratamentos. As variáveis mais discriminantes foram o número de folhas (39,47%) e seu comprimento (20,79%) e a altura da planta (17,06%); largura foliar e massa da raiz poderão ser descartadas em futuros estudos em cultivos de alface (0% de contribuição). Os resultados indicam que a execução de um único experimento, comum em pesquisas com alface, não permite conclusões acuradas, devendo ser um critério a ser considerado no planejamento experimental.

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1 Introduction

Lettuce (*Lactuca sativa* L.), an annual herbaceous species, can be cultivated in a protected environment or at open field, allowing its cultivation in almost the whole world, with China, United States of America and India standing out as the largest producers, with 16.3, 3.7 and 1.3 million megagrams (FAO, 2021). Brazil has 108.4 thousand establishments that grow lettuce, with production of 671.5 thousand megagrams, highlighting the Southeast, South and Northeast regions, for concentrating 64.1, 16.2 and 10.5% of the national production, respectively (IBGE, 2020).

Considering the increasing demand, the development of new cultivars adapted to different agroecosystems (Carvalho *et al.*, 2020), with more attractive nutritional properties and tolerant to diseases (Jacinto *et al.*, 2020) are objectives of the breeding program. The evaluation of cultivars in different production systems (Silva *et al.*, 2011, Souza *et al.*, 2020) and also, the use of soil covers (Frias *et al.*, 2017) are management practices that have been researched. However, the short experimental period of the researches does not lead to accurate information, considering the environmental variations within and between years.

In scientific research, the repetition of the experiment allows to evaluate the influence of environmental variation on morphoagronomic characters of plants. It also allows to increase the experimental precision, because, in the joint analysis, the residual degrees of freedom are increased, making the F-calculated values more precise. However, carrying out experiments demands physical, human and financial resources that, in most cases, are scarce (Diel *et al.*, 2020). Therefore, it is important, in the experimental planning phase, to define the number of repetitions of the experiment, associating precision in the results and efficiency in carrying out the research.

Despite its importance, there are no official rules about how many times experiment must be repeated. By allowing the estimation of the number of measurements that should be carried out in each experimental unit, the repeatability analysis has been used in annual and perennial species (Resende, 2002). However, it can be useful in estimating the number of experiments that should be performed. With this, trustworthy results are obtained, passive to generalizations, time and labor reduction and the optimization of financial resources.

Few studies were dedicated to indicating the number of repetitions of experiments, highlighting the one by Cargnelutti Filho *et al.* (2006), who evaluated the effectiveness of nine experiments with *Phaseolus vulgaris* L. cultivars, in which seven would already be enough to identify superior ones; by Benin *et al.* (2014), who evaluated 285 experiments of *Triticum* spp. installed in 31 locations, showing that the number of trials, 8 to 14, depending on the location, would be sufficient; and Mendes & Ramalho (2018), who evaluated 25 *P. vulgaris* lines in 36 environments, different growing seasons and years, in which they recommended a

minimum of 12 environments to observe the phenotypic stabilization of the genotypes. In relation to the lettuce crop, there are no references in the national or international literature with the use of repeatability analyses.

The selection of the variables-response is one of the main steps, because problems related to the type of variable (quali-quantitative), the presence of correlation and multicollinearity, heterocedasticity can derail the use or reduce the efficiency of biometric models, with reflections on the conclusions. Therefore, the selection of discriminatory variables reduces time and resources (human, physical and financial) to the project, making it more efficient. Singh's criterion informs the relative importance of the variable, indicating those that are less discriminating, enabling its discard (Cruz *et al.*, 2020). When associated with the analysis of correlation and multivariate method, such as Tocher, it will allow a more accurate decision.

Despite the edaphoclimatic differences in the producing regions and the various types of lettuce cultivated, our results may awaken in researchers the need to repeat the experiment, making the project more efficient, with precise and accurate results. This research aimed to estimate the number of experiments and the importance of morphoagronomic variables-response of lettuce cv. 'Veneranda'.

2 Material and methods

The research was conducted between 2020 and 2021 on a commercial property under the organic production system (MAPA n° 13966), located in the village of Flexeiras (9° 47' 50.92" S; 36° 36' 14.63" W), with 237 m a.s.l, rural area of Arapiraca, BSh Köppen climate, semi-arid region of the State of Alagoas, Brazil. The soil of the experimental area was classified as Acriferric Yellow Latosol.

The experimental area, flat topography, homogeneous color, without a history of agricultural use, after systematized, no factors responsible for external variation were found, being therefore considered homogeneous, installing the experiments under the completely randomized design, with four treatments (SoilTain DW® geosynthetic blanket, white and black polypropylene mulching, and uncovered soil) and five repetitions. The experimental plot consisted of 42 plants, of which 20 were considered as working area and the others, border. Four experiments were carried out: 1) 13/Jun to 17/Jul/2020 (autumn-winter); 2) 07/Aug to 14/Sep/2020 (winter); 3) 02/Dec/2020 to 05/Jan/2021 (summer); and 4) 04/Feb to 10/Mar/2021 (summer end). All experiments were installed in the same area. The climate data during the experimental period is shown in Table 1.

Lettuce cv. 'Veneranda', crispy type, light green, was used because it is widely cultivated in the region. The SoilTain DW® geosynthetic blanket, used in water filtration in water treatment plants, has a black color, 14 mm thickness, high strength and tenacity, with UV protection, inert to biological degradation and resistant to

chemical attack, it is a solid residue that one wishes to test its viability as an alternative mulching. Synthetic mulchings, white and black, waterproof, 20 μm thickness, have been used by producers without prior research. Uncovered soil, as a control treatment, predominates in the region's crops.

Table 1. Mean climate data from the experimental periods in Arapiraca/AL, Brazil.

Tabela 1. Dados médios climáticos dos períodos experimentais em Arapiraca/AL, Brasil.

Variables	Experiments periods			
	Jun- Jul/2020	Aug- Sep/2020	Dec- Jan/2021	Feb- Mar/2021
Rainfall (mm) ¹	131.5	53,3	4,3	14.0
Minimum temperature (°C) ²	19.2	18.7	21.9	22.7
Maximum temperature(°C) ²	27.9	28.5	33.3	34.8
Global solar radiation (Mj m ⁻²) ²	1.16	1.32	1.65	1.66

Source: ¹Arapiraca Department of Agriculture; ²Inmet (2021).

Fonte: ¹Secretaria de Agricultura de Arapiraca; ²Inmet (2021).

The result of the chemical analysis of the soil (0 to 20 cm) presented the following characteristics: pH 6.8 (H₂O); P: 106 mg dm⁻³ (Mehlich); Na and K (Mehlich), Ca + Mg and Al (1N KCl), and H (calcium acetate, pH 7.0): 0.22, 0.23, 5.7, 0 and 0.6 cmolc dm⁻³, respectively; organic matter: 2.18%; Fe, Cu, Zn and Mn (Mehlich): 162.7, 7.23, 45.55 and 75.94 mg dm⁻³, respectively. Following the recommendations of Cavalcanti (2008), 30 and 40 kg of N/ha were applied at planting and in topdressing, respectively, and 60 kg K₂O/ha at planting, to meet the demand of the crop, using compost organic consisting of castor bean cake and tanned cattle manure (1:1), which presented the following chemical characteristics: organic matter: 42.8%; N: 3.12%; P₂O₅: 0.57%; K₂O: 0.43%; Ca: 1.36%; Mg: 0.81%; Fe, Zn, Cu, Mn with concentrations of 5,200, 93, 25 and 105 mg dm⁻³, respectively.

Soil preparation was carried out with the aid of a rotating hoe coupled to a 6.5 CV tractor, at a depth of 0.15 m. Subsequently, the beds were leveled and prepared in dimensions of 1.0 x 2.8 m, and 0.10 m in height, adopting a spacing between plants of 0.25 x 0.25 m, equivalent to the initial stand of 123,200 plants/ha. The seedlings were produced in 200-cell trays, with volume of 18 cm³ each, manually placing one seed per cell, in commercial substrate Bioplant 401®. The transplanting to the field took 25 days after germination in each experiment.

The foundation fertilization was carried out over the beds. The irrigation system was drip irrigation, with two 16 mm thick polyethylene tapes distributed in the beds, with emitters spaced 0.2 m and flow rate of 1.6 L/h, twice a day (morning and afternoon), for 20 minutes, except on rainy days, following the management adopted by the producer. The irrigation tapes were placed under the ground covers. Weed control was manual, in the control treatment, without soil cover.

The analyzed variables were obtained at the end of each experiment, in which the stand (plants/ha), height (in cm, determined from the collar to the outermost leaf of the plants, with the aid of graduated ruler), the number of commercial leaves/plant, the length and width of all commercial leaves (in cm, using graduated ruler), from three randomly selected plants in the working area of the plot. Shoot fresh mass, root mass (in kg, determined with the aid of a digital scale) and yield for each treatment (in Mg/ha, stand of each treatment x average shoot fresh mass) were evaluated using all the plants in the working area of the plot.

The individual analysis of variance was performed, aiming to evaluate the homoscedasticity of the experiments. From the Hartley test, F-maximum, which assesses the relationship between the highest and the lowest mean square error, a maximum ratio of 4.66 was obtained for shoot mass, which, according to Ferreira (2018), indicates homogeneity of variances residuals, and the four experiments should be included in the joint analysis.

The statistical model $Y_{ij} = \mu + (R/a)_{ik} + t_i + a_j + ta_{ij} + \varepsilon_{ijk}$ was adopted, in which: Y_{ij} corresponds to the phenotypic value of treatment i in environment j ; μ : general mean; $(R/a)_{jk}$: effect of repetitions ($k = 1, 2, \dots, r$) inside environments ($j = 1, 2, \dots, q$); t_i : treatment effect ($i = 1, 2, \dots, p$); a_j : environment effects ($j = 1, 2, \dots, q$); $(ta)_{ij}$: effect of the treatment \times environments interaction; ε_{ijk} : experimental error.

Analysis of variance was performed for each variable between the experiments, adopting the Scott-Knott test in the grouping of means ($p < 0.05$). From the repeatability analysis, through the principal components analysis (covariance and correlation methods), the coefficients and the number of experiments were determined, setting a determination coefficient of 0.85. According to Resende (2002), this parameter varies from 0 to 1 and, when ≥ 0.60 , it has a high magnitude; between ≥ 0.30 and < 0.60 , mean magnitude; and < 0.30 , low magnitude. For the relative contribution (%), the method of Singh, described by Cruz *et al.* (2020), was adopted using the generalized Mahalanobis distance (D^2) as a dissimilarity measure. Pearson's correlations were performed between the morphoagronomic variables. The similarity between the treatments (soil covers) was determined by the Tocher cluster method using D^2 distance, to assist in discarding variables.

3 Results and Discussion

There was a significant effect ($p < 0.05$) for the interaction soil cover \times experiments for all variables, except for root mass, indicating that these factors, together, promote changes in the behavior of lettuce cv. 'Veneranda' (Anova data not shown). The CV ranger from 1.7% for leaf width to 18.2% for yield (Table 2). According to Lúcio *et al.* (2011), when considering the green mass of plants, there is greater variability within the experimental unit due to the variation in the water content of each plant, directly influencing the coefficient of

variation. This information is corroborated by Paixão *et al.* (2016), in which there was reduction in 10.7% in the CV between green mass and dry mass of lettuce.

Table 2. Average values of each variable evaluated in lettuce cv. ‘Veneranda’ in each of the experiments. Average of four treatments and five repetitions.

Tabela 2. Valores médios de cada variável avaliada na alface cv. Veneranda em cada período experimental. Média de quatro tratamentos e cinco repetições.

Variables	Experiments periods				CV (%)
	Jun-Jul/20	Aug-Sep/20	Dec/20-Jan/21	Feb-Mar/21	
Stand (plants/ha)	103,400.0 b	107,387.5 a	109,037.5 a	106,287.5 a	2.4
Height of plants (cm)	19.2 b	23.5 a	18.8 b	18.9 b	6.7
N° leaves	13.9 c	18.4 a	17.1 b	19.1 a	8.2
Leaf length (cm)	16.2 b	17.7 a	15.5 c	14.5 d	2.6
Leaf width (cm)	13.9 c	15.5 a	14.3 b	13.5 d	1.7
Shoot mass (g)	129.3 d	233.2 a	171.2 b	147.8 c	16.7
Root mass (g)	9.3 b	12.3 a	10.2 b	8.9 b	11.4
Yield (Mg/ha)	13.4 d	25.0 a	18.7 b	15.9 c	18.2

Means followed with the same letter horizontally belong to the same group by the Scott-Knott test ($p < 0.05$). CV: coefficient of variation.

Médias seguidas com letras iguais na horizontal pertencem ao mesmo grupo pelo teste Scott-Knott ($p < 0,05$). CV: coeficiente de variação.

The execution of the four experiments allowed observing the environmental effect on the morphoagronomic characters of lettuce (Table 2). It was observed that the 2nd experiment promoted the highest mean values in all variables. During this experimental period, 53.3 mm of rainfall was recorded (Table 1), so that the crop’s water demand, estimated at 124 mm during the cycle (Magalhães *et al.* 2015), was supplied by irrigation. The minimum and maximum temperatures, 18.7 and 28.4°C, respectively, associated with luminosity can explain the better performance of the ‘Veneranda’ cultivar in this period, allowing a high yield (Table 2).

In the 1st experiment rained 131.5 mm, however, poorly distributed, in which, in the first four days, precipitation of 53% (66 mm) was observed, reflecting in the reduction of the stand (seedlings dead), shoot mass and yield (Table 2). According to the sigmoid growth curve, the phenological phase of vegetative development, which presents greater leaf area, photosynthesis and transpiration, corresponds to the moment of greater water demand.

The stadium starts to the 16th day after planting (crop coefficient, Kc: 1.0), according to Santana *et al.* (2016), which evaluated the ‘Vera’ cultivar in Uberaba/MG. Silva *et al.* (2015) observed reduction of 91.3% and 66.6% in the yield of the ‘Vera’ and ‘Babá de Verão’ cultivars, in the rainy and dry periods, respectively. The authors attributed this effect to excess water, for altering the chemical, physical and biological balance of the soil, in addition to promoting physical damage to leaves due to drops and splashes of rain, and greater susceptibility to the onset of diseases.

Lettuce is a species of C₃ photosynthetic metabolism which, despite the existence of genes that confer thermotolerance (Yoong *et al.* 2016) and the ‘Veneranda’ cultivar is tolerant to bolting, typical at high temperatures, it is possible that the increase in temperature above 30°C associated with global solar radiation (Table 1), common to the region’s semi-arid climate, may have promoted photorespiration and thermal stress, reflecting in higher energy expenditure and reduction of the characters evaluated in the 3rd and 4th experiment.

Considering that this is an organic production system, without the addition of synthetic fertilizers, it is possible that the low mineralization of organic matter has not met the nutritional demand of the ‘Veneranda’ cultivar. Also in the organic system, Silva *et al.* (2015) observed yield in the dry period of 5.9 and 7.6 Mg/ha of ‘Vera’ and ‘Babá de Verão’ cultivars, respectively. Higher yields were observed by Meneses *et al.* (2016), in Sergipe/Brazil, using mineral and organic fertilizers and black mulching, with yield of 49.0 Mg/ha for ‘Vera’ cultivar.

In general, the repeatability coefficients were of medium magnitude, according to the classification by Resende (2002). Using the covariance method, the coefficients ranged from 0.31 for shoot mass to 0.86 for root mass. By the correlation method, it ranged from 0.25 (low magnitude) for the number of leaves to 0.81 for the root mass (Table 3). The lower the repeatability coefficient, the lower the regularity of the variable studied between production cycles (experiments). Therefore, the variables root mass and plant height showed greater regularity (Table 2), reflecting in the high magnitude repeatability coefficients and in the low number of measurements (Table 3). This information is in agreement with Cargnelutti Filho *et al.* (2006), who observed an inverse relationship between the repeatability coefficient and the number of experiments.

Table 3. Estimates of repeatability (R) and determination (R²) coefficients and number of experiments by principal component analysis using covariance and correlation methods.

Tabela 3. Estimativas dos coeficientes de repetibilidade (R) e de determinação (R²) e número de experimentos pela análise dos componentes principais usando os métodos da covariância e correlação.

Variables	Principal component analysis					
	Covariance			Correlation		
	R	R ²	N° experiments	R	R ²	N° experiments
Stand	0.40	0.73	5.7	0.41	0.73	5.7
Height of plants	0.75	0.92	1.8	0.43	0.75	7.5
N° leaves	0.61	0.84	4.9	0.25	0.77	16.9
Leaf length	0.57	0.84	4.2	0.53	0.82	4.9
Leaf width	0.39	0.72	6.1	0.48	0.78	4.2
Shoot mass	0.31	0.75	8.8	0.30	0.73	9.3
Root mass	0.86	0.96	0.6	0.81	0.95	0.9
Yield	0.50	0.80	3.9	0.46	0.77	4.7

According to the coefficients of determination associated with the estimates of the repeatability coefficients, all presented high magnitude ($R^2 > 0.72$), regardless of the method used and the variable analyzed (Table 3), indicating that the number of experiments will allow us to indicate, with accuracy, the best treatment (soil cover).

The yield is the most important response-variable. Using the covariance method, a repeatability coefficient of 0.5 (mean magnitude) was obtained, indicating average regularity between production cycles (Table 2), in which 3.9 (4.0) experiments are sufficient to indicate the best treatment, with 80% reliability (R^2). Increasing the number of experiments will imply little gain in accuracy. However, it will reflect an increase in project costs and labor.

The 'Veneranda' cultivar consists of a genetically stable material. Although, in cases where the genotypes are still in the breeding phase, with genetic variability, it is expected that the values of the repeatability coefficient have a low magnitude, reflecting a greater number of measurements and experiments, since this parameter (R) varies with the environmental conditions and with the genetic properties of the population studied.

The most important characters in the discrimination of treatments were the number of leaves, leaf length and plant height, respectively, accumulating 77.32% of the total data variation, according to Singh's criteria (Table 4).

Table 4. Relative importance (%), according to Singh's criteria, and Pearson correlations of morphoagronomic characters of lettuce cv. 'Veneranda'.

Tabela 4. Importância relativa (%), segundo critério de Singh, e correlações de Pearson de caracteres morfoagronômicos da alface cv. Veneranda.

	Stand	Height	Leaves		Mass		Yield	
			Nº	Length	Width	Shoot		Root
Stand	1.98¹	0.25	0.29	0.23	0.28	0.30	0.38	0.47
Height		17.06	0.41	0.77**	0.75**	0.81*	0.92*	0.80*
NL			39.47	0.04	0.28	0.62*	0.22	0.64*
Length				20.79	0.88**	0.67*	0.86**	0.66*
Width					0.00	0.82*	0.99**	0.81*
SM						11.58	0.96**	0.98*
RM							0.00	0.94*
Yield								9.12

¹Relative importance in diagonal (bold). ** and *: significant to 1 and 5%, respectively. NL: number of leaves; SM: shoot mass; RM: root mass.

¹Importância relativa na diagonal (negrito). ** e *: significativo a 1 e 5%, respectivamente. NL: Número de folhas; SM: massa da parte aérea; RM: massa da raiz.

The leaf width and root mass variables presented zero contribution values (Table 4), in agreement with Azevedo *et al.* (2013), which also obtained a low relative contribution for the variables shoot mass (1.85%) and root mass (4.64%), however, similar for plant height (26.7%). For the number of leaves, Silveira *et al.* (2019) observed a contribution of 25.3%, similar to the results of this research, indicating that this is an important variable when evaluating morphoagronomic characters of lettuce.

Using Tocher's multivariate clustering method, three dissimilar groups were formed: I: geosynthetic blanket and black mulching; II: white mulching; and III: uncovered soil. The 1st group, both dark covers, had a similar influence on the morphoagronomic characters of lettuce cv. 'Veneranda', mainly due to the effect on soil temperature, making it higher, as observed by Oliveira *et al.* (2021), also in Arapiraca/AL, with soil temperature of 41.5°C (geosynthetic blanket), observed reduction of stand and yield of *Capsicum annuum* L. This temperature increase occurs due to less reflection and greater absorption and transmission of solar energy on dark surfaces (Jahan *et al.*, 2018).

When performing the Tocher cluster, discarding the leaf width and root mass variables, there was no change in the formation and original composition of the groups, indicating that these characters could be discarded in future studies of soil cover. In addition to this aspect, the variable leaf width is directly related to shoot mass and yield; root mass is directly related to plant height, leaf length, shoot mass and yield, considering that these variables have a high magnitude and positive linear correlation (Table 4).

Despite the edaphoclimatic conditions of the producing regions and the diversity of types of lettuce that are cultivated in the World, the results showed that the interaction of soil cover × experiments influence the morphoagronomic characters of the 'Veneranda' cultivar, with reflections on the number of repetitions of the experiments and also, on the importance of the variables-response. This information may help researchers during the experimental planning, by demonstrating the need to repeat the experiments and that the selection of variables will be able to discriminate, more assertively, the evaluated treatments, maximizing physical, human and financial resources.

4 Conclusion

The estimated number of experiments varies from 1 to 9, according to the studied response-variable. For yield, four experiments are enough. The most discriminating variables are the number of leaves, leaf length and plant height. Leaf width and root mass may be discarded in future evaluations. The results show that a single evaluation cycle (experiment) is not enough to promote accurate estimates of morphoagronomic variables in lettuce cv. 'Veneranda'. This information can be useful when experiments planning with lettuce.

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