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PLANEJAMENTO EXPERIMENTAL PARA MUDAS DE COUVE-FLOR E PLANTAS DE ALFACE: DEFINIÇÃO DO TAMANHO AMOSTRAL ÓTIMO

Frederico Westphalen, RS
2023

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Dissertação apresentada ao Curso de Pós-Graduação em Agronomia – Agricultura e Ambiente, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Mestre em Agronomia.**

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Karina Chertok Bittencourt

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DEDICATÓRIA

Aos meus queridos pais, Ana Gabriela Bittencourt Gimenez e José Alberto Chertok Voskoboynik.

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"A verdadeira viagem de descobrimento não consiste em procurar novas paisagens,

mas em ter novos olhos."

Marcel Proust

RESUMO

PLANEJAMENTO EXPERIMENTAL PARA MUDAS DE COUVE-FLOR E PLANTAS DE ALFACE: DEFINIÇÃO DO TAMANHO AMOSTRAL ÓTIMO

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ORIENTADOR: Marcos Toebe

A definição do tamanho amostral é uma importante etapa do planejamento de experimentos, pois a coleta de uma amostra suficientemente representativa é essencial para obter resultados confiáveis. Entretanto, o tamanho de amostra ótimo pode variar segundo a espécie, caracteres avaliados e estatísticas posteriormente estimadas. Nesse sentido, pesquisas com culturas olerícolas de importância econômica, como a couve-flor e a alface, comumente têm utilizado tamanhos amostrais pequenos, em vista da escassez de trabalhos com foco no seu dimensionamento amostral. Dessa forma, o presente trabalho teve como objetivo geral otimizar o planejamento experimental de experimentos com as culturas da couve-flor e da alface por meio do dimensionamento amostral para diferentes estatísticas e caracteres. Assim, um experimento em ambiente protegido com mudas de couve-flor foi conduzido na área experimental da Universidade Federal do Pampa, Campus Itaqui, e um experimento a campo com 26 genótipos de alface foi conduzido na área experimental da Universidade Federal de Santa Maria, Campus Frederico Westphalen. Em mudas de couve-flor, os seguintes caracteres foram avaliados: número de folhas, altura de planta, comprimento da raiz e comprimento total (altura de planta + comprimento de raiz), e em plantas de alface, a produtividade por planta (massa fresca em gramas), o número de folhas, a altura de planta, diâmetro do colo e o diâmetro médio da cabeça foram avaliados. Estatísticas de precisão foram estimadas, obtendo-se a amplitude dos seus intervalos de confiança a 95%. Cem cenários amostrais foram simulados para cada estatística e caractere utilizando reamostragem *bootstrap* com reposição, e tamanhos amostrais ótimos foram definidos ajustando os intervalos de confiança de 95% a modelos da família potência e encontrando o ponto máximo de curvatura. Além disso, quatro métodos para obter o ponto de máxima curvatura foram comparados, e equações preditivas para estatísticas de precisão com base no tamanho amostral foram propostas. A amplitude do intervalo de confiança a 95% das estatísticas reduziu conforme o tamanho amostral aumentou, até um ponto de estabilização. O método de distâncias perpendiculares foi considerado o mais eficiente para definir o ponto de máxima curvatura. Os tamanhos amostrais variaram conforme estatísticas e caracteres, sendo esta variação maior entre estatísticas. A estatística F destacou-se por obter tamanhos amostrais maiores em todos os estudos. As equações preditivas apresentaram excelente qualidade de ajuste, permitindo conhecer os valores médios, máximos e mínimos de estatísticas de precisão a partir da seleção de um tamanho amostral específico. Assim, as informações trazidas por estes estudos contribuem para otimizar o planejamento experimental das culturas da couve-flor e da alface e serão úteis para pesquisadores da área que desejam avaliar a precisão experimental por meio das estatísticas e caracteres descritos.

Palavras-chave: *Brassica oleracea*, *Lactuca sativa*, estatísticas de precisão, horticultura, reamostragem *bootstrap*.

ABSTRACT

EXPERIMENTAL PLANNING FOR CAULIFLOWER SEEDLINGS AND LETTUCE PLANTS: DEFINITION OF THE OPTIMAL SAMPLE SIZE

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ADVISOR: Marcos Toebe

Defining the sample size is an important step in the planning of experiments, as collecting a sufficiently representative sample is essential to obtain reliable results. However, the optimal sample size may vary depending on the species, evaluated characters, and the subsequently estimated statistics. In this sense, research on economically important vegetable crops, such as cauliflower and lettuce, has commonly used small sample sizes, given the scarcity of studies focusing on their sample dimensioning. Therefore, the present work aimed to optimize the experimental planning of experiments with cauliflower and lettuce crops through sample sizing for different statistics and characters. Thus, a greenhouse experiment with cauliflower seedlings was conducted in the experimental area of the Federal University of Pampa, Itaqui Campus, and a field experiment with 26 lettuce genotypes was conducted in the experimental area of the Federal University of Santa Maria, Frederico Westphalen Campus. For cauliflower seedlings, the following characters were assessed: number of leaves, plant height, root length, and total length (plant height + root length), and for lettuce plants, the yield per plant (fresh weight in grams), number of leaves, plant height, stem diameter, and mean head diameter were evaluated. Precision statistics were estimated, obtaining the 95% confidence interval width. One hundred sampling scenarios were simulated for each statistic and character using bootstrap resampling with replacement, and optimal sample sizes were defined by adjusting the 95% confidence intervals to models of the power family and finding the maximum curvature point. Furthermore, four methods for obtaining the maximum curvature point were compared, and predictive equations for precision statistics based on sample size were proposed. The 95% confidence interval width of the statistics reduced as the sample size increased, until a point of stabilization. The perpendicular distance method was considered the most efficient for defining the maximum curvature point. The sample sizes varied according to statistics and characters, with this variation being greater between statistics. The F statistic stood out for obtaining larger sample sizes in all studies. The predictive equations presented excellent fitting quality, which allows for knowing the mean, maximum, and minimum values of precision statistics based on the selection of a specific sample size. Thus, the information provided by these studies contributes to optimizing the experimental planning of cauliflower and lettuce crops and may be useful for researchers in the field who wish to evaluate experimental precision through the statistics and characters described.

Keywords: *Brassica oleracea*, *Lactuca sativa*, precision statistics, horticulture, bootstrap resampling.

SUMÁRIO

1 INTRODUÇÃO

O planejamento prévio à realização de experimentos é de extrema importância para prever e evitar problemas durante a sua condução, além de possibilitar a otimização de recursos e mão-de-obra destinados aos mesmos (FREEMAN et al., 2013). Escolhas relacionadas ao uso de delineamentos experimentais, número de tratamentos e repetições e definição e delimitação da área, são realizadas antes da implantação de um experimento. Entretanto, dentre os aspectos englobados pelo planejamento experimental, uma importante decisão que normalmente é tomada de forma empírica é a definição do tamanho amostral a ser utilizado para a coleta de dados (SOUZA et al., 2022). Na pesquisa agronômica, esta definição refere-se, na maioria das vezes, ao número de plantas que serão avaliadas ao final do período experimental. Sendo assim, quando delineamentos experimentais são utilizados, é necessário considerar as restrições que estes impõem (STORCK et al., 2016), determinando, portanto, um número de plantas a serem avaliadas dentro de cada unidade experimental (SOUZA et al., 2022).

A importância do tamanho amostral tem sido destacada na literatura em várias ocasiões (CONFALONIERI et al., 2009; ANDERSON et al., 2017; POLITI et al., 2023), uma vez que a representatividade da amostra pode ser comprometida em razão da utilização de tamanhos amostrais inadequados. Tamanhos de amostra considerados insuficientes, isto é, extremamente pequenos, podem prejudicar a confiabilidade dos resultados obtidos por meio da análise dos dados oriundos desta amostra (PIÑERA-CHAVEZ et al., 2020). Por outro lado, a seleção de um número excessivo de observações resulta em maior tempo de avaliação, o qual pode afetar diretamente a mensuração de variáveis, principalmente se tratando de organismos vivos, além de gerar uma maior necessidade de recursos e mão-de-obra (TOEBE et al., 2018). Dessa forma, a definição de um tamanho amostral ótimo tem sido o foco de diversos trabalhos ao longo do tempo (TOEBE et al., 2019; CARGNELUTTI FILHO e TOEBE, 2020, 2021, 2022; SHIMANDA et al., 2020; SOUZA et al., 2023a, 2023b, 2023c, 2023d). Entretanto, tais pesquisas destacam que este número pode variar de acordo com a cultura utilizada e, inclusive, as características avaliadas, as condições experimentais e as análises posteriores.

No estudo de Toebe et al. (2019), ao determinar o tamanho de amostra para estimar coeficientes de correlação linear de Pearson em espécies de crotalária, constataram que os tamanhos de amostra ótimos variaram de 10 a 440 vagens, dependendo da espécie, variável e magnitude do coeficiente de correlação. Além disso, para esta mesma cultura, tamanhos de amostra maiores foram encontrados para estimar a média, em comparação ao coeficiente de variação experimental (TOEBE et al., 2018). Em milho, Cargnelutti Filho e Toebe (2020) definiram um tamanho amostral total de 260 plantas por ensaio para ajustar modelos de regressão múltipla de forma precisa, enquanto para a análise de componentes principais, os mesmos autores encontraram um tamanho de amostra de 267 plantas (CARGNELUTTI FILHO e TOEBE, 2021) e de 270 plantas para análise de correlação canônica (CARGNELUTTI FILHO e TOEBE, 2022). Para experimentos realizados com a cultura da soja, ao definir o tamanho amostral por unidade experimental, Souza et al. (2022) concluíram que 18 plantas foram suficientes para estimar estatísticas de precisão, à exceção da estatística F, para a qual 41 plantas por parcela foram necessárias. Por outro lado, a amostragem de 17 a 20 plantas de soja foi considerada suficiente para otimizar o teste de Bartlett em soja (SOUZA et al., 2023c), enquanto o teste de não aditividade de Tukey requereu de 14 a 19 plantas por unidade experimental (SOUZA et al., 2023e). Estes exemplos ilustram a importância da definição de tamanhos amostrais ótimos para diferentes culturas e técnicas, considerando a mensuração de diferentes variáveis, a fim de aumentar a base de recomendações que contribuam para a padronização da amostragem, aumentando, assim, a confiabilidade dos resultados das pesquisas.

Nesse sentido, diversas culturas ainda carecem de recomendações de tamanhos amostrais ótimos, principalmente visando à realização de análises específicas, que incluem a estimativa de diferentes estatísticas, encontrando-se na literatura um menor número de estudos de dimensionamento amostral com foco em espécies olerícolas. Dentre estes estudos, destacam-se os trabalhos de Sari et al. (2017) com tomate cereja (*Solanum lycopersicum* var. *cerasiforme*), Krysczun et al. (2018) com a cultura da beringela (*Solanum melongena*) e Alves et al. (2022) com genótipos de pimenta (*Capsicum chinense*). Entretanto, outras olerícolas de importância econômica, como as culturas da couve-flor (*Brassica oleracea* var. *botrytis*) e da alface (*Lactuca sativa*), têm recebido menos atenção em estudos de dimensionamento amostral. Ambas as espécies são amplamente cultivadas no mundo, principalmente devido ao seu alto valor nutricional e sua utilização no preparo de diversos pratos (BHATTACHARJEE e SINGHAL, 2018; YANG et al., 2022). Por esse motivo, estas olerícolas são alvo de inúmeros estudos agronômicos que visam ao aumento da produtividade das mesmas (WU et al., 2012; RAY e MISHRA, 2019; MUSTAFA et al., 2023; YAVUZ et al., 2023). Contudo, observa-se uma grande variação do número de plantas amostradas para a realização destas pesquisas, ressaltando a necessidade de padronização do mesmo.

Somado a isso, é importante destacar que metodologias mais robustas têm sido recentemente utilizadas em estudos de dimensionamento amostral (SOUZA et al., 2022, 2023b), incluindo a simulação de um grande número de cenários amostrais por meio da reamostragem *bootstrap* (EFRON, 1979) e a posterior definição de um ponto máximo de curvatura, para o qual vários métodos podem ser utilizados. Esta abordagem apresenta vantagens em relação a outros métodos onde o tamanho amostral é determinado por meio de equações pré-definidas, como a equação de Cochran (1977), anteriormente utilizada para o dimensionamento amostral para a cultura da alface (SANTOS et al., 2010), pois permite a definição do tamanho de amostra ótimo para várias técnicas e estatísticas. Ainda, outros avanços envolvendo o planejamento experimental demonstram-se promissores, como as equações preditivas propostas por Souza et al. (2022), as quais permitem conhecer o valor de estatísticas de precisão a partir de um dado tamanho de amostra em ensaios com soja. Estas equações possuem potencial de calibração e aplicação para outras culturas e estatísticas (SOUZA et al., 2022, 2023b). Nesse contexto, estudos com foco no dimensionamento amostral para olerícolas de importância econômica, aliados à investigação de diferentes metodologias e da predição da precisão experimental, tornam-se úteis para os pesquisadores da área, funcionando como um guia para otimizar o planejamento experimental, garantindo maior confiabilidade aos resultados obtidos.

2 HIPÓTESES

Considerando o abordado no tópico anterior, formularam-se as seguintes hipóteses:

- O ponto máximo de curvatura utilizado como referência para a determinação do tamanho amostral ótimo diferirá segundo a metodologia utilizada para encontrá-lo.
- Estatísticas de precisão experimental exibirão uma resposta distinta entre si à variação do número de plantas amostradas por unidade experimental.
- A amplitude dos intervalos de confiança a 95% poderá ser modelada para o dimensionamento amostral por unidade experimental para estatísticas de precisão em mudas de couve-flor e plantas de alface.
- A construção de modelos preditivos a partir de reamonstragem *bootstrap* permitirá prever a precisão experimental para mudas de couve-flor e plantas de alface.

3 OBJETIVO GERAL

Este trabalho teve como objetivo otimizar o planejamento experimental de experimentos com as culturas da couve-flor e da alface por meio do dimensionamento amostral para diferentes estatísticas e caracteres.

4 OBJETIVOS ESPECÍFICOS

- Comparar métodos utilizados para definir o tamanho amostral ótimo por unidade experimental para mudas de couve-flor.
- Analisar a resposta da análise de variância à variação do tamanho amostral por unidade experimental em experimentos com mudas de couve-flor.
- Definir o tamanho amostral por unidade experimental ótimo para estimar estatísticas de precisão experimental para as culturas de couve-flor e da alface.
- Propor e adaptar modelos preditivos para estimar a precisão de experimentos com couve-flor e alface.

5 ARTIGO 1 – WHAT IS THE BEST WAY TO DEFINE SAMPLE SIZE FOR CAULIFLOWER SEEDLINGS?

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5.1 ABSTRACT

The aim of this study was to compare four methods for defining the ideal sample size per experimental unit to estimate the overall experimental mean for the total length, shoot length, root length, and the number of leaves of cauliflower seedlings. An experiment was carried out where the number of leaves, shoot, root, and total length were measured, and the general, perpendicular distance, linear response plateau, and spline methods were tested. While the general method may under or overestimate sample size and the sampling of 10 seedlings suggested by the spline method is still too far from the stabilization point of the curve, the perpendicular distance and linear plateau response methods are recommended for obtaining results corresponding to narrower confidence interval widths. Therefore, according to the perpendicular distance method, at least 15 seedlings per experimental unit are required to estimate the overall experimental mean of cauliflower seedlings reliably for the traits here measured.

Keywords: *Brassica oleracea*, horticulture, experimental planning, maximum curvature point.

5.2 RESUMO

O objetivo deste estudo foi comparar quatro métodos para definir o tamanho amostral ideal por unidade experimental para estimar a média experimental geral para o comprimento total, comprimento de parte aérea, comprimento de raiz e número de folhas de mudas de couve-flor. Um experimento foi conduzido no qual foram medidos o número de folhas, o comprimento total, de parte aérea e de radícula, e foram testados os métodos: geral, de distâncias perpendiculares, resposta linear platô e spline. Enquanto o método geral pode sub ou superestimar o tamanho amostral e a amostragem de 10 mudas sugerida pelo método spline ainda se encontra muito distante do ponto de estabilização da curva, os métodos de distâncias perpendiculares e resposta linear platô são recomendados por obter resultados que corresponderam a amplitudes de intervalos de confiança menores. Portanto, de acordo com o método de distâncias perpendiculares, pelo menos 15 mudas por unidade experimental são necessárias para estimar a média experimental geral de mudas de couve-flor confiavelmente para os traços aqui mensurados.

Palavras-chave: *Brassica oleracea*, horticultura, planejamento experimental, máximo ponto de curvatura.

5.3 SCIENTIFIC NOTE

Different methodologies have been proposed for defining sample size based on the maximum curvature point (Federer 1955), such as the general, perpendicular distance, linear plateau response, and spline methods (Silva & Lima 2017). However, Cargnelutti Filho et al. (2021) showed that different methods obtained quite different results when defining optimal plot size for several crops, making the selection of the method the first crucial step in sample size definition since, if not appropriate, it may lead to unrepresentative numbers. Also, little attention has been given to the definition of sample size per experimental unit, that is, considering experimental restrictions, which are present in experiments that use experimental designs, being this the case for experiments performed with most horticultural crops.

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is an example of a widely studied horticultural crop that has been the object of several experiments thorough the years in which different sample sizes have been chosen empirically, once the lack of standardization for this number can be easily visualized in the literature. While Thomson et al. (2013) assessed 20 cauliflower plants per plot, Tempesta et al. (2019) used a sample of 5 plants from each experimental unit, and Costa et al. (2020) collected only 1 plant per cultivar. Thus, a recommendation of the number of plants to be collected per experimental unit based on the comparison of methods may be extremely useful to researchers that perform experiments with cauliflower seedlings, facilitating experimental planning and the obtention of more reliable results. Therefore, the aim of this study was to compare four methods for defining the ideal sample size per experimental unit to estimate the overall experimental mean for the total length, shoot length, root length, and the number of leaves of cauliflower seedlings.

The experiment was conducted in a greenhouse at the Federal University of Pampa (UNIPAMPA), Itaqui, Rio Grande do Sul, Brazil. Cultivar Teresópolis Gigante was sown using three substrates (50% Mecplant® + 50% Carolina Padrão®, 75% Mecplant® + 25% rice husk, and 75% Carolina Padrão® + 25% rice husk), in 72 and 128 cell-trays (3x2 two-factor scheme) with four replications, in a completely randomized design. Thirty days later, twenty seedlings were randomly collected from each experimental unit, considering higher sample numbers are rarely used in cauliflower studies (Thomson et al. 2013; Tempesta et al. 2019; Costa et al. 2020). Then, the following traits were measured: a) Number of Leaves (NL) in units, b) Shoot Length (SL), from neck to leaflet insertion, in cm; c) Root Length (RL), from neck to root apex, in cm; and d) Total Length (TL), as the sum of SL and RL, in cm. Other experiments with 1, 2, …, 100 seedlings per experimental unit were simulated using bootstrap resampling, with 10,000 resamples with repositioning (Efron 1979).

The statistical analyses were performed using R software (R Development Core Team 2021) in several of its functions, and R package soilphysics (Silva & Lima 2015) according to the applications carried out to determine the sample size by Silva & Lima (2017). After subdividing the database per experimental unit, these sample sizes were subjected to analysis of variance, performed according to the following model: $Y_{ijk} = m + T_i + S_j + (TS)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the value observed in the response variable in plot *ijk*, *m* is the overall mean, T_i is the fixed effect of level *i* (i = 1 and 2) of the tray-cell-size factor, *Sj* is the fixed effect of level *j* (j = 1, 2, 3) of the substrate factor, $(TS)_{ij}$ is the interaction fixed effect of level *i* of the tray-cell-size factor with level *j* of the substrate factor and ε_{ijk} is the experimental error effect (Storck et al. 2016). Thereafter, m effect was extracted from this model in each resampling, using specific routines with *sample*() and *aov*() functions.

Resamplings for each planned sample scenario were subjected to descriptive analysis defining minimum, percentiles of 2.5, mean, percentiles of 97.5, and maximum values. 95% confidence interval width ($CI_{95\%}$) was estimated from the difference between percentiles of 97.5 and percentiles of 2.5. Next, $CI_{95\%}$ estimates were fitted using *nls*() function through the following power model: $CI_{95\%} = \alpha \times n^{\beta} + \varepsilon$, where α is the coefficient of interception, n, sample size, β, exponential rate of decay, and ε, random effect error. Posteriorly, four methods for determining the maximum curvature point were used: the general, perpendicular distances, linear plateau response, and spline methods, according to Silva & Lima (2017), using *maxcurv*() function from soilphysics package (Silva & Lima 2015). This point was considered the representative sample size.

In the reference experiment, the effects of the substrate, tray-cell size, and substrate × tray-cell size interaction factors were significant. As expected, for all traits, $CI_{95\%}$ decreased exponentially as sample size increased up to a stabilization point (Fig. 1), that is, the sampling of 1 seedling corresponds to a much wider $CI_{95\%}$ compared with the sampling of 100 seedlings per experimental unit. This reflects the higher the number of seedlings collected, the more representative the sample (Siegel 2016), once too small sample sizes may subject results to over or underestimation (Cargnelutti Filho et al. 2018). Nevertheless, the mean property of m was constant for all traits (4.62 units for NL, 7.82 cm for SL, 8.51 cm for RL, and 16.34 cm for TL), which was also observed by Toebe et al. (2018), who reported this statistic as a non-biased estimator. Moreover, power models presented satisfactory fitting-quality (Moinester & Gottfried 2014), verified through the coefficient of determination (R^2), root mean square error (RMSE), and d index (Table 1).

Although the optimal sample size variated slightly between traits, the four methods led to quite different results (Fig. 2). Whilst the general method considered two seedlings were enough, the others required either 10 (spline), 15 (perpendicular distance), or even 19 seedlings per experimental unit (linear plateau response) to estimate *m* reliably. However, considering the $CI_{95\%}$ observed when sampling only two seedlings, such a low number would most likely lead to unreliable estimates. Also, the sampling of 10 seedlings per experimental unit is still too far from the stabilization point of the curve, meaning the general and spline methods may not be ideal choices for the conditions under study.

Moreover, although both perpendicular distance and linear plateau response methods presented representative sample numbers, the precision gain obtained by the linear plateau response in relation to the perpendicular distance method is too little to justify selecting the first over the latter. Thus, although both could be used reliably, the results obtained using the perpendicular distance method may come up as more efficient under the practical perspective of researchers for being closer to the minimum sufficient number of seedlings to be sampled per experimental unit enough to reach high precision, avoiding collecting greater samples, as recommended by the linear plateau response, which often requires more resources and manpower. Therefore, we highly encourage the sampling of at least 15 cauliflower seedlings per experimental unit in order to estimate the overall experimental mean reliably for the traits here measured.

In this sense, even if the sampling recommendations here proposed might be highly practical and efficient for optimizing experimental planning of experiments with cauliflower seedlings, considering the majority of them use experimental restrictions, these should not be applied to other horticultural crops without performing preliminary studies, as well as the method comparison here presented should only serve as a basis to researchers who aim at defining sample size for other species from the Brassicaceae family.

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5.5 TABLE

Table 1 - Coefficient of determination (R^2), root mean square error (RMSE) and d index of the power models, and maximum curvature points and sample sizes for the overall experimental mean of the number of leaves (NL), shoot length (SL), root length (RL) and total length (TL) of cauliflower seedlings.

Trait	Power model	R^2	RMSE	d index
NL	$CI_{95\%} = 0.5019 \times n^{-0.4985}$	0.9995	0.0015	0.9999
SL	$CI_{95\%} = 3.7533 \times n^{-0.5466}$	0.9588	0.1009	0.9892
RL	$CI_{95\%} = 1.2576 \times n^{-0.5017}$	0.9998	0.0022	0.9998
TL	$CI_{95\%} = 4.1439 \times n^{-0.5534}$	0.9837	0.0703	0.9957

Trait	Maximum curvature method	Maximum Curvature	Maximum $CI_{95\%}$	Sample size
NL	General method	1.0000	0.5019	1
NL	Spline method	9.9940	0.1593	10
NL	Perpendicular distance method	14.4865	0.1324	15
NL	Linear plateau response method	18.9407	0.1158	19
SL	General method	1.4951	3.0126	2
SL	Spline method	9.4000	1.1028	10
SL	Perpendicular distance method	13.9518	0.8887	14
SL	Linear plateau response method	16.9607	0.7987	17
RL	General method	1.0000	1.2576	1
RL	Spline method	9.9542	0.3971	10
RL	Perpendicular distance method	14.4469	0.3294	15
RL	Linear plateau response method	18.8022	0.2886	19
TL	General method	1.5941	3.2014	2
TL	Spline method	9.3205	1.2048	10
TL	Perpendicular distance method	13.8726	0.9668	14
TL	Linear plateau response method	16.7023	0.8724	17

5.6 FIGURES

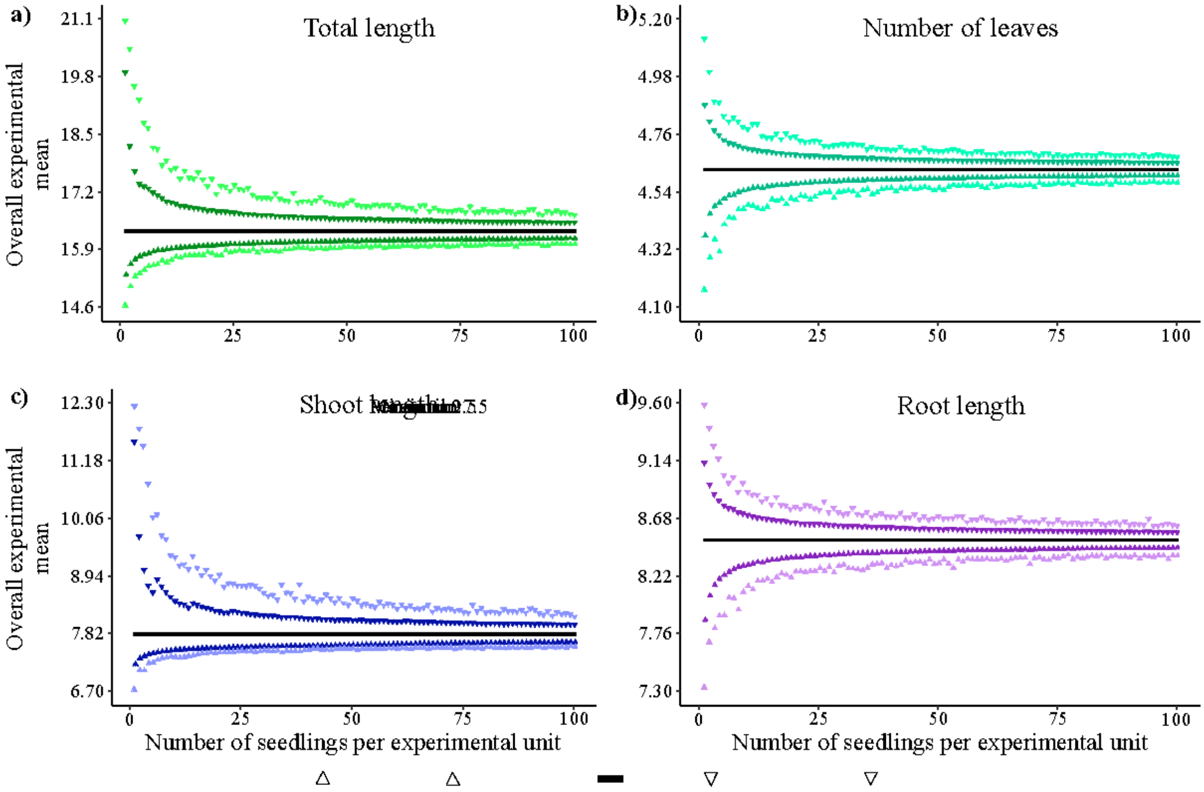

Figure 1 - Minimum, 2.5 percentile, mean, 97.5 percentile, and maximum values of the overall experimental mean of cauliflower seedlings.

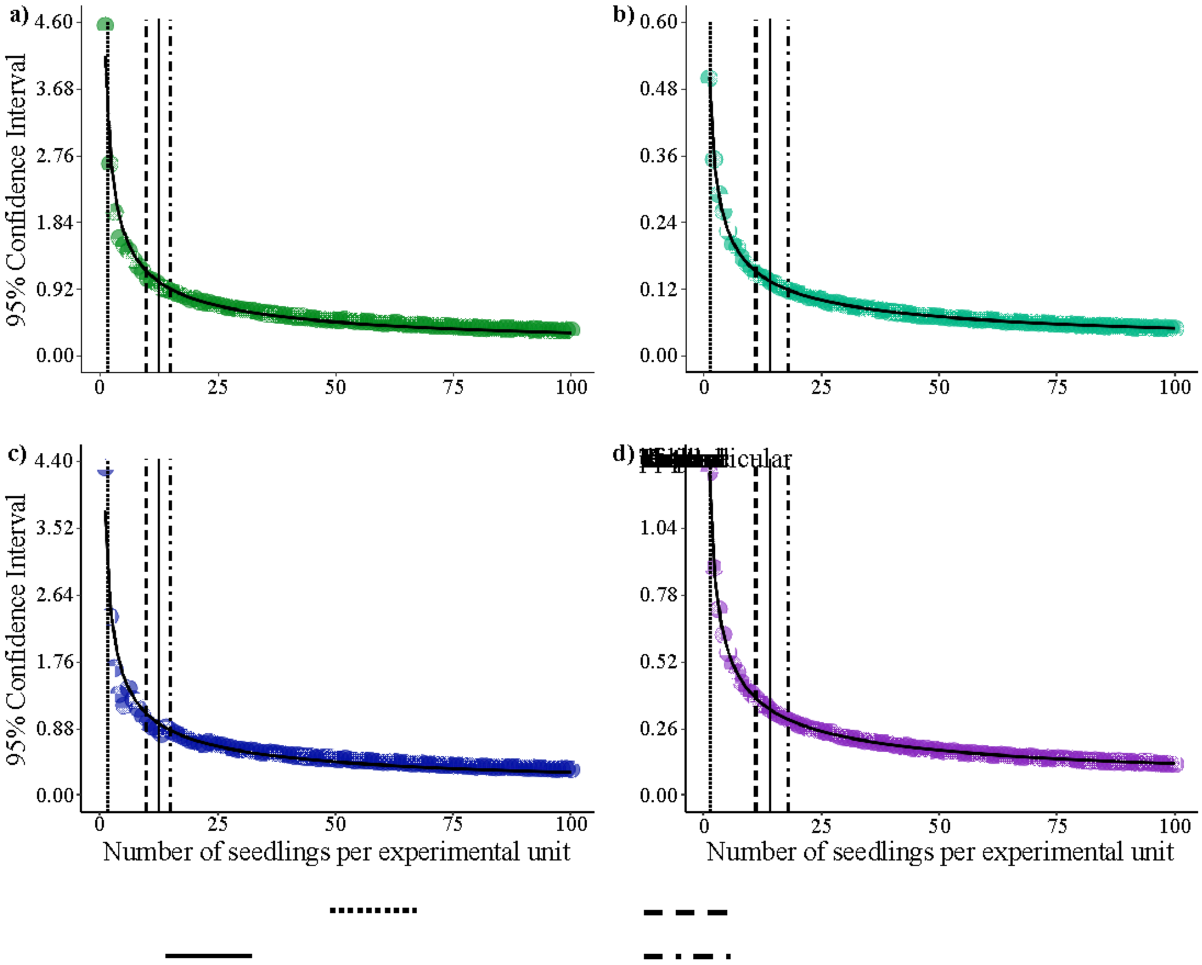

Figure 2 - Sample size determination via power model and maximum curvature points for estimating the overall experimental mean of cauliflower seedlings.

6 ARTIGO 2 – SAMPLE SIZE AFFECTS THE PRECISION OF THE ANALYSIS OF VARIANCE IN EXPERIMENTS WITH CAULIFLOWER SEEDLINGS

(Formatação da revista Ciência Rural)

Publicado no periódico: Ciência Rural

Situação: Publicado

6.1 ABSTRACT

The aim of this study was to verify whether sample size would affect the precision of the analysis of variance in experiments with cauliflower seedlings. An experiment was carried out where the number of leaves, shoot, root and total length were measured. For each variable, resamplings with repositions were performed in sample scenarios of 1, 2, …, 100 seedlings per experimental unit and sample size was defined for the variance components through Schumacher models and maximum curvature points. The mean squares of the analysis of variance suffer direct interference of the number of sampled seedlings. The sampling of 16 seedlings per experimental unit is enough to estimate the analysis of variance reliably, promoting satisfactory precision gains compared to the sampling of only one seedling per experimental unit.

Key words: *Brassica oleracea*, horticulture, experimental planning, precision gain.

6.2 RESUMO

O objetivo deste estudo foi verificar se o tamanho de amostra afeta a precisão da análise de variância em experimentos com mudas de couve-flor. Um experimento foi conduzido onde o número de folhas, comprimento de parte aérea, raiz e total foram mensurados. Para cada variável, reamostragens com reposição foram realizadas em cenários amostrais de 1, 2, ..., 100 mudas por unidade experimental e o tamanho de amostra foi definido para os componentes de variância por meio de modelos de Schumacher e pontos de máxima curvatura. Os quadrados médios da análise de variância sofrem interferência direta do número de mudas amostradas. A amostragem de 16 mudas por unidade experimental é suficiente para estimar a análise de variância de forma confiável, promovendo satisfatórios ganhos de precisão ao comparar-se com a amostragem de apenas uma muda por unidade experimental.

Palavras-chave: *Brassica oleracea*, horticultura, planejamento experimental, ganho de precisão.

6.3 SCIENTIFIC NOTE

In a previous work, four methods based on the maximum curvature point were compared to determine the optimal sample size per experimental unit to estimate the overall experimental mean of cauliflower (*Brassica oleracea* L. var. botrytis) seedlings (BITTENCOURT et al., 2022), where a reduction in the 95% confidence interval width ($CI_{95\%}$) of the statistic was verified as sample size increased, up to a stabilization point. Thus, the methods that found values closer to the stabilization point of the curve were chosen, once precision gain up from this point would no longer be enough to justify increasing the number of sampled plants (CARGNELUTTI FILHO et al., 2018; SOUZA et al., 2022). That example highlights the importance of quantifying precision gain when defining sample size, which would not only facilitate the decision on the number of plants to be sampled per experimental unit but would also guarantee a minimum acceptable precision to the results. However, the previous approach focused only on the overall experimental mean without exploring other components of the analysis of variance.

The analysis of variance is widely performed to summarize data in experiments with experimental designs (WELHAM et al., 2015). Nonetheless, in order to find actual significant differences through the F test that follows, mean squares must be estimated reliably, avoiding type I and II errors (ANDERSON et al., 2017). For this, sample size plays a crucial role, as verified by SOUZA et al. (2022) for soybean crop, based on its impact for estimating other statistics in experiments performed with crotalaria and maize (TOEBE et al., 2018; CARGNELUTTI FILHO & TOEBE, 2021). Therefore, considering that studies connecting sample size and the precision gain of the analysis of variance have not been found in the literature for horticultural crops such as cauliflower, the aim of this study was to verify whether sample size would affect the precision of the analysis of variance in experiments with cauliflower seedlings.

The experiment was carried out at the Federal University of Pampa (UNIPAMPA), Itaqui, Rio Grande do Sul, Brazil. Cauliflower cultivar Teresopolis Gigante was sown using three substrate mixtures (50% Mecplant® + 50% Carolina Padrão®, 75% Mecplant® + 25% rice husk, and 75% Carolina Padrão® + 25% rice husk), and trays with 72 and 128 cells, forming a 3x2 two-factor scheme, in a completely randomized design with four repetitions. Seedlings were kept in a greenhouse for a period of thirty days. During the sampling, twenty seedlings were randomly collected from each experimental unit, considering the sample numbers used in cauliflower experiments (THOMSON et al., 2013; TEMPESTA et al., 2019; COSTA et al., 2020). Then, the following traits were measured: a) Number of Leaves (NL) in units, b) Shoot Length (SL), from neck to leaflet insertion, in cm; c) Root Length (RL), from neck to root apex, in cm; and d) Total Length (TL), as the sum of SL and RL, in cm. Other experiments with 1, 2, …, 100 seedlings per experimental unit were simulated using bootstrap resampling, with 10,000 resamples with reposition (EFRON, 1979).

The statistical analyses were performed using native functions and packages from R software (R DEVELOPMENT CORE TEAM, 2022). First, the database was stratified into experimental units, and in each sample size, an analysis of variance was performed through the following mathematical model: $Y_{ijk} = m + T_i + S_j + (TS)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} is the value observed in the response variable in plot *ijk*, *m* is the overall mean, T_i is the fixed effect of level *i* (i = 1 and 2) of the tray-cell-size factor, *Sj* is the fixed effect of level *j* (j = 1, 2, 3) of the substrate factor, $(TS)_{ij}$ is the interaction fixed effect of level *i* of the tray-cell-size factor with level *j* of the substrate factor and ε_{ijk} is the experimental error effect. Thereafter, the mean squares of T_i, *Sj*, $(TS)_{ij}$, and ε_{ijk} were extracted in the sample scenarios per experimental unit. This process was carried out using *sample*() and *aov*() functions.

Resamplings for each planned sample scenario were subjected to descriptive analysis defining minimum, percentiles of 2.5, mean, percentiles of 97.5, and maximum values. The

95% confidence interval width ($CI_{95\%}$) was estimated as the difference between percentiles of 97.5 and percentiles of 2.5. Posteriorly, the precision gain criterion was estimated in percentage, assuming that the greater the $CI_{95\%}$, the lower the precision of the analysis-of-variance mean squares' estimates (SOUZA et al., 2022). Thus, the sample size of one seedling per experimental unit (CI_1) was taken as a reference, where the $CI_{95\%}$ is maximum and the precision is minimum. The following formula was used to estimate precision gain:

$$PG = 100 - \left(\frac{CI_i}{CI_1}\right) * 100$$

where CIi is the 95% confidence interval width, obtained from the sample sizes of 2, 3, ..., 100 seedlings per experimental unit [for further information *vide* CARGNELUTTI FILHO et al. (2018) and SOUZA et al. (2022)].

Finally, the precision gain was fitted using *nls*() function through Schumacher's model (SCHUMACHER, 1939): $PG_i = \alpha \times exp\left(\beta \times n^{-1}\right) + \varepsilon$, where PG_i is the i^{th} precision gain observation per statistic, in each n sample size, α and β are parameters of the model, *exp* is the exponential function and ε_i is the error of random effect. A maximum curvature point was defined over the fitted models through the perpendicular distances' method (SILVA & LIMA, 2017), as recommended by BITTENCOURT et al. (2022) for cauliflower, using *maxcurv*() function from soilphysics package (SILVA & LIMA, 2015).

The variance components fluctuated in response to the variation of the number of seedlings sampled per experimental unit, also varying for each specific trait (Figure 1). In all cases, $CI_{95\%}$ tends to reduce gradually as the number of sampled seedlings is increased, which means estimates become more accurate (TOEBE et al., 2018; BITTENCOURT et al., 2022; SOUZA et al., 2022). On the other hand, small sample sizes (≤ 5 seedlings per experimental unit) result in greater $CI_{95\%}$, making the mean squares estimates more biased. These results are similar to the ones observed by SOUZA et al. (2022) when analyzing the response of variance components in soybean.

From this response, it was observed that the precision of the analysis-of-variance mean squares was increased as sample size increased, establishing a direct relationship between result reliability and the number of seedlings used for data collection, especially considering the influence of the analysis of variance in the determination of significant differences between treatments. In general, the sufficient sample sizes for obtaining reliable estimates of the analysis of variance varied from 13 to 16 cauliflower seedlings per experimental unit, with precision gains oscillating from ≥ 76.52% to ≤ 93.42%, depending on the variance component and trait analyzed (Table 1 and Figure 2). These values were obtained through the parametrization of precise Schumacher models (SCHUMACHER, 1939), with coefficients of determination (R^2) ≥ 0.78, root mean square error (RMSE) oscillating from 1.43 to 4.83, and d index ≥ 0.93. Furthermore, in sample sizes ≤ 3, a considerable precision gain is observed every time there is an increase in the number of sampled seedlings. This response remains until the sampling number reaches 10 seedlings per experimental unit, up from where precision gain starts becoming lower and lower, until finally reaching the maximum curvature point, that is, the ideal sample size for each trait and variance component.

In that perspective, considering all traits and variance components jointly, the minimum sampling number of 16 seedlings per experimental unit can be recommended as sufficient to make accurate mean square estimates for the analysis of variance of experiments with cauliflower seedlings, corroborating the results obtained by BITTENCOURT et al. (2022), who suggested the sampling of at least 15 cauliflower seedlings per experimental unit to estimate the overall experimental mean. The collection of greater samples normally demands more resources and manpower that are not justified by the little precision gain obtained (TOEBE et al., 2015), and in some cases, oversampling may even result in greater variations between experimental units that can inflate the error mean square (SOUZA et al., 2022), harming the detection of significant differences between treatments due to the occurrence of type II error (ANDERSON et al., 2017). Importantly, the practical results here obtained should be applied cautiously in cauliflower seedlings' experiments with experimental designs, and should not be used for other horticultural crops without performing preliminary studies, serving only as a support to researchers that conduct experiments with other species from the Brassicaceae family.

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6.5 TABLE

Table 1 - Coefficient of determination (R^2), root mean square error (RMSE) and d index of the Schumacher models, precision gains, and sample sizes for the analysis of variance of the number of leaves (NL), shoot length (SL), root length (RL) and total length (TL) of cauliflower seedlings.

Trait	Statistic*	Schumacher model	R^2	RMSE	d	Precision gain (%)	Sample size
NL	EMS	$PG_i = 92.7268 \times exp(-2.0653 \times n^{-1})$	0.98	1.70	0.99	80.34	15
NL	TMS	$PG_i = 90.8951 \times exp(-2.6275 \times n^{-1})$	0.99	1.59	0.99	76.82	16
NL	SMS	$PG_i = 90.9115 \times exp(-2.7168 \times n^{-1})$	0.99	1.43	0.99	76.55	16
NL	IMS	$PG_i = 92.1433 \times exp(-2.2303 \times n^{-1})$	0.98	1.65	0.99	79.22	15
TL	EMS	$PG_i = 101.0929 \times exp(-0.9950 \times n^{-1})$	0.79	4.69	0.93	93.11	13
TL	TMS	$PG_i = 95.2857 \times exp(-1.4891 \times n^{-1})$	0.94	2.61	0.98	85.09	14
TL	SMS	$PG_i = 97.0627 \times exp(-1.3535 \times n^{-1})$	0.93	2.96	0.98	87.37	13
TL	IMS	$PG_i = 98.4280 \times exp(-1.2088 \times n^{-1})$	0.89	3.52	0.97	89.40	13
SL	EMS	$PG_i = 101.2845 \times exp(-0.9737 \times n^{-1})$	0.78	4.83	0.93	93.42	13
SL	TMS	$PG_i = 97.7196 \times exp(-1.1904 \times n^{-1})$	0.88	3.64	0.96	88.85	13
SL	SMS	$PG_i = 98.8328 \times exp(-1.1430 \times n^{-1})$	0.87	3.86	0.96	90.14	13
SL	IMS	$PG_i = 100.1760 \times exp(-1.0428 \times n^{-1})$	0.82	4.39	0.94	91.96	13
RL	EMS	$PG_i = 94.5164 \times exp(-1.9269 \times n^{-1})$	0.98	1.66	0.99	82.45	15
RL	TMS	$PG_i = 90.8445 \times exp(-2.6214 \times n^{-1})$	0.99	1.54	0.99	76.79	16
RL	SMS	$PG_i = 90.6974 \times exp(-2.6674 \times n^{-1})$	0.99	1.55	0.99	76.52	16
RL	IMS	$PG_i = 91.0810 \times exp(-2.5692 \times n^{-1})$	0.99	1.52	0.99	77.17	16

* EMS: error mean square; TMS: tray cell size mean square; SMS: substrate mean square; IMS: interaction mean square.

6.6 FIGURES

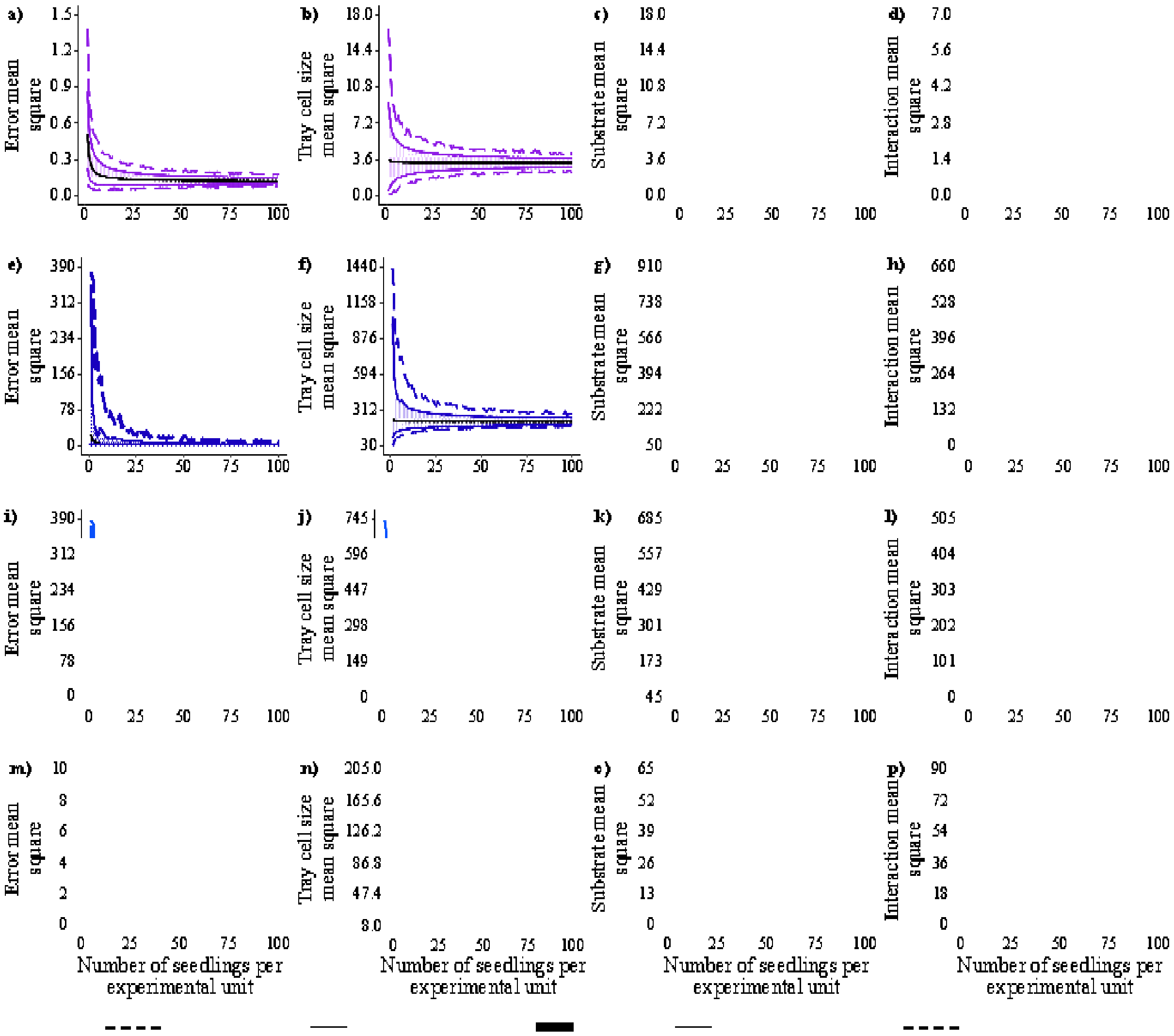

Figure 1 - Minimum, 2.5 percentile, mean, 97.5 percentile and maximum values of the mean squares of the error, tray cell size, substrate, and tray cell size × substrate interaction in the number of leaves (a, b, c, and d), total length (e, f, g, and h), shoot length (i, j, k, and l), and root length (m, n, o, and p) of cauliflower seedlings.

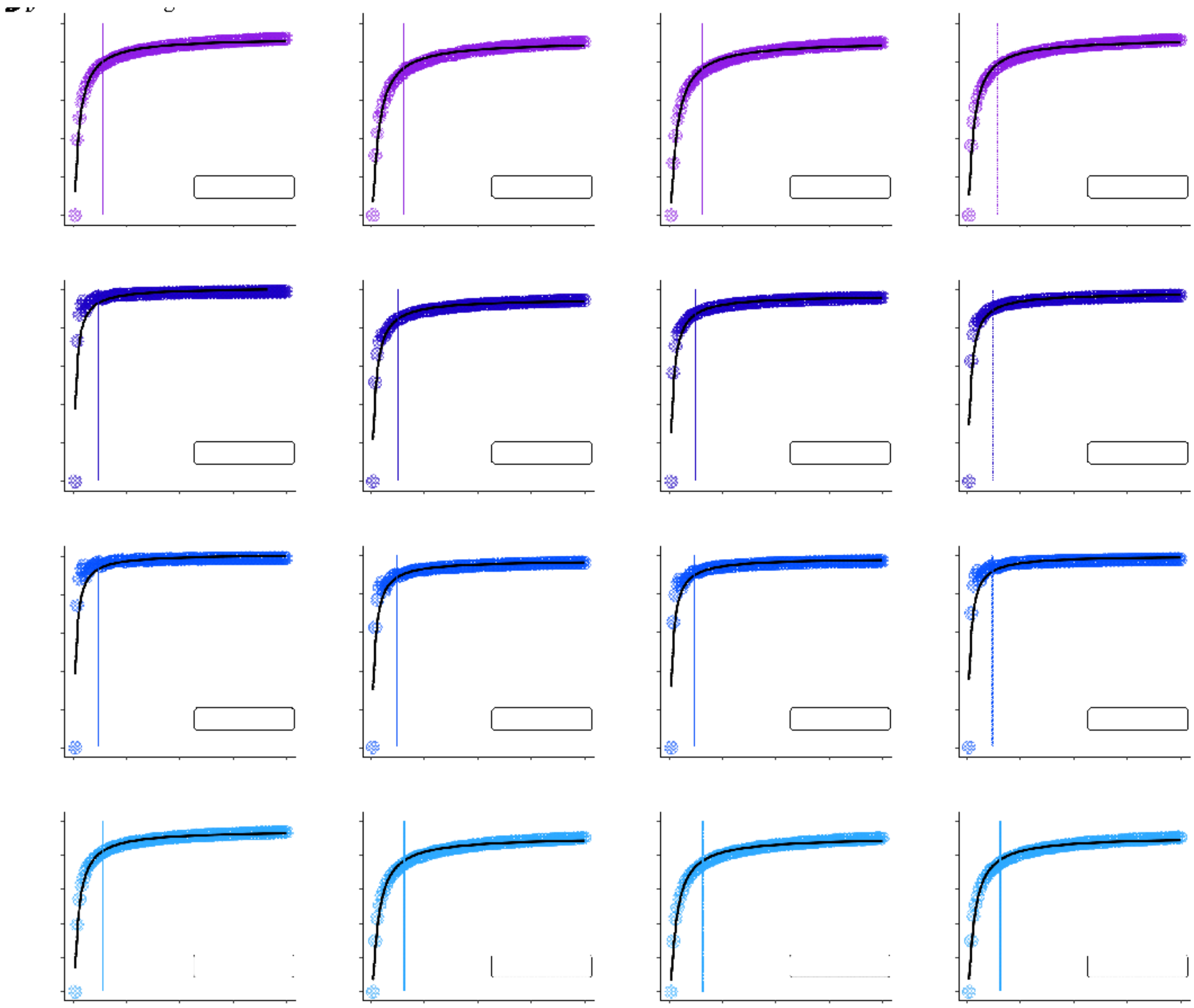

Figure 2 - Sample size determination via Schumacher model and maximum curvature points for estimating the mean squares of the error, tray cell size, substrate, and tray cell size × substrate interaction in the number of leaves (a, b, c, and d), total length (e, f, g, and h), shoot length (i, j, k, and l), and root length (m, n, o, and p) of cauliflower seedlings.

7 ARTIGO 3 – HOW MANY CAULIFLOWER SEEDLINGS ARE NECESSARY TO ESTIMATE EXPERIMENTAL PRECISION STATISTICS RELIABLY?

(Formatação da revista Scientia Horticulturae)

Publicado no periódico: Scientia Horticulturae
Situação: Publicado

7.1 ABSTRACT

The aims of this study were to determine the adequate sample size of cauliflower seedlings per experimental unit to estimate experimental precision statistics and to forecast experimental precision statistics. From a reference experiment in which 20 seedlings per experimental unit were sampled and the number of leaves, shoot length, root length, and total length were measured, a bootstrapping procedure was applied with replacement, recreating experiments with sample sizes ranging from 1 to 100 seedlings per experimental unit, obtaining 10,000 resamples for each size. For each resample, 13 precision statistics were estimated, for which the 95% confidence interval widths were calculated, fitting these values to the sampling scenarios through traditional power models. The sample size was then determined through the maximum curvature point through the perpendicular distances method for each precision statistic and trait, for which a modified power model was used for forecasting. The reference experiment showed high experimental precision, which varied according to the trait and precision statistic considered. An exponential decrease in the confidence interval width was observed as the sample number was increased, and the optimal sample size varied slightly among statistics and traits, where the maximum value found was 20 seedlings per experimental unit, representing the sufficient sample size for estimating experimental precision statistics in experiments with cauliflower seedlings. Also, the forecasting models showed excellent fitting quality and can be used and adapted by researchers performing experiments on seedling production of cauliflower or other horticultural crops.

Keywords: *Brassica oleracea*, experimental planning, sample size, forecasting.

7.2 INTRODUCTION

Seedling production is a crucial step in the productive process of most horticultural crops (Postemsky et al., 2016; Zhou et al., 2019; Ors et al., 2021). This is because seedling quality can be a determinant to achieve cultivation success, once this stage directly affects field performance and yield (Yan et al., 2019). In this regard, vegetables from the Brassicaceae family respond positively to protected cultivation during the seedling stage before transplanting, mainly due to temperature width reduction, moisture maintenance, and reduction in the incidence of pests and diseases (Muimba-Kankolongo, 2018). Among these, cauliflower (*Brassica oleracea* L. var. *botrytis* L.) is one of the most important vegetables grown in the world, especially because of its high nutritional value (Bhattacharjee and Singhal, 2018). Hence, several studies have been carried out over the years on management techniques to optimize the growth and quality of cauliflower seedlings (Leśniewicz et al., 2010; Wu et al., 2012; Ray and Mishra, 2017; Wu et al., 2019). However, experimental precision is a key factor in research that has been little investigated for experiments with this crop, namely in terms of experimental planning and sample size determination, considering the latter can be decisive for the validation of research results that aim to provide such technical recommendations.

Many studies use precision statistics to evaluate the impact of the experimental error, that is, the variation between experimental units that had received the same treatment (Cargnelutti Filho et al., 2009; Górdon-Mendoza and Camargo-Buitrago, 2015; Storck et al., 2016). Some of the most important are the coefficient of experimental variation, the variation index, the F-test value, and the least significant difference. Although the coefficient of experimental variation (Pimentel-Gomes, 1990) is the most commonly addressed among these statistics, other indicators were proposed in order to compensate for the criticism regarding its strong association with the experimental mean (Döring et al., 2015; Döring and Reckling, 2018; Cargnelutti Filho et al., 2018), such as the variation index (Pimentel-Gomes, 1991) and the F-test value (Resende and Duarte, 2007). Furthermore, several studies have reported the least significant difference as an appropriate precision indicator as well (Lúcio et al., 1999; Cargnelutti Filho et al., 2009; Górdon-Mendoza and Camargo-Buitrago, 2015; Cargnelutti Filho et al., 2018). Nevertheless, estimating these values reliably also requires acute experimental planning, which includes the use of representative sample sizes (Souza et al., 2022).

The first step in the planning of experiments should be defining the number of plants that will be evaluated per experimental unit, that is, sample size (Confalonieri et al., 2009). Setting the ideal sample size is fundamental because, if under or overestimated, it may result in incorrect inferences (Confalonieri et al., 2009; Alvez-Silva et al., 2018) and, as a consequence, misleading results and recommendations. Bittencourt et al. (2022), Souza et al. (2022), and Souza et al. (2023) defined sample size per experimental unit, that is, considering experimental restrictions, which facilitates the application of such recommendations in experiments that use experimental designs, which is extremely common in seedling trials, where trays are normally considered experimental units. Thus, this approach should be acknowledged when finding the ideal sample size for this type of experiment. Thereafter, once the sample size is defined, an interesting next step would be forecasting what will be the experimental precision reached as a function of this number (Souza et al., 2022).

In this sense, Souza et al. (2022) proposed for the first time, for soybean experiments, a methodology based on inverse predictive equations to estimate experimental precision statistics using its 95% confidence interval width, in which the desired sample size is added jointly to a power-model-based formula. As highlighted by these authors, this method should be tested for other crops as well since it can be an aidful tool to optimize experimental planning and bring support to researchers. On that basis, we tested the following hypothesis: (*i*) experimental precision is associated with sample size so that a representative sampling increases the precision of inferences, such as experimental precision statistics, estimated in experiments with cauliflower seedlings; and (*ii*) the experimental precision of experiments with cauliflower seedlings can be forecasted from a sample size defined *a priori*. Therefore, the aims of this study were to determine the adequate sample size of cauliflower seedlings per experimental unit to estimate experimental precision statistics and to forecast experimental precision statistics.

7.3 MATERIAL AND METHODS

1.1.1. Experimental design

The experiment was carried out in a greenhouse at the Federal University of Pampa (UNIPAMPA), Itaqui Campus, in the state of Rio Grande do Sul, Brazil, on May 23rd, 2015. Seeds of cauliflower cultivar Teresopolis Gigante were sown in three substrates (50% Mecplant® + 50% Carolina Padrão®, 75% Mecplant® + 25% rice husk, or 75% Carolina Padrão® + 25% rice husk), and for each substrate, polystyrene trays of 72 and 128 cells were used, where cells had a volume of 113 cm^3 and 36 cm^3, respectively. Thus, a two-factor scheme (3 x 2) with four replications was set in a completely randomized design, totaling 24 experimental units (12 trays of 72 cells and 12 trays of 128 cells). These factors were selected for being frequently evaluated for seedling production.

After 30 days, twenty seedlings from each experimental unit were randomly collected considering a useful area of 40 cells and 84 cells, for the trays with 72 and 128 cells, respectively, where a border area of 1 cell was excluded from each far end. Thus, the following traits were measured: a) the number of leaves (NL), in units; b) shoot length (SL), from neck to leaflet insertion, in cm; c) root length (RL), from neck to root apex, in cm; and d) total length (TL), as the sum of SL and RL, in cm.

1.1.1. Bootstrapping procedure and precision statistics

From the experiment with 20 seedlings measured per experimental unit (reference experiment), other experiments with 1, 2, …, 100 seedlings per experimental unit were carried out, following the methodology by Bittencourt et al. (2022), Souza et al. (2022), and Souza et al. (2023). For this, 100 sample sizes were planned, fluctuating from 1 to 100 seedlings per experimental unit, with an increase of 1 seedling at a time. These sample sizes were initially subjected to the analysis of variance procedure, for which the database was previously subdivided per experimental unit and the average value of each experimental unit in each scenario was obtained, that is, in the sampling scenario of 2 seedlings per experimental unit, an average was calculated for each experimental unit considering the 2 sampled seedlings. This analysis was performed according to the following mathematical model:

$$Y_{ijk} = m + T_i + S_j + (TS)_{ij} + \varepsilon_{ijk}$$

where Y_{ijk} is the value observed in the response trait in plot *ijk*, *m* is the overall mean, T_i is the fixed effect of level i (i = 1 and 2) of the tray cell size factor, *Sj* is the fixed effect of level j (j = 1, 2, 3) of the substrate factor, $(TS)_{ij}$ is the fixed effect of the interaction of level i of the tray cell size factor with level j of the substrate factor and ε_{ijk} is the effect of the experimental error, supposedly normal and independently distributed with an average of zero and a common variance σ^2 (Storck et al., 2016). This procedure was performed with 10,000 resamples with replacement (Efron, 1979).

In each resampling, after obtaining the variance components, 13 experimental precision statistics were estimated according to studies by Pimentel-Gomes et al. (1990), Pimentel-Gomes et al. (1991), Lúcio (1997), Resende and Duarte (2007), Cargnelutti Filho et al. (2009) and Souza et al. (2022). Calculation formulas are shown in Table 1. Thus, the sum of combinations performed as a function of the number of seedlings evaluated per experimental unit totalizes a database of 1,000,000 simulated experiments for each trait, considering 100 sample scenarios and 10,000 experiments for each scenario. These statistical analyses were performed with specific routines using the *sample()* and *aov()* functions.

1.1.1. Sample size definition

The sample size was defined by finding the 95% confidence interval width ($CI_{95\%}$) of the statistics, as suggested by Roth & Greenland (2018), followed by the determination of the maximum curvature point. Descriptive statistics such as minimum values, percentiles of 2.5, means, percentiles of 97.5, and maximum values were estimated for each statistic in each bootstrap resample of the sampling scenarios per experimental unit. The $CI_{95\%}$ was obtained through the following equation:

$$CI_{95\%} = UP - LP$$

where UP is the upper percentile (97.5 percentile) and LP is the lower percentile (2.5 percentile) of the bootstrap estimates.

Posteriorly, the $CI_{95\%}$ estimates were adjusted to the scenarios of 1, 2, ..., 100 seedlings per experimental unit through the traditional power model. Thus, the following model was used, which was parameterized through the *nls()* function:

$$CI_{95\%} = \alpha \times n^{\beta} + \varepsilon$$

where α is the coefficient of interception, n is the sample size, β is the exponential rate of decay, and ε is the error of random effect. Next, the maximum curvature point was determined through the perpendicular distances method (Lorentz et al., 2012) adapted by Silva and Lima

(2017), as suggested by Bittencourt et al. (2022), using the *maxcurv (method = "pd")* function, for each precision statistic and trait. This point was considered the adequate sample size for the precision statistics under study.

1.1.1. Forecasting experimental precision statistics

Experimental precision statistics were predicted through the methodology proposed by Souza et al. (2022), using a modified power model by Olivoto et al. (2018), for NL, SL, RL, and TL. Thus, the following model was used for each precision statistic in each trait:

$$CI_{95\%} = \delta^{PS} \times \alpha \times n^{\beta} + \varepsilon$$

where δ is the adjustment factor of the coefficient of interception proposed by Olivoto et al. (2018), α is the coefficient of interception, n is the sample size, β is the exponential rate of decay, PS is the mean value of each precision statistic of the bootstrap resamples per sample size and ε is the error of random effect. The parameterization of the models was performed with 700,000 values, equivalent to 70% of the resamples for each precision statistic. The other 300,000 values (30%) were used for validation.

In the validation phase, simple linear regressions were applied between the observed and predicted $CI_{95\%}$ per experimental precision statistic for each trait. The fitting quality of the 104 generated models (13 precision statistics × 4 response traits × 2 types of power models) was analyzed through the coefficient of determination, Willmott's agreement index, and root mean square error, using the hydrogof package. Finally, the modified power models were inverted, similar to the methodology by Souza et al. (2022), generating new formulas for forecasting experimental precision statistics, according to the following equations:

$$CI_{95\%} = \delta^{PS} \times \alpha \times n^{\beta} + \varepsilon \quad (I)$$

$$\delta^{PS} = \frac{CI_{95\%}}{\alpha \times n^{\beta}} \quad (II)$$

$$PS = log_{\delta}\left(\frac{CI_{95\%}}{\alpha \times n^{\beta}}\right) \quad (III)$$

Statistical analyses were performed with Microsoft Office Excel and R software (R Development Core Team, 2022).

7.4 RESULTS

1.1.1. High experimental precision was obtained in the reference experiment

The reference experiment, performed with 20 seedlings per experimental unit, presented F-test values varying from 22.28 (SL) to 92.53 (RL) for the tray cell size factor and from 19.08 (RL) to 57.25 (SL) for the substrate factor (Table 2). The lowest F values were obtained for the interaction factor, which fluctuated from 3.97 (SL) to 22.58 (RL). As for CVe and VI, NL presented the lowest values (7.23% and 3.61%, respectively), and SL, had the highest (16.85% and 8.43%).

The least significant difference shows the tray cell size factor allowed treatments to differ more easily than the substrate factor since LSDt presented a lower overall mean (0.96) compared to LSDs (1.43). The same was observed regarding the mean values of the least significant difference of the interaction for LSDts (1.66) compared to LSDst (2.02). Accordingly, for the least significant difference as a percentage of the average, the lowest means were observed for LSDt%, followed by LSDs%, LSDts%, and LSDst%, subsequently.

1.1.1. Confidence interval width decreased exponentially in response to sample size increase

For all statistics and traits, $CI_{95\%}$ tended to an exponential decrease as sample size was increased, that is, a much greater width was observed when only 1 seedling was sampled than when 100 seedlings were taken, in general. A summary of this response can be seen in Fig. 1, where some statistics and traits were randomly chosen (for more information see Supplementary Fig. 1, 2, 3, 4, 5, 6, 7, and 8). This can be exemplified by considering the confidence upper limit of Ft for the NL, where the sampling of only 10 seedlings per experimental unit promoted a value of 41.33, which decreased to 35.63 when 90 seedlings were considered. However, the confidence lower limit presented an opposite response, where the value obtained for 10 seedlings (11.73) was lower than the one obtained for 90 seedlings per experimental unit (22.11), which contributed to creating a “funnel” shape.

As for CVe and VI, higher mean values were obtained for the measurement traits, where SL was the one that presented the highest means, with values of 24.98% and 12.49%, respectively, for 1 seedling per experimental unit, which decreased to 17.24% and 8.62% when 100 seedlings were sampled (Supplementary Fig. 2d and 2e). The counting trait (NL) presented the lowest CVe and VI, with means of 15.19% and 7.60%, respectively, for 1 seedling per experimental unit. Accordingly, such values decreased to 7.35% and 3.67%, respectively, for a sample number of 100 seedlings (Supplementary Fig. 1d and 1e).

For all characteristics, the highest least significant difference mean values correspond to the substrate factor within the tray cell size factor (LSDst), followed by the tray cell size within the substrate (LSDts), and subsequently, the substrate factor (LSDs). Thus, the tray cell size presented the lowest value (LSDt). Also, lower means were observed for the NL compared with the other traits. The same pattern was observed for the least significant difference as a percentage of the average (LSDt%, LSDs%, LSDts%, and LSDst%).

1.1.1. Sample size varied slightly among traits and experimental precision statistics

All experimental precision statistics of all traits obtained good fitting quality indicators, of which all R^2 values were higher than 0.84 and all d indexes were higher than 0.90 (Tables 3, 4, 5, and 6). For TL, RMSE varied from 0.37 to 6.20 among statistics. The lowest RMSE values were obtained for NL, which fluctuated from 0.01 to 4.92, whereas the highest were obtained for RL and SL, which reached values up to 7.36 and 7.37, respectively.

A little variation in the optimal sample size per experimental unit was observed among traits and experimental precision statistics (Supplementary Fig. 5, 6, 7, and 8). While both NL and RL required 16 to 19 seedlings per experimental unit, the ideal sample number for SL oscillated from 14 to 20 seedlings, and from 13 to 19 for TL. Hence, considering all traits and statistics, 20 cauliflower seedlings per experimental unit was the maximum value obtained.

1.1.1. Fitting quality and validation of the forecasting models

The modified power models for forecasting also showed excellent fitting quality as indicated by R^2, RMSE, and d index (Tables 3, 4, 5, and 6). All R^2 values were higher than 0.91 and all d indexes were higher than 0.97. RMSE varied from 0.01 to 1.08 among statistics for the NL, and from 0.01 to 1.46 for RL. Higher RMSE values were obtained for SL and TL, where the first one fluctuated from 0.08 to 6.88, and the latter, from 0.22 to 2.37. Furthermore, the predicted $CI_{95\%}$ of the modified power models was closer to the observed

$CI_{95\%}$ in comparison to the traditional power models (Fig. 2, 3, 4, and 5, and Supplementary Fig. 9, 10, 11, and 12). Interestingly, smaller sample sizes, which naturally correspond to greater $CI_{95\%}$, tended to express an inferior predictive capacity, gradually increasing predictability as the number of seedlings increases until stabilizing, when the sufficient sample size is reached, equally to the observed by Souza et al. (2022).

7.5 DISCUSSION

In this study, we defined the ideal sample size per experimental unit for estimating experimental precision statistics in experiments with cauliflower seedlings, based on seedling traits that are commonly measured by researchers and producers when assessing seedling quality (Hussain et al., 2016; Collela et al., 2019; Chrysargyris et al., 2020; Soares et al., 2020). Furthermore, we proposed forecasting models for such precision statistics along with a step-by-step for its application. Hence, our findings intend to optimize experimental planning, not only regarding the importance of a sufficient sample size to make reliable statistical inferences (Confalonieri et al., 2009; Butturi-Gomes et al., 2014; Alvez-Silva et al., 2018; Khosravi et al., 2020), but also facilitating the decision-making on this number to achieve the desired experimental precision through an easily applicable forecasting tool (Souza et al., 2022).

As observed in Table 2, a much higher Ft value was obtained for RL compared to the other traits. This reflects the strong relationship between tray cell size and root growth since trays with bigger cells allow increases in RL, which may be limited as that space is reduced (Poorter et al., 2012; Williams et al., 2016). As for Fs, however, SL and NL obtained higher values than RL, suggesting the substrate factor influences these characteristics the most. An explanation for this is that the type of substrate used for seedling production directly affects water and nutrient uptake (Carmona et al., 2012; Zhang et al., 2012), and although this also affects root growth, the latter may be limited by tray cell size, as mentioned, so that the resources absorbed by the roots are still being directed to shoot growth and leave emission (Taiz et al., 2017). Consequently, when associating both factors (tray cell size and substrate) RL is still the trait with a more evident response, thus obtaining the highest Fi.

Moreover, when comparing CVe and VI results, VI values were logically much lower, equaling half the CVe values. Thus, the effect of the number of replicates, which is taken into account only for VI calculation (Pimentel-Gomes, 1991), is easily visualized. Importantly, this parameter included in the VI calculation structure serves as a mechanism to reduce its association with the experimental mean (Cargnelutti Filho et al., 2009; Górdon-Mendoza and Camargo-Buitrago, 2015; Cargnelutti Filho et al., 2018, Souza et al., 2022), for which CVe has been strongly criticized (Döring and Reckling, 2018). Nevertheless, for both these statistics, NL was the one trait that obtained the lowest values. This could be partially explained by the natural tendency of this trait to present a greater uniformity *per se* in comparison to the others, considering the seedling production period. Accordingly, all least significant differences were lower for the NL as well, which simply reflects how low variations among replicates allow treatments to differ more easily. Overall, these results highlight experimental precision may variate depending not only on which trait is analyzed but also on the experimental precision statistic that is considered, suggesting such statistics should be used jointly to increase reliability.

Once the $CI_{95\%}$ of these statistics was analyzed in response to the proposed sample scenarios, an exponential decrease was perceived as the number of seedlings sampled per experimental unit was increased. Nonetheless, this is not the first time this particularity was reported (Cargnelutti Filho et al., 2013; Toebe et al., 2014; Toebe et al., 2015; Toebe et al., 2018; Souza et al., 2022). Simply put, it means the smaller the sample number, the farther the lower and upper limits are from the real values, thus creating wider intervals that make room for over and/or underestimating results. For instance, the sampling of only one seedling per experimental unit would most likely lack representativeness and consequently, lead to unreliable estimates.

However, although determining the ideal sample size of a certain crop is essential to reach more accurate results, it is also a useful instrument to avoid oversampling, which optimizes evaluation time and manpower (Toebe et al., 2018; Bittencourt et al., 2022; Souza et al., 2022). Therefore, since sample size variates according to the experimental precision statistic and trait, the maximum value obtained among all represents the sufficient number of seedlings to estimate all of these statistics for all four characteristics in a reliable manner, which is, in this case, 20 cauliflower seedlings per experimental unit.

Aside from that recommendation, experimental planning can be enhanced even more by predicting experimental precision as a function of the chosen sample size. For that purpose, Souza et al. (2022) proposed forecasting equations for experiments with soybean crops, based on the aforementioned $CI_{95\%}$ decreasing mechanism in response to sample size increase. As shown in Tables 3, 4, 5, and 6, we have now proposed an adaptation of this method for cauliflower seedlings. The first step to applying it is selecting the precision statistic that will be predicted for a given trait and arbitrating a hypothetical sample size (n). Note that this value should be close to the one recommended for the statistic in question for more accurate predictability. Next, by adding this number to the respective power model $\left[CI_{95\%} = \alpha \times n^{\beta} + \varepsilon\right]$, the $CI_{95\%}$ of the statistic can be obtained. Finally, both these values can be added to the corresponding forecasting equation $\left[PS = log_{\delta}\left(\frac{CI_{95\%}}{\alpha \times n^{\beta}}\right)\right]$ in order to estimate the value of the desired experimental precision statistic for the chosen sample size. Additional equations are shown in Supplementary Tables 1, 2, 3, 4, 5, 6, 7, and 8 for estimating the upper and lower limits of the predicted value of the statistics. A scheme of this process is outlined in Fig. 6.

The calibration and testing of this method for experiments with cauliflower seedlings that measure different traits and/or study different factors are highly encouraged, as well as for experiments on the seedling production of other horticultural crops, given the high fitting quality achieved by the models, despite its possible limitations. In this sense, researchers that aim at optimizing experimental planning through sample size definition and forecasting should perform the required preliminary tests in order to adapt or modify the equations proposed here.

7.6 CONCLUSIONS

A slight variation was observed among traits and precision statistics regarding sample size definition. Considering all traits and statistics under study, the sampling of 20 cauliflower seedlings per experimental unit was enough for estimating experimental precision statistics reliably. The proposed modified power models were successful in forecasting experimental precision statistics and presented excellent fitting quality. Therefore, these equations may be applied or modified by researchers performing similar experiments. The recommendations and information presented here represent an extremely useful tool for optimizing the planning of experiments with cauliflower seedlings and may be adapted, serving as a basis for other horticultural crops.

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7.8 TABLES

Table 1. Codes, calculation structures and references of the experimental precision statistics.

Precision statistic	Code	Equation[(*)]	Reference
F-test value for the tray cell size	Ft	$\frac{TCSMS}{EMS}$	Storck et al. (2016)
F-test value for the substrate	Fs	$\frac{SMS}{EMS}$	Storck et al. (2016)
F-test value for the interaction	Fi	$\frac{IMS}{EMS}$	Storck et al. (2016)
Coefficient of experimental variation	CVe	$100\frac{\sqrt{EMS}}{m}$	Pimentel-Gomes (1990)
Variation index	VI	$\frac{CVe}{\sqrt{r}}$	Pimentel-Gomes (1991)
Least significant difference for the tray cell size	LSDt	$q_{\alpha(i;Edf)}\sqrt{\frac{EMS}{jr}}$	Lúcio (1997)
Least significant difference for the substrate	LSDs	$q_{\alpha(j;Edf)}\sqrt{\frac{EMS}{ir}}$	Lúcio (1997)
Least significant difference for the tray cell size within the substrate	LSDts	$q_{\alpha(i;Edf)}\sqrt{\frac{EMS}{r}}$	Lúcio (1997)
Least significant difference for the substrate within the tray cell size	LSDst	$q_{\alpha(j;Edf)}\sqrt{\frac{EMS}{r}}$	Lúcio (1997)
Least significant difference as a percentage of the average for the tray cell size	LSDt%	$100\frac{LSDt}{m}$	Lúcio (1997)
Least significant difference as a percentage of the average for the substrate	LSDs%	$100\frac{LSDs}{m}$	Lúcio (1997)
Least significant difference as a percentage of the average for the tray cell size within the substrate	LSDts%	$100\frac{LSDts}{m}$	Lúcio (1997)
Least significant difference as a percentage of the average for the substrate within the tray cell size	LSDst%	$100\frac{LSDst}{m}$	Lúcio (1997)

(*) TCSMS: tray cell size mean squares; SMS: substrate mean squares; EMS: error mean squares; IMS: mean squares of the interaction between substrate and tray cell size; *m*: overall experimental mean; r: number of replicates; i: number of levels of the tray cell size factor; j: number of levels of the substrate factor; $q_{\alpha(i \text{ or } j;Edf)}$: critical value for Tukey's test performed at 5%; Edf: degrees of freedom of the error.

Table 2. Values of experimental precision statistics obtained from the reference experiment.

Precision statistic(*)	Trait			
	Number of leaves	Shoot length	Root length	Total length
Ft	28.84	22.28	92.53	61.85
Fs	47.44	57.25	19.08	53.57
Fi	5.68	3.97	22.58	12.45
CVe	7.23	16.85	10.71	11.67
VI	3.61	8.43	5.36	5.84
LSDt	0.29	1.13	0.78	1.64
LSDs	0.43	1.68	1.16	2.43
LSDts	0.50	1.96	1.36	2.83
LSDst	0.60	2.38	1.65	3.44
LSDt%	6.20	14.45	9.19	10.01
LSDs%	9.22	21.50	13.67	14.89
LSDts%	10.73	25.03	15.92	17.34
LSDst%	13.04	30.41	19.33	21.06

(*) The codes of the precision statistics are shown in Table 1.

Table 3. Power models, forecasting equations, and sample size recommendation for estimating experimental precision statistics when measuring the number of leaves of cauliflower seedlings.

Precision statistic[(*)]	Power model	Maximum $CI_{95\%}$	Maximum Curvature	$R^{2(£)}$	RMSE	d	Sample size
Ft	$CI_{95\%} = 38.8809\times n^{-0.1974}$	21.8789	18.4077	0.84	2.92	0.90	19
Fs	$CI_{95\%} = 68.9120\times n^{-0.1935}$	39.1965	18.4671	0.85	4.92	0.90	19
Fi	$CI_{95\%} = 10.8721\times n^{-0.2318}$	5.5697	17.9126	0.88	0.72	0.93	18
CVe	$CI_{95\%} = 11.0005\times n^{-0.3926}$	3.7266	15.7540	0.99	0.13	0.99	16
VI	$CI_{95\%} = 5.5003\times n^{-0.3926}$	1.8633	15.7540	0.99	0.07	0.99	16
LSDt	$CI_{95\%} = 0.4209\times n^{-0.3865}$	0.1447	15.8332	0.99	0.01	0.99	16
LSDs	$CI_{95\%} = 0.6262\times n^{-0.3865}$	0.2153	15.8332	0.99	0.01	0.99	16
LSDts	$CI_{95\%} = 0.7290\times n^{-0.3865}$	0.2507	15.8332	0.99	0.01	0.99	16
LSDst	$CI_{95\%} = 0.8856\times n^{-0.3865}$	0.3045	15.8332	0.99	0.01	0.99	16
LSDt%	$CI_{95\%} = 9.4351\times n^{-0.3926}$	3.1963	15.7540	0.99	0.11	0.99	16
LSDs%	$CI_{95\%} = 14.0376\times n^{-0.3926}$	4.7555	15.7540	0.99	0.17	0.99	16
LSDts%	$CI_{95\%} = 16.3421\times n^{-0.3926}$	5.5362	15.7540	0.99	0.19	0.99	16
LSDst%	$CI_{95\%} = 19.8521\times n^{-0.3926}$	6.7253	15.7540	0.99	0.23	0.99	16
	Modified power model	Forecasting equation			R^2	RMSE	d
Ft	$CI_{95\%}=1.0997^{Ft}\times 12.3654\times n^{-0.5766}$	$Ft = log_{1.0997}\left(\frac{CI_{95\%}}{12.3654\times n^{-0.5766}}\right)$			0.99	0.57	0.99
Fs	$CI_{95\%}=1.0733^{Fs}\times 13.5348\times n^{-0.6021}$	$Fs = log_{1.0733}\left(\frac{CI_{95\%}}{13.5348\times n^{-0.6021}}\right)$			0.99	1.08	0.99
Fi	$CI_{95\%}=1.6605^{Fi}\times 2.5268\times n^{-0.5747}$	$Fi = log_{1.6605}\left(\frac{CI_{95\%}}{2.5268\times n^{-0.5747}}\right)$			0.99	0.15	0.99
CVe	$CI_{95\%} =0.9693^{CVe}\times 16.5426\times n^{-0.4412}$	$CVe = log_{0.9693}\left(\frac{CI_{95\%}}{16.5426\times n^{-0.4412}}\right)$			0.99	0.07	0.99
VI	$CI_{95\%} =0.9396^{VI}\times 8.2713\times n^{-0.4412}$	$VI = log_{0.9396}\left(\frac{CI_{95\%}}{8.2713\times n^{-0.4412}}\right)$			0.99	0.04	0.99
LSDt	$CI_{95\%} =0.3916^{LSDt}\times 0.6842\times n^{-0.4441}$	$LSDt = log_{0.3916}\left(\frac{CI_{95\%}}{0.6842\times n^{-0.4441}}\right)$			0.99	0.01	0.99
LSDs	$CI_{95\%} =0.5325^{LSDs}\times 1.0179\times n^{-0.4441}$	$LSDs = log_{0.5325}\left(\frac{CI_{95\%}}{1.0179\times n^{-0.4441}}\right)$			0.99	0.01	0.99
LSDts	$CI_{95\%} =0.5820^{LSDts}\times 1.1850\times n^{-0.4441}$	$LSDts = log_{0.5820}\left(\frac{CI_{95\%}}{1.1850\times n^{-0.4441}}\right)$			0.99	0.01	0.99
LSDst	$CI_{95\%} =0.6404^{LSDst}\times 1.4396\times n^{-0.4441}$	$LSDst = log_{0.6404}\left(\frac{CI_{95\%}}{1.4396\times n^{-0.4441}}\right)$			0.99	0.01	0.99
LSDt%	$CI_{95\%} =0.9644^{LSDt\%}\times 14.1886\times n^{-0.4412}$	$LSDt\% = log_{0.9644}\left(\frac{CI_{95\%}}{14.1886\times n^{-0.4412}}\right)$			0.99	0.06	0.99
LSDs%	$CI_{95\%} =0.9759^{LSDs\%}\times 21.1097\times n^{-0.4412}$	$LSDs\% = log_{0.9759}\left(\frac{CI_{95\%}}{21.1097\times n^{-0.4412}}\right)$			0.99	0.09	0.99
LSDts%	$CI_{95\%} =0.9793^{LSDts\%}\times 24.5753\times n^{-0.4412}$	$LSDts\% = log_{0.9793}\left(\frac{CI_{95\%}}{24.5753\times n^{-0.4412}}\right)$			0.99	0.10	0.99
LSDst%	$CI_{95\%} =0.9829^{LSDst\%}\times 29.8536\times n^{-0.4412}$	$LSDst\% = log_{0.9829}\left(\frac{CI_{95\%}}{29.8536\times n^{-0.4412}}\right)$			0.99	0.13	0.99

[(*)] The codes of the precision statistics are shown in Table 1. [(£)] Coefficient of determination (R²), Root Mean Square Error (RMSE) and Willmott's agreement index (d).

Table 4. Power models, forecasting equations, and sample size recommendation for estimating experimental precision statistics when measuring shoot length of cauliflower seedlings.

Precision statistic[(*)]	Power model	Maximum $CI_{95\%}$	Maximum Curvature	$R^{2(£)}$	RMSE	d	Sample size
Ft	$CI_{95\%} = 51.4615 \times n^{-0.1602}$	32.1192	18.9622	0.89	1.81	0.97	19
Fs	$CI_{95\%} = 162.7358 \times n^{-0.1286}$	111.0982	19.4573	0.88	7.37	0.93	20
Fi	$CI_{95\%} = 7.8452 \times n^{-0.2274}$	4.0673	17.9720	0.98	0.13	0.99	18
CVe	$CI_{95\%} = 138.1435 \times n^{-0.5256}$	34.2907	14.1696	0.98	3.90	0.99	15
VI	$CI_{95\%} = 69.0717 \times n^{-0.5256}$	17.1453	14.1696	0.95	1.95	0.99	15
LSDt	$CI_{95\%} = 13.5909 \times n^{-0.6290}$	2.6971	13.0804	0.93	0.46	0.98	14
LSDs	$CI_{95\%} = 20.2206 \times n^{-0.6290}$	4.0127	13.0804	0.93	0.68	0.98	14
LSDts	$CI_{95\%} = 23.5401 \times n^{-0.6290}$	4.6715	13.0804	0.93	0.80	0.98	14
LSDst	$CI_{95\%} = 28.5961 \times n^{-0.6290}$	5.6748	13.0804	0.93	0.97	0.98	14
LSDt%	$CI_{95\%} = 118.4853 \times n^{-0.5256}$	29.4110	14.1696	0.95	3.34	0.99	15
LSDs%	$CI_{95\%} = 176.2823 \times n^{-0.5256}$	43.7577	14.1696	0.95	4.97	0.99	15
LSDts%	$CI_{95\%} = 205.2223 \times n^{-0.5256}$	50.9414	14.1696	0.96	5.79	0.99	15
LSDst%	$CI_{95\%} = 249.3009 \times n^{-0.5256}$	61.8828	14.1696	0.96	7.05	0.99	15

	Modified power model	Forecasting equation	R^2	RMSE	d
Ft	$CI_{95\%} = 1.0671^{Ft} \times 13.4733 \times n^{-0.1999}$	$Ft = log_{1.0671}\left(\frac{CI_{95\%}}{13.4733 \times n^{-0.1999}}\right)$	0.97	0.98	0.99
Fs	$CI_{95\%} = 1.0167^{Fs} \times 48.8494 \times n^{-0.0911}$	$Fs = log_{1.0167}\left(\frac{CI_{95\%}}{48.8494 \times n^{-0.0911}}\right)$	0.91	4.87	0.97
Fi	$CI_{95\%} = 1.4190^{Fi} \times 2.7183 \times n^{-0.3071}$	$Fi = log_{1.4190}\left(\frac{CI_{95\%}}{2.7183 \times n^{-0.3071}}\right)$	0.99	0.08	0.99
CVe	$CI_{95\%} = 1.0923^{CVe} \times 15.4262 \times n^{-0.3621}$	$CVe = log_{1.0923}\left(\frac{CI_{95\%}}{15.4262 \times n^{-0.3621}}\right)$	0.96	3.81	0.99
VI	$CI_{95\%} = 1.1932^{VI} \times 7.7131 \times n^{-0.3621}$	$VI = log_{1.1932}\left(\frac{CI_{95\%}}{7.7131 \times n^{-0.3621}}\right)$	0.96	1.91	0.99
LSDt	$CI_{95\%} = 14.9987^{LSDt} \times 0.0861 \times n^{-0.1282}$	$LSDt = log_{14.9987}\left(\frac{CI_{95\%}}{0.0861 \times n^{-0.1282}}\right)$	0.97	0.31	0.99
LSDs	$CI_{95\%} = 6.1726^{LSDs} \times 0.1280 \times n^{-0.1282}$	$LSDs = log_{6.1726}\left(\frac{CI_{95\%}}{0.1280 \times n^{-0.1282}}\right)$	0.97	0.47	0.99
LSDts	$CI_{95\%} = 4.7753^{LSDts} \times 0.1491 \times n^{-0.1282}$	$LSDts = log_{4.7753}\left(\frac{CI_{95\%}}{0.1491 \times n^{-0.1282}}\right)$	0.97	0.54	0.99
LSDst	$CI_{95\%} = 3.6220^{LSDst} \times 0.1811 \times n^{-0.1282}$	$LSDst = log_{3.6220}\left(\frac{CI_{95\%}}{0.1811 \times n^{-0.1282}}\right)$	0.97	0.66	0.99
LSDt%	$CI_{95\%} = 1.1085^{LSDt\%} \times 13.2309 \times n^{-0.3621}$	$LSDt\% = log_{1.1085}\left(\frac{CI_{95\%}}{13.2309 \times n^{-0.3621}}\right)$	0.96	3.27	0.99
LSDs%	$CI_{95\%} = 1.0717^{LSDs\%} \times 19.6850 \times n^{-0.3621}$	$LSDs\% = log_{1.0717}\left(\frac{CI_{95\%}}{19.6850 \times n^{-0.3621}}\right)$	0.96	4.86	0.99
LSDts%	$CI_{95\%} = 1.0613^{LSDts\%} \times 22.9168 \times n^{-0.3621}$	$LSDts\% = log_{1.0613}\left(\frac{CI_{95\%}}{22.9168 \times n^{-0.3621}}\right)$	0.96	5.66	0.99
LSDst%	$CI_{95\%} = 1.0502^{LSDst\%} \times 27.8389 \times n^{-0.3621}$	$LSDst\% = log_{1.0502}\left(\frac{CI_{95\%}}{27.8389 \times n^{-0.3621}}\right)$	0.96	6.88	0.99

(*) The codes of the precision statistics are shown in Table 1. (£) Coefficient of determination (R^2), Root Mean Square Error (RMSE) and Willmott's agreement index (d).

Table 5. Power models, forecasting equations, and sample size recommendation for estimating experimental precision statistics when measuring root length of cauliflower seedlings.

Precision statistic(*)	Power model	Maximum $CI_{95\%}$	Maximum Curvature	$R^{2(£)}$	RMSE	d	Sample size
Ft	$CI_{95\%} = 100.4150\times n^{-0.2149}$	53.8472	18.1700	0.85	7.36	0.91	19
Fs	$CI_{95\%} = 29.2548\times n^{-0.2440}$	14.5040	17.7344	0.90	1.74	0.94	18
Fi	$CI_{95\%} = 32.2181\times n^{-0.2259}$	16.7718	17.9918	0.87	2.19	0.92	18
CVe	$CI_{95\%} = 13.9265\times n^{-0.4488}$	4.1231	15.0608	0.99	0.07	0.99	16
VI	$CI_{95\%} = 6.9633\times n^{-0.4488}$	2.0616	15.0608	0.99	0.04	0.99	16
LSDt	$CI_{95\%} = 1.0258\times n^{-0.4463}$	0.3054	15.1004	0.99	0.01	0.99	16
LSDs	$CI_{95\%} = 1.5265\times n^{-0.4463}$	0.4544	15.1004	0.99	0.01	0.99	16
LSDts	$CI_{95\%} = 1.7768\times n^{-0.4463}$	0.5290	15.1004	0.99	0.01	0.99	16
LSDst	$CI_{95\%} = 2.1584\times n^{-0.4463}$	0.6426	15.1004	0.99	0.01	0.99	16
LSDt%	$CI_{95\%} = 11.9447\times n^{-0.4488}$	3.5364	15.0608	0.99	0.06	0.99	16
LSDs%	$CI_{95\%} = 17.7714\times n^{-0.4488}$	5.2614	15.0608	0.99	0.09	0.99	16
LSDts%	$CI_{95\%} = 20.6889\times n^{-0.4488}$	6.1252	15.0608	0.99	0.11	0.99	16
LSDst%	$CI_{95\%} = 25.1325\times n^{-0.4488}$	7.4408	15.0608	0.99	0.13	0.99	16

	Modified power model	Forecasting equation	R^2	RMSE	d
Ft	$CI_{95\%}=1.0312^{Ft}\times 28.2462\times n^{-0.5898}$	$Ft = log_{1.0312}\left(\frac{CI_{95\%}}{28.2462\times n^{-0.5898}}\right)$	0.99	1.46	0.99
Fs	$CI_{95\%}=1.1455^{Fs}\times 8.4549\times n^{-0.5688}$	$Fs = log_{1.1455}\left(\frac{CI_{95\%}}{8.4549\times n^{-0.5688}}\right)$	0.99	0.31	0.99
Fi	$CI_{95\%}=1.1290^{Fi}\times 8.7612\times n^{-0.5712}$	$Fi = log_{1.1290}\left(\frac{CI_{95\%}}{8.7612\times n^{-0.5712}}\right)$	0.99	0.39	0.99
CVe	$CI_{95\%} =0.9877^{CVe}\times 17.5093\times n^{-0.4752}$	$CVe = log_{0.9877}\left(\frac{CI_{95\%}}{17.5093\times n^{-0.4752}}\right)$	0.99	0.04	0.99
VI	$CI_{95\%} =0.9756^{VI}\times 8.7547\times n^{-0.4752}$	$VI = log_{0.9756}\left(\frac{CI_{95\%}}{8.7547\times n^{-0.4752}}\right)$	0.99	0.02	0.99
LSDt	$CI_{95\%} =0.8192^{LSDt}\times 1.3440\times n^{-0.4774}$	$LSDt = log_{0.8192}\left(\frac{CI_{95\%}}{1.3440\times n^{-0.4774}}\right)$	0.99	0.01	0.99
LSDs	$CI_{95\%} =0.8745^{LSDs}\times 1.9996\times n^{-0.4774}$	$LSDs = log_{0.8745}\left(\frac{CI_{95\%}}{1.9996\times n^{-0.4774}}\right)$	0.99	0.01	0.99
LSDts	$CI_{95\%} =0.8912^{LSDts}\times 2.3279\times n^{-0.4774}$	$LSDts = log_{0.8912}\left(\frac{CI_{95\%}}{2.3279\times n^{-0.4774}}\right)$	0.99	0.01	0.99
LSDst	$CI_{95\%} =0.9096^{LSDst}\times 2.8278\times n^{-0.4774}$	$LSDst = log_{0.9096}\left(\frac{CI_{95\%}}{2.8278\times n^{-0.4774}}\right)$	0.99	0.01	0.99
LSDt%	$CI_{95\%} =0.9857^{LSDt\%}\times 15.0177\times n^{-0.4752}$	$LSDt\% = log_{0.9857}\left(\frac{CI_{95\%}}{15.0177\times n^{-0.4752}}\right)$	0.99	0.03	0.99
LSDs%	$CI_{95\%} =0.9904^{LSDs\%}\times 22.3433\times n^{-0.4752}$	$LSDs\% = log_{0.9904}\left(\frac{CI_{95\%}}{22.3433\times n^{-0.4752}}\right)$	0.99	0.05	0.99
LSDts%	$CI_{95\%} =0.9917^{LSDts\%}\times 26.0115\times n^{-0.4752}$	$LSDts\% = log_{0.9917}\left(\frac{CI_{95\%}}{26.0115\times n^{-0.4752}}\right)$	0.99	0.05	0.99

LSDst%	$\mathrm{CI}_{95\%} = 0.9932^{LSDst\%} \times 31.5983 \times n^{-0.4752}$	$LSDst\% = log_{0.9932}\left(\frac{CI_{95\%}}{31.5983\times n^{-0.4752}}\right)$	0.99	0.07	0.99

(*) The codes of the precision statistics are shown in Table 1. (£) Coefficient of determination (R^2), Root Mean Square Error (RMSE) and Willmott's agreement index (d).

Table 6. Power models, forecasting equations, and sample size recommendation for estimating experimental precision statistics when measuring total length of cauliflower seedlings.

Precision statistic(*)	Power model	Maximum $\mathrm{CI}_{95\%}$	Maximum Curvature	$R^{2(£)}$	RMSE	d	Sample size
Ft	$\mathrm{CI}_{95\%} = 113.0647 \times n^{-0.1986}$	63.4013	18.4077	0.89	6.20	0.94	19
Fs	$\mathrm{CI}_{95\%} = 105.5724 \times n^{-0.1831}$	61.8007	18.6255	0.88	6.03	0.93	19
Fi	$\mathrm{CI}_{95\%} = 25.3622 \times n^{-0.2270}$	13.1609	17.9918	0.93	1.22	0.96	18
CVe	$\mathrm{CI}_{95\%} = 74.4849 \times n^{-0.6120}$	13.2587	15.3144	0.98	1.91	0.99	16
VI	$\mathrm{CI}_{95\%} = 37.2424 \times n^{-0.6120}$	7.6571	13.2587	0.98	0.95	0.99	14
LSDt	$\mathrm{CI}_{95\%} = 12.8180 \times n^{-0.6668}$	2.3534	12.7041	0.97	0.37	0.99	13
LSDs	$\mathrm{CI}_{95\%} = 19.0706 \times n^{-0.6668}$	3.5015	12.7041	0.97	0.55	0.99	13
LSDts	$\mathrm{CI}_{95\%} = 22.2014 \times n^{-0.6668}$	4.0763	12.7041	0.98	0.64	0.99	13
LSDst	$\mathrm{CI}_{95\%} = 26.9698 \times n^{-0.6668}$	4.9519	12.7041	0.98	0.78	0.99	13
LSDt%	$\mathrm{CI}_{95\%} = 63.8855 \times n^{-0.6120}$	13.1351	13.2587	0.96	1.64	0.99	14
LSDs%	$\mathrm{CI}_{95\%} = 95.0486 \times n^{-0.6120}$	19.5423	13.2587	0.96	2.43	0.99	14
LSDts%	$\mathrm{CI}_{95\%} = 110.6529 \times n^{-0.6120}$	22.7506	13.2587	0.96	2.83	0.99	14
LSDst%	$\mathrm{CI}_{95\%} = 134.4192 \times n^{-0.6120}$	27.6371	13.2587	0.96	3.44	0.99	14

	Modified power model	Forecasting equation	R^2	RMSE	d
Ft	$\mathrm{CI}_{95\%} = 1.0515^{Ft} \times 13.2247 \times n^{-0.4254}$	$Ft = log_{1.0515}\left(\frac{CI_{95\%}}{13.2247\times n^{-0.4254}}\right)$	0.99	1.46	0.99
Fs	$\mathrm{CI}_{95\%} = 1.0549^{Fs} \times 11.7756 \times n^{-0.3478}$	$Fs = log_{1.0549}\left(\frac{CI_{95\%}}{11.7756\times n^{-0.3478}}\right)$	0.98	1.84	0.99
Fi	$\mathrm{CI}_{95\%} = 1.2687^{Fi} \times 3.0933 \times n^{-0.4318}$	$Fi = log_{1.2687}\left(\frac{CI_{95\%}}{3.0933\times n^{-0.4318}}\right)$	0.99	0.27	0.99
CVe	$\mathrm{CI}_{95\%} = 1.1354^{CVe} \times 6.8408 \times n^{-0.3703}$	$CVe = log_{1.1354}\left(\frac{CI_{95\%}}{6.8408\times n^{-0.3703}}\right)$	0.98	1.31	0.99
VI	$\mathrm{CI}_{95\%} = 1.2892^{VI} \times 3.4204 \times n^{-0.3703}$	$VI = log_{1.2892}\left(\frac{CI_{95\%}}{3.4204\times n^{-0.3703}}\right)$	0.98	0.66	0.99
LSDt	$\mathrm{CI}_{95\%} = 2.8947^{LSDt} \times 0.6893 \times n^{-0.3409}$	$LSDt = log_{2.8947}\left(\frac{CI_{95\%}}{0.6893\times n^{-0.3409}}\right)$	0.98	0.22	0.99
LSDs	$\mathrm{CI}_{95\%} = 2.0430^{LSDs} \times 1.0255 \times n^{-0.3409}$	$LSDs = log_{2.0430}\left(\frac{CI_{95\%}}{1.0255\times n^{-0.3409}}\right)$	0.98	0.33	0.99
LSDts	$\mathrm{CI}_{95\%} = 1.8472^{LSDts} \times 1.1938 \times n^{-0.3409}$	$LSDts = log_{1.8472}\left(\frac{CI_{95\%}}{1.1938\times n^{-0.3409}}\right)$	0.98	0.38	0.99
LSDst	$\mathrm{CI}_{95\%} = 1.6572^{LSDst} \times 1.4503 \times n^{-0.3409}$	$LSDst = log_{1.6572}\left(\frac{CI_{95\%}}{1.4503\times n^{-0.3409}}\right)$	0.98	0.46	0.99
LSDt%	$\mathrm{CI}_{95\%} = 1.1596^{LSDt\%} \times 5.8674 \times n^{-0.3703}$	$LSDt\% = log_{1.1596}\left(\frac{CI_{95\%}}{5.8674\times n^{-0.3703}}\right)$	0.98	1.13	0.99

LSDs%	$CI_{95\%} = 1.1047^{LSDs\%} \times 8.7294 \times n^{-0.3703}$	$LSDs\% = log_{1.1047}\left(\frac{CI_{95\%}}{8.7294 \times n^{-0.3703}}\right)$	0.98	1.68	0.99
LSDts%	$CI_{95\%} = 1.0893^{LSDts\%} \times 10.1626 \times n^{-0.3703}$	$LSDts\% = log_{1.0893}\left(\frac{CI_{95\%}}{10.1626 \times n^{-0.3703}}\right)$	0.98	1.95	0.99
LSDst%	$CI_{95\%} = 1.0729^{LSDst\%} \times 12.3453 \times n^{-0.3703}$	$LSDst\% = log_{1.0729}\left(\frac{CI_{95\%}}{12.3453 \times n^{-0.3703}}\right)$	0.98	2.37	0.99

(*) The codes of the precision statistics are shown in Table 1. (£) Coefficient of determination (R^2), Root Mean Square Error (RMSE) and Willmott's agreement index (d).

7.9 FIGURE CAPTIONS

Fig. 1. Minimum, 2.5 percentile, mean, 97.5 percentile and maximum values of the precision statistics F-test value for the interaction (a), coefficient of experimental variation (b), variation index (c), and least significant difference as a percentage of the average for the tray cell size within the substrate (d), when measuring the number of leaves, shoot length, root length and total length of cauliflower seedlings, respectively, at the planned sample sizes from 1 to 100 seedlings per experimental unit.

Fig. 2. Observed and predicted values of the 95% confidence interval width ($CI_{95\%}$) for the validation of the modified power models for the precision statistics F-test value for the tray cell size (a), F-test value for the substrate (b), F-test value for the interaction (c), coefficient of experimental variation (d), variation index (e), least significant difference for the tray cell size (f), least significant difference for the substrate (g), least significant difference for the tray cell size within the substrate (h), least significant difference for the substrate within the tray cell size (i), least significant difference as a percentage of the average for the tray cell size (j), least significant difference as a percentage of the average for the substrate (k), least significant difference as a percentage of the average for the tray cell size within the substrate (l), and least significant difference for the substrate within the tray cell size (m), when measuring the number of leaves of cauliflower seedlings at the planned sample sizes from 1 to 100 seedlings per experimental unit.

Fig. 3. Observed and predicted values of the 95% confidence interval width ($CI_{95\%}$) for the validation of the modified power models for the precision statistics F-test value for the tray cell size (a), F-test value for the substrate (b), F-test value for the interaction (c), coefficient of experimental variation (d), variation index (e), least significant difference for the tray cell size (f), least significant difference for the substrate (g), least significant difference for the tray cell size within the substrate (h), least significant difference for the substrate within the tray cell size (i), least significant difference as a percentage of the average for the tray cell size (j), least significant difference as a percentage of the average for the substrate (k), least significant difference as a percentage of the average for the tray cell size within the substrate (l), and least significant difference for the substrate within the tray cell size (m), when measuring shoot length of cauliflower seedlings at the planned sample sizes from 1 to 100 seedlings per experimental unit.

Fig. 4. Observed and predicted values of the 95% confidence interval width ($CI_{95\%}$) for the validation of the modified power models for the precision statistics F-test value for the tray cell size (a), F-test value for the substrate (b), F-test value for the interaction (c), coefficient of experimental variation (d), variation index (e), least significant difference for the tray cell size (f), least significant difference for the substrate (g), least significant difference for the tray cell size within the substrate (h), least significant difference for the substrate within the tray cell size (i), least significant difference as a percentage of the average for the tray cell size (j), least significant difference as a percentage of the average for the substrate (k), least significant difference as a percentage of the average for the tray cell size within the substrate (l), and least significant difference for the substrate within the tray cell size (m), when measuring root

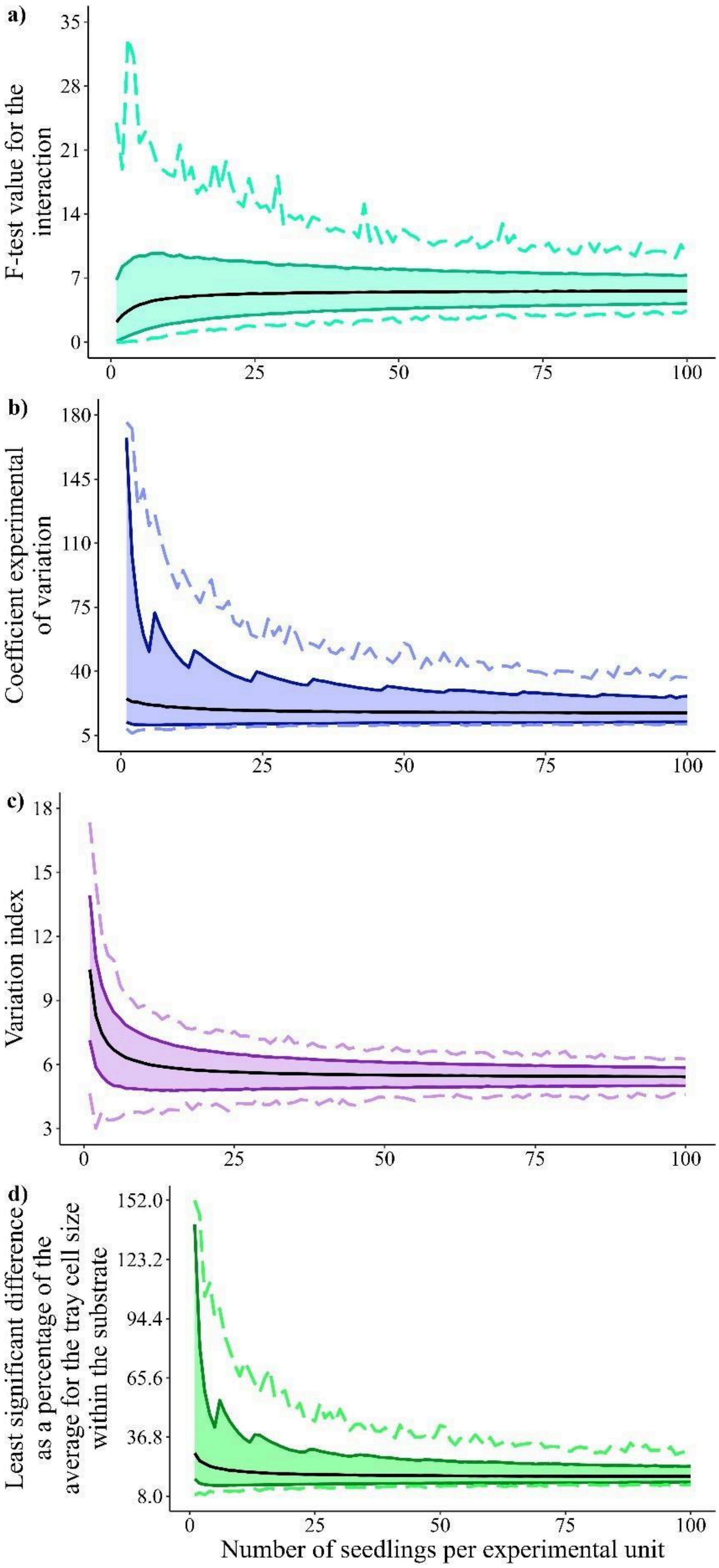
length of cauliflower seedlings at the planned sample sizes from 1 to 100 seedlings per experimental unit.

Fig. 5. Observed and predicted values of the 95% confidence interval width ($CI_{95\%}$) for the validation of the modified power models for the precision statistics F-test value for the tray cell size (a), F-test value for the substrate (b), F-test value for the interaction (c), coefficient of experimental variation (d), variation index (e), least significant difference for the tray cell size (f), least significant difference for the substrate (g), least significant difference for the tray cell size within the substrate (h), least significant difference for the substrate within the tray cell size (i), least significant difference as a percentage of the average for the tray cell size (j), least significant difference as a percentage of the average for the substrate (k), least significant difference as a percentage of the average for the tray cell size within the substrate (l), and least significant difference for the substrate within the tray cell size (m), when measuring the total length of cauliflower seedlings at the planned sample sizes from 1 to 100 seedlings per experimental unit.

Fig. 6. Step-by-step scheme of the forecasting methodology for experimental precision statistics, considering an exponential decreasing response of the 95% confidence interval width ($CI_{95\%}$) as a function of the increase in the number of seedlings sampled per experimental unit, from the choice of the desired sample size (n) and its inclusion in a power model to estimate $CI_{95\%}$, which will be used in an inverted modified power model.

[*] The codes of the precision statistics are shown in Supplementary Table 1. [**] α is the coefficient of interception; β is the exponential rate of decay; δ is the adjustment factor proposed by Olivoto et al. (2018); ε is the error of random effect.

7.10 FIGURES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F-test value for the interaction
35
28
21
14
7
0
0
25
50
75
100
b)
Coefficient experimental of variation
180
145
110
75
40
5
0
25
50
75
100
c)
Variation index
18
15
12
9
6
3
0
25
50
75
100
d)
Least significant difference as a percentage of the average for the tray cell size within the substrate
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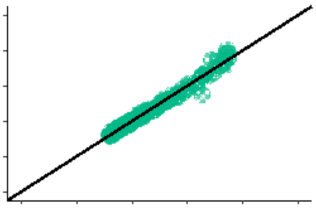
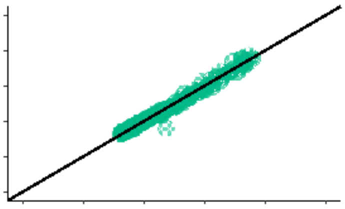
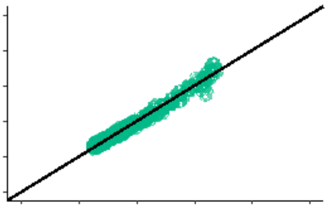
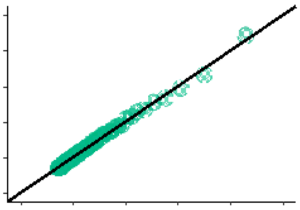
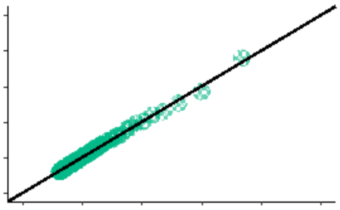
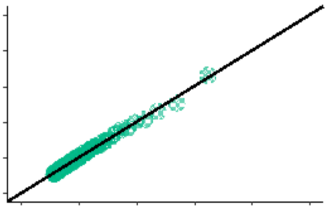
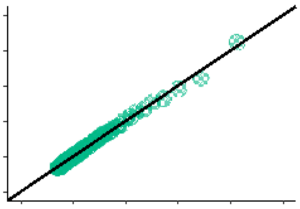
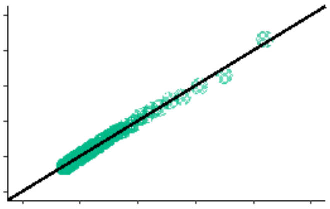
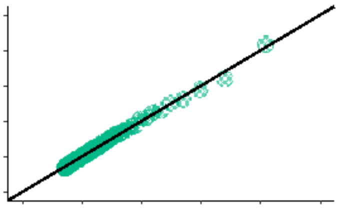
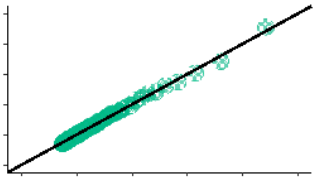
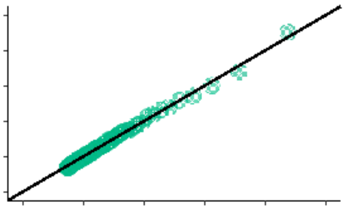
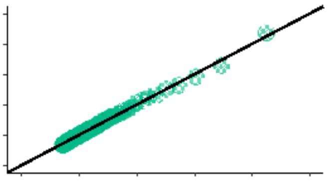
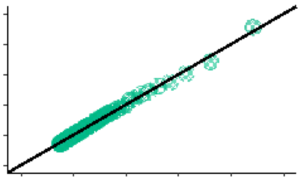
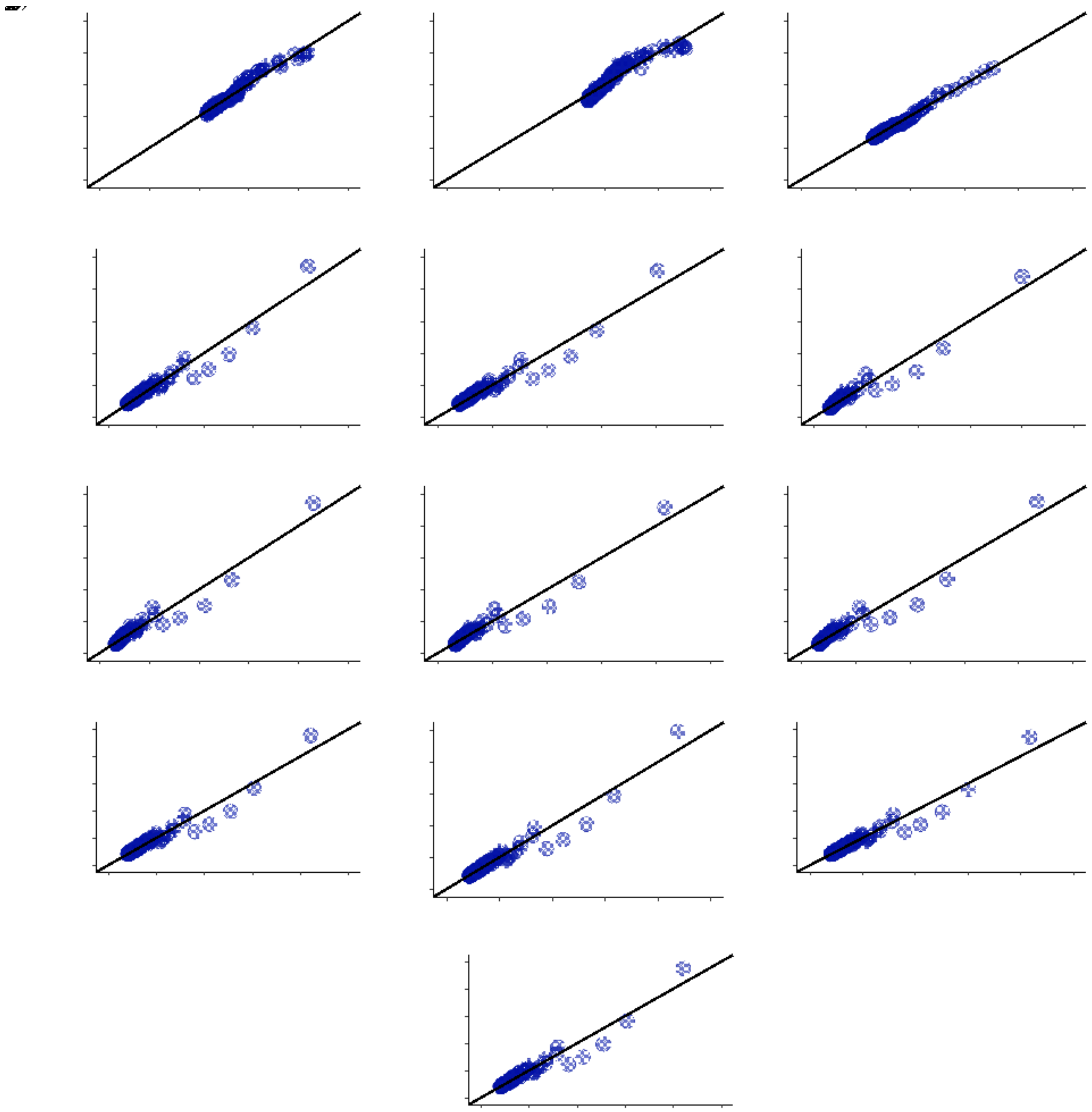
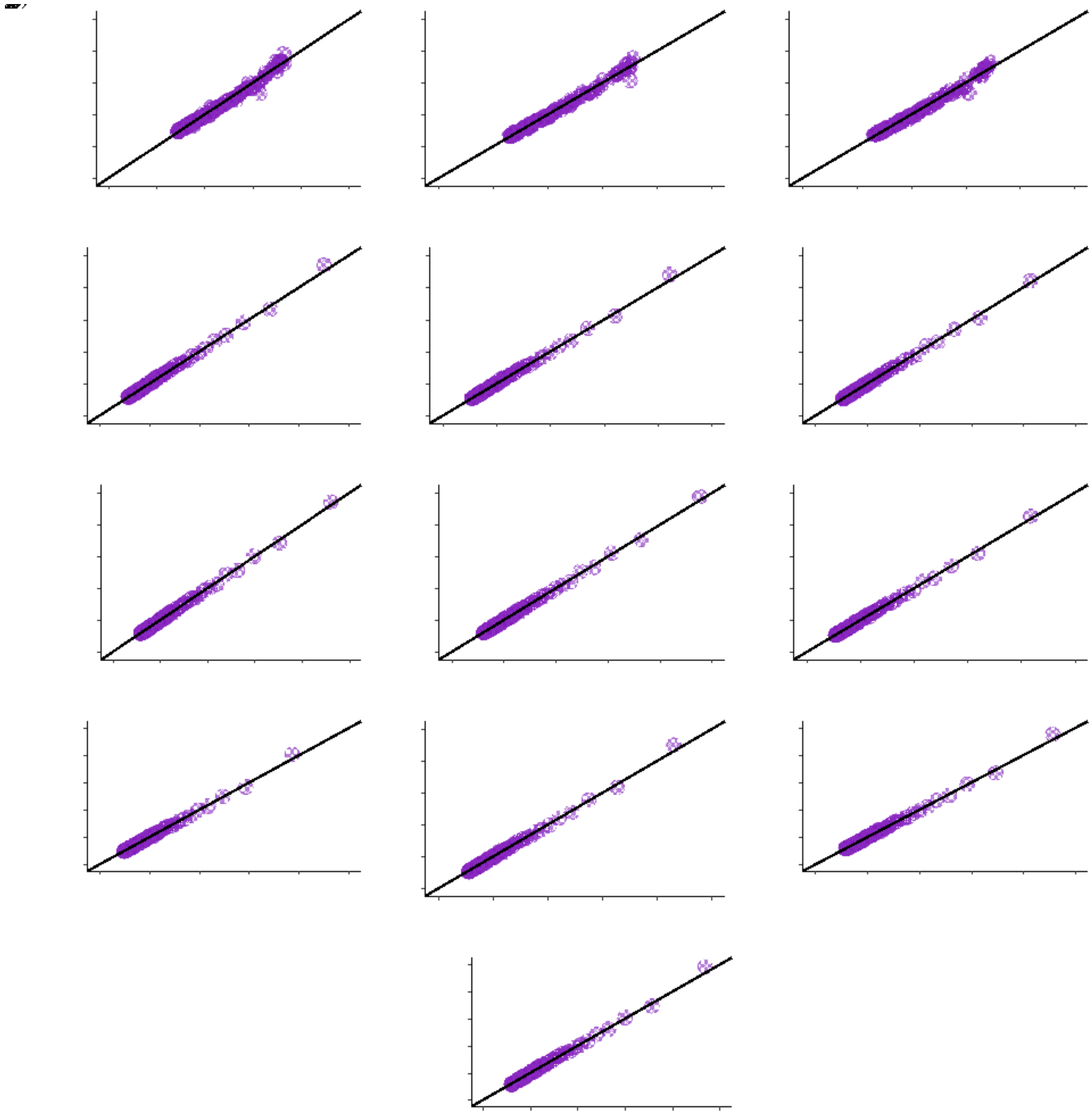
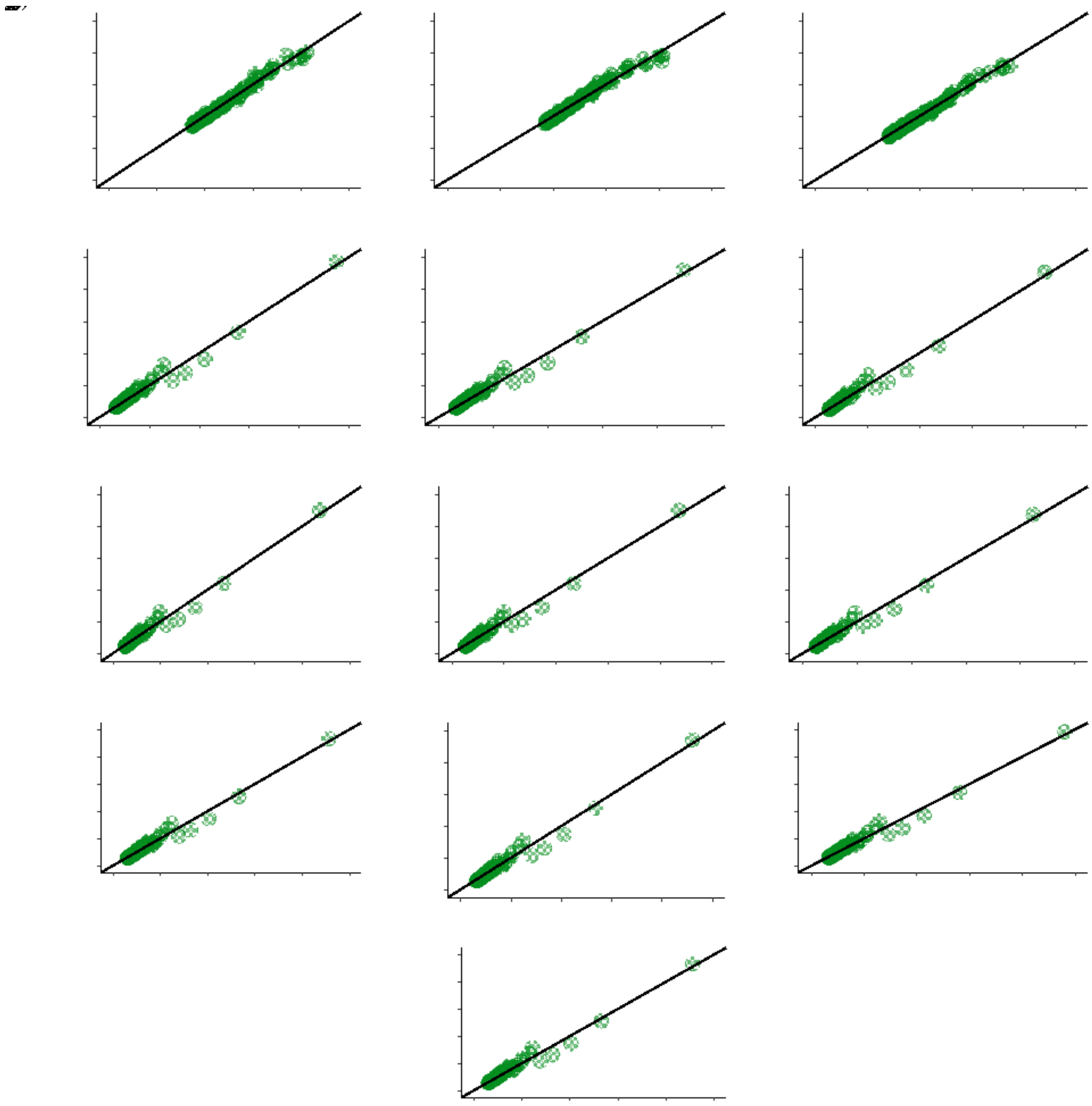
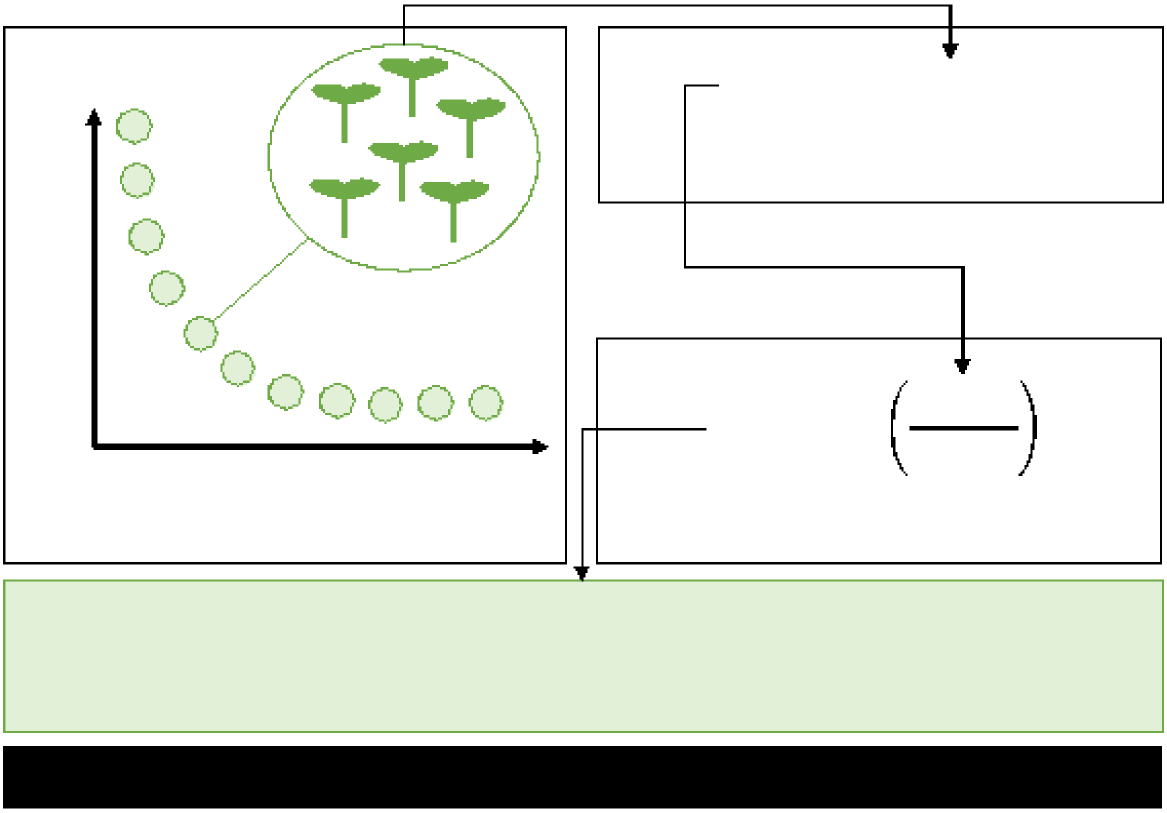
123.2
94.4
65.6
36.8
8.0
0
25
50
75
100
Number of seedlings per experimental unit









7.11 SUPPLEMENTARY MATERIAL

Supplementary material for this article is available online.

8 ARTIGO 4 – SAMPLE SIZE MATTERS: PRECISION STATISTICS ARE AFFECTED BY THE NUMBER OF PLANTS ASSESSED IN EXPERIMENTS WITH LETTUCE

(Formatação da revista Scientia Horticulturae)

Publicado no periódico: Scientia Horticulturae
Situação: Submetido

8.1 ABSTRACT

Evidence has shown that precision statistics results are influenced by sample size. However, several horticultural crops such as lettuce have not received enough attention in sample-size studies. Thus, this study aimed (i) to find the optimal sample size to estimate precision indicators in experiments that measure lettuce yield and (ii) to provide equations to forecast such precision indicators based on sample size. With data from an experiment with 26 lettuce genotypes, where 15 plants per experimental unit were sampled and lettuce yield per plant was measured, a bootstrap procedure with replacement was performed, simulating scenarios from 1 to 100 plants per experimental unit. Next, 16 precision statistics were estimated in each scenario, obtaining their 95% confidence interval widths. These values were fitted using shifted power models and sample size was found via maximum curvature point. Finally, forecasting formulas were adapted using an inverted modified power model, and the contribution of the statistics was analyzed through the principal component analysis. The 95% confidence interval widths decreased in response to sample size increase, and sample size varied among statistics. The optimal sample size to estimate precision statistics for lettuce yield per plant was 19 plants per experimental unit, considering the statistics with the greatest contributions to the total variation. Excellent fitting quality was obtained for the forecasting models, which were successful in predicting the value of statistics based on sample size.

Keywords: experimental planning, *Lactuca sativa L.*, precision indicators.

8.2 INTRODUCTION

Precision statistics, also known as precision indicators, are important tools utilized to measure experimental precision (Górdon-Mendoza and Camargo-Buitrago, 2015; Storck et al., 2016). When analyzing result reliability, researchers normally rely on indexes such as the coefficient of variation (CV) (Pimentel-Gomes, 1990) and the variation index (VI) (Pimentel-Gomes, 1991), which facilitate making comparisons between the precision of different evaluations or trials, besides being widely used due to their simple calculation structures. Variance components such as mean squares are also important to measure the amount of error (error mean square) and the variability between genotypes or treatments (genotype or treatment mean square), besides being used in the calculation of the F-test value (F) (Resende and Duarte, 2007). The latter is determinant in finding differences between treatments, as well as the least significant difference (LSD), for which these statistics are also used as indicators of experimental precision (Lúcio et al., 1997; Cargnelutti Filho et al., 2009; Górdon-Mendoza and Camargo-Buitrago, 2015). Moreover, when studying genotypes, namely in plant breeding programs, other criteria become important, such as selective accuracy (SA) and heritability (H) (Resende and Duarte, 2007). However, the values of all of these indicators can be massively influenced by the sample size selected for data collection (Souza et al., 2022; Bittencourt et al., 2023; Souza et al., 2023), which can put into question their own reliability depending on the sampling conditions.

Defining the sample size to be used in an experiment should be part of the initial phase of experimental planning, once the results can be negatively affected by sampling an insufficient number of observations, and the process of data collection, as well as the measurements of variables, can be harmed when this number is too high (Confalonieri et al., 2009; Alvez-Silva et al., 2018). Hence, several studies on sample size have focused on optimizing this number by finding the ideal sample size for different situations, which in the field of agronomy essentially means different crops (Cargnelutti Filho et al., 2013; Toebe et al., 2015; Toebe et al., 2018; Souza et al., 2022; Bittencourt et al., 2023; Souza et al., 2023). Importantly, these studies have evidenced that the optimal sample size may also vary within the same species depending on the trait, environmental conditions, and even the selected statistic. In this sense, the lack of sample size recommendations in the literature for crops of economic importance, under distinct possible scenarios, makes it difficult for researchers to follow a pattern that will guarantee a minimum acceptable precision without leading to resource wastage.

Namely, sample size recommendations for horticultural crops are scarcer in comparison to other species, making this group of plants a preferential target for sample size studies, as recently highlighted by Bittencourt et al. (2023). In the latter, sample size was defined for cauliflower seedlings considering precision indicators, and a forecasting methodology for such statistics was adapted from Souza et al. (2022), which allows researchers to know the value of a given statistic based on the number of plants selected per experimental unit. This approach has contributed to the field, optimizing the planning of experiments, and its calibration for different crops and conditions is encouraged.

On that basis, among the many horticultural crops, lettuce (*Lactuca sativa L.*) is the most consumed and cultivated leafy vegetable around the world (Noumedem et al., 2017), mainly for being a source of vitamins, minerals, and bioactive compounds that are beneficial to human health (Yang et al., 2022). Because of that, several studies on lettuce are performed every year aiming at increasing its yield and nutritional quality. However, in many cases, small sample sizes are used during evaluations, which may vary from three plants per experimental unit (Mustafa et al., 2023) to six (Qiao et al., 2023) or 10 plants (Yavuz et al., 2023) per treatment. This lack of standardization regarding an optimal sample size for the crop may be compromising the reliability of results from lettuce research, and consequently, the technical recommendations that derive from those. Therefore, the aims of this study were (i) to find the optimal sample size to estimate precision indicators in experiments that measure lettuce yield and (ii) to provide equations to forecast such precision indicators based on sample size.

8.3 MATERIAL AND METHODS

8.3.1 Reference experiment conduction and data collection

A reference experiment with 26 lettuce genotypes (Table 1) was carried out in the experimental area of the Federal University of Santa Maria (UFSM), in the Frederico Westphalen Campus, Rio Grande do Sul, Brazil (27º23’50” S latitude, 53º25’37” W longitude, at an altitude of 522 m). The region’s climate is humid subtropical, with no dry season defined, belonging to the cfa type (Wrege et al., 2012), and its soil is classified as Dystrophic Red Latosol (Santos et al., 2018). Seedling production was carried out in a greenhouse, under controlled temperature (15-20ºC) and humidity (60-70%). The seeds were sown on November 7th, 2022, in expanded polystyrene trays of 128 cells containing a vermiculite-based commercial substrate, watered daily, and kept in the greenhouse for 72 days.

Afterward, the seedlings were transplanted to the field and arranged in a completely randomized block design with two repetitions, totaling 52 experimental units. Each experimental unit contained 24 lettuce plants, spaced by 0.30 m within each row and between rows. Base fertilization was performed according to soil analysis and recommendations for the crop (CQFS - Comissão de Química e Fertilidade do Solo, 2016), and cover fertilization was carried out 34 days after transplant. Sixty-five days after transplant, yield was measured considering fresh weight per plant in grams (g), by randomly selecting 15 plants from each experimental unit.

8.3.2 Bootstrap resampling and estimate of precision statistics

All statistical analyses were performed using R software (R Development Core Team, 2023) and Microsoft Office Excel. First, a bootstrapping procedure was performed, in which experiments with different sampling scenarios were carried out based on the reference experiment, following the methodology of Bittencourt et al. (2022) and Souza et al. (2022). Hence, a total of 100 sample sizes were obtained, varying from 1 to 100 plants per experimental unit.

Next, an analysis of variance was performed for each sample size, after subdividing the data into experimental units, and the average value of each unit in each sampling scenario was obtained. The following mathematical model was used:

$$Y_{ij} = m + G_i + \beta_j + \varepsilon_{ij}$$

in which Y_{ij} represents the observed value in the response trait in plot ij, m is the overall mean, G_i is the fixed effect of level i (i = 1, 2, …, 26) of the genotype factor, β_j is the random effect of level j (j = 1 and 2) of the block, and ε_{ij} is the effect of the experimental error, which is supposedly normal and independently distributed with an average of zero and a common variance σ^2 (Storck et al., 2016).

The procedure was carried out with 10,000 resamples with replacement (Efron, 1979). Hence, a database of 1,000,000 simulated experiments was obtained, considering the 100 sampling scenarios and 10,000 resamples for each scenario. Afterward, 16 precision statistics were estimated for each resampling. The codes, calculation structures, and references of each statistic are shown in Table 2. These statistical analyses were performed with specific routines, including the *sample*() and *aov*() functions.

8.3.3 Definition of the optimal sample size per experimental unit

To find the optimal sample size for each statistic, the 95% confidence interval width ($CI_{95\%}$) of the statistics was calculated (Rothman and Greenland, 2018), and the maximum curvature point was defined, using the perpendicular distances method (Silva and Lima, 2017), as suggested by Bittencourt et al. (2022) and Souza et al. (2022). First, the minimum, 2.5 percentile, mean, 97.5 percentile, and maximum values of each statistic were obtained in each resample of every sample scenario (Supplementary Table 2). Then, the following equation was used to calculate the $CI_{95\%}$:

$$CI_{95\%} = UP - LP$$

in which UP is the upper percentile (97.5 percentile) and LP is the lower percentile (2.5 percentile).

The resulting $CI_{95\%}$ values were fitted to the sampling scenarios of 1 to 100 plants per experimental unit using a shifted power model, which was parameterized with the *nls*() function:

$$CI_{95\%} = \alpha \times (n - \beta)^{c} + \varepsilon$$

in which α represents the coefficient of interception, n, the sample size, β and c, coefficients of second and third order, and ε, the random-effect error.

Then, the maximum curvature point was found for each statistic using the *maxcurv* function through the perpendicular distances method, and this point was considered the optimal sample size. Additionally, a principal component analysis was performed, by using the *svd*() function and setting a mean of zero and a unitary variance to all statistics. This was done to verify the contribution of the precision statistics to the total variation in the sampling scenarios so that the final decision on the optimal sample size was made based on the statistics with the greatest contributions.

8.3.4 Forecasting precision statistics

The methodology proposed by Souza et al. (2022) was adapted to forecast precision statistics, using a modified power model proposed by Olivoto et al. (2018). Hence, the following model was used for each statistic:

$$CI_{95\%} = \delta^{PS} \times \alpha \times n^{\beta} + \varepsilon$$

in which δ is the adjustment factor of the coefficient of interception (Olivoto et al., 2018), α is the coefficient of interception, n is the given sample size, β is the exponential rate of decay, *PS* is the mean value of the precision statistic, and ε is the random-effect error. Also, the $CI_{95\%}$ of the parameters of the model were obtained using the *confint*() function. The model was parameterized using 700,000 values, which represents 70% of the resamples for each precision statistic. The remaining 300,000 values were used for validation.

The validation process consisted of applying simple linear regressions between the observed and predicted $CI_{95\%}$ values of each precision statistic. The models' fitting quality was assessed through the following indicators: coefficient of determination (R^2), Willmott's agreement index (d index), and root mean square error (RMSE). The *hydrogof* package was used for the analyses. Finally, the forecasting formulas were generated from the inversion of the modified power models, as follows:

$$CI_{95\%} = \delta^{PS} \times \alpha \times n^{\beta} + \varepsilon \quad (I)$$

$$\delta^{PS} = \frac{CI_{95\%}}{\alpha \times n^{\beta}} \quad (II)$$

$$PS = log_{\delta}\left(\frac{CI_{95\%}}{\alpha \times n^{\beta}}\right)_{(III)}$$

8.4 RESULTS

8.4.2 Experimental precision of the reference experiment

High mean square values were obtained for blocks and genotypes in comparison to the error mean square in the reference experiment, which resulted in high F values, so that the use of blocks in the experimental design was considered efficient (F = 11.20; p-value = 0.00259), and significant differences between genotypes were observed (F = 2.13; p-value = 0.03250). The overall mean of the fresh weight was 128.94 g, which led to a CVe of 34.66% and a VI of 24.51%. Also, a CVg of 26.00% and a CVr of 0.75 were obtained. The variance values oscillated from 998.35 (Vres) to 1123.78 (Vgen) and 2122.13 (Vphen). Moreover, an LSD5 of 183.63 and an LSD5% of 142.42% were obtained, and for SA and H, values of 0.73 and 0.53 were found, respectively.

8.4.3 Response of the statistics' confidence interval width to sample size variation

The $CI_{95\%}$ of all statistics decreased exponentially as the number of plants sampled per experimental unit increased. As shown in Fig. 1, wider confidence intervals are observed in scenarios where small sample sizes were simulated compared to those where a larger number of plants was selected. For example, the $CI_{95\%}$ of the CVe reduced from 24.37 when only one plant per experimental unit was sampled to 2.91 for 100 plants per experimental unit. This is the result of a reduction in the upper limit values and an increase in the lower limit values of the statistics. Moreover, the mean property of some precision statistics reduced with sample size increase, such as the mean squares, the least significant differences, Vphen, Vres, CVe, and VI, while the means of others such as F, Vgen, CVg, CVr, SA, and H increased. This indicates an increase in precision in scenarios of greater sample sizes. On the other hand, the mean property of the mean remained stable.

8.4.4 Fitting quality of the shifted power models and optimal sample sizes

The shifted power models obtained good fitting quality for all precision statistics, as expressed by the fitting quality indicators shown in Table 3. R^2 and d index were close to 1 for all statistics. R^2 values varied from 0.9980 (SA) to 0.9999 (GMS and Vphen), while d index values varied from 0.9995 (SA and H) to 0.9999, which was obtained for most statistics. Also, low RMSE values were obtained, which oscillated from 0.0038 for SA to 104.5083 for BMS, possibly due to the high value obtained for this statistic.

The optimal sample sizes found varied among statistics (Fig. 2). The lowest sample size was obtained for GMS, EMS, Vphen, Vgen, and Vres, which corresponds to 14 plants per experimental unit. An optimal size of 15 plants per experimental unit was found to estimate the mean, BMS, and CVg, while SA required 16 plants, CVe, LSD5, LSD5%, and VI required 17 plants, and CVg, CVr, and H required 19 plants. The maximum sample size obtained was 21 plants per experimental unit to estimate F. In this sense, the statistics that contributed the most to the total variation, which was retained in the first principal component (PC1), are the ones with a contribution percentage higher than the one marked by the dashed line, as shown in Fig. 3. Thus, although the F statistic had a high contribution, the remaining statistics can be reliably estimated with the sampling of 19 lettuce plants per experimental unit, which is the second highest optimal sample size obtained among the statistics with the greatest contributions.

8.4.5 Fitting quality of the forecasting models for precision statistics

Good fitting quality was also obtained for the modified power models used for forecasting precision statistics (Table 4). R^2 values varied from 0.9967 for H to 0.9998 for the majority of the statistics, and d index values varied from 0.9992 (H) to 0.9999 (most statistics), whereas the RMSE values fluctuated from 0.0045 for SA to 106.5006 for BMS. Moreover, the upper and lower limits of the parameters of the models were obtained (Table 5). These values can be used in the forecasting formulas to know the range of the optimal sample sizes for each statistic. Regarding the validation process (Fig. 4), the predicted $CI_{95\%}$ values were close to the observed $CI_{95\%}$ of the models. It was also noted that predictability was affected by the number of plants sampled in each scenario, as a lower forecasting capacity was observed at small sample sizes (wide confidence intervals), which was also pointed out by Bittencourt et al. (2023) and Souza et al. (2022).

8.5 DISCUSSION

This study addressed an important issue concerning the planning of experiments by finding an optimal sample size per experimental unit (Alvez-Silva et al., 2018; Bittencourt et al., 2023; Souza et al., 2022) for a widely grown and consumed horticultural crop, such as lettuce (Noumedem et al., 2017). Focusing on lettuce yield, the response of different precision statistics to sample size variation was analyzed. Besides, forecasting equations to predict the value of those statistics based on sample size were proposed by adapting the methodology of

Souza et al. (2022). Thus, the information presented here can be useful to researchers performing experiments with the crop, facilitating the selection of a feasible sample size that still guarantees acceptable precision.

A satisfactory precision was obtained in the reference experiment, as demonstrated by the precision statistics results (Table 1). The slightly higher CVe value obtained, in comparison to other indexes such as VI, was most likely influenced by the low overall experimental mean, given the strong association between these two statistics, for which the CVe has been previously criticized (Döring and Reckling, 2018). Also, CVe, LSD5, and LSD5% higher values may be an effect of these statistics being more associated with residual variances than with genetic variances (Górdon-Mendoza and Camargo-Buitrago, 2015; Souza et al., 2023a). Thus, a better understanding of the experimental precision can be achieved by considering all statistics jointly (Souza et al. 2022; Bittencourt et al., 2023), especially in studies that compare genotypes. The values obtained by the F (higher than 1.96 and lower than 5.26) and SA (higher than 0.70 and lower than 0.90) statistics, for example, indicate high precision according to the scale of Resende and Duarte (2007). Another point worth mentioning is the significant difference existent between the lettuce genotypes selected for the experiment. This represents an advantage when defining an optimal sample size for the crop, once the sample sizes found apply to a wide range of genotypes.

Once sample size is highly associated with result reliability, the $CI_{95\%}$ of each statistic was analyzed as a function of the number of plants sampled per experimental unit. The $CI_{95\%}$ decrease in response to sample size increase (Fig. 1) is a normally observed pattern in sample size studies (Cargnelutti Filho et al., 2013; Olivoto et al., 2018; Toebe et al., 2018; Souza et al., 2023a). This occurs because the smallest sample sizes are not representative enough, causing the upper and lower limits to be distant from the real values (Rothman and Greenland, 2018). Therefore, when using these sizes, there is a high risk of under or overestimation of results (Souza et al., 2022). Such under or overestimation can be better visualized when focusing on the mean property of the statistics. Notably, the statistics that indicate high precision at high values (the higher, the better), such as F, SA, H, CVg, and CVr, had their mean values underestimated at low sample sizes, and the opposite was observed for the statistics that indicate high precision at low values (the lower, the better), such as CVe, VI, GMS, EMS, Vphen, Vres, LSD5, and LSD5%, which had their mean values overestimated. Some exceptions were noted with BMS and Vgen, for which that pattern was not as attenuated. Still, this observation highlights the strong relationship between precision and sample size. In this sense, finding an optimal sample size means narrowing the $CI_{95\%}$ until its value stabilizes, but also avoiding the selection of an unnecessarily high number of plants, which could be thought of as oversampling (Toebe et al., 2015; Souza et al., 2023a; Souza et al., 2023b).

As expected, although similar sample sizes were found among the statistics, some of them required the sampling of more plants per experimental unit than others (Fig. 2). Interestingly, the F statistic was the one that required the most robust sampling, which was also observed in the studies by Souza et al. (2022), Souza et al. (2023a), and Bittencourt et al. (2023). This specificity should be taken into account, especially in cases where this statistic is used as the only precision indicator in order to guarantee an accurate estimate. However, the second greatest optimal sample size among the statistics that contributed the most to the total variation retained in the first principal component was attributed to H (Fig. 3), the statistic with the greatest contribution, which required 19 plants per experimental unit, thus making this number the optimal sample size to estimate precision statistics reliably when measuring lettuce yield per plant.

Furthermore, the forecasting equations here proposed, adapted from the work of Souza et al. (2022), can be used to facilitate the decision on the number of plants to sample per experimental unit based on the desired precision (Table 4). This prediction implicates arbitrating a sample size per experimental unit in order to know the value of a given statistic. Thus, such a number can be added to the shifted power model parametrized for the statistic in question $[CI_{95\%} = \alpha\times(n - \beta)^{c} + \varepsilon]$ by replacing n so that the $CI_{95\%}$ of the statistic can be calculated. Next, by adding the $CI_{95\%}$ and the same sample size (n) to the forecasting equation $\left[PS = log_{\delta}\left(\frac{CI_{95\%}}{\alpha\times n^{\beta}}\right)\right]$, the precision statistic value can be found. The following example illustrates the application of the forecasting methodology using the VI statistic, and arbitrating a sample size of 17 plants per experimental unit, based on the recommendations here made: 1) The $CI_{95\%}$ is given by $CI_{95\%} = 20.6933\times[17 - (-\ 0.4427)]^{0.4994}$, resulting in 4.9633; 2) By applying the forecasting equation, VI is given by $VI = log_{0.9801}\left(\frac{4.9633}{33.7778\times17^{-0.4986}}\right)$, resulting in a mean VI of 25.13%.

Moreover, those formulas can also be applied by using the upper and lower limits of the parameters of the shifted power (Supplementary Table 1) and modified power (Table 5) models. In this way, a maximum and minimum value can be set for the statistic, considering a confidence interval for its result. Thus, in the aforementioned example, the value of VI can vary from 22.69% to 27.63%, when sampling 17 plants per experimental unit. Importantly, the forecasting formulas here shown must be calibrated and tested before being applied to other lettuce traits, statistics, or experiments performed in extremely different environmental conditions than the one in this study.

8.6 CONCLUSIONS

The optimal sample sizes found varied among precision statistics. Taking into account the statistics with the greatest contributions to the total variation, 19 plants per experimental unit were enough to estimate experimental precision statistics for lettuce yield per plant reliably, with the exception of F, which required 21 plants per experimental unit. The proposed forecasting equations were successful in predicting the value of the precision statistics based on sample size. The sample size recommendations and models demonstrated in the present study contribute to improving and optimizing the planning of lettuce experiments that measure yield per plant.

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8.8 TABLES

Table 1. Phenotypical description of the 26 lettuce genotypes used in the reference experiment regarding type, variety, leaf color, cycle (days), and brand.

Code	Genotype	Variety	Leaf Color	Cycle (days)	Brand
G1	Gamboa	Butterhead	Green	60	ISLA
G2	Creta	Erect	Purple	60	ISLA
G3	Sibéria	Iceberg	Green	80	ISLA
G4	Trindade	Iceberg	Light green	75	ISLA
G5	Mimosa Brava	Salad bowl	Green	55	ISLA
G6	Itapuã Super	Looseleaf	Light green	55	ISLA
G7	Grand Rapids	Looseleaf	Light Green	60	ISLA
G8	Simpson	Looseleaf	Green	70	ISLA
G9	Hanson	Crisphead	Light green	80	ISLA
G10	Cerbiatta	Oakleaf	Green	72	ISLA
G11	Friseé Atalaia	Frisée	Purple	55	ISLA
G12	Baba de verão	Butterhead	Light green	60	ISLA
G13	Itaúna Frisée	Frisée	Green	55	ISLA
G14	Batávia cacimba	Curly red-leaf	Dark red	60	ISLA
G15	Regina de verão	Loose butterhead	Yellowish green	65	ISLA
G16	Mimosa Prado	Mimosa	Dark red	55	ISLA
G17	Brunela	Frisée	Light green	55	Feltrin
G18	Giovana	Mimosa	Light green	45	Feltrin
G19	Betania	Mimosa	Purple	55	Feltrin
G20	Stella	Butterhead	Light green	60	Feltrin
G21	Cinderela	Curly green-leaf	Light green	70	Feltrin
G22	Delícia Americana	Crisphead	Light green	80	ISLA

G23	Americana GL 659	Iceberg	Green	75	Feltrin
G24	Fortaleza	Butterhead	Green	60	ISLA
G25	Repolhuda todo ano	Butterhead	Light green	70	Feltrin
G26	Mônica	Curly green-leaf	Green	65	Feltrin

Table 2. Codes, calculation structures, and references of the precision statistics.

Precision statistic	Code	Equation(*)	Reference
Block mean square	BMS	$\sigma^2 + i\sigma_b^2$	Storck et al. (2016)
Genotype mean square	GMS	$\sigma^2 + \frac{r}{i-1}\sum_i G_i^2$	Storck et al. (2016)
Error mean square	EMS	σ^2	Storck et al. (2016)
F-test value	F	$\frac{GMS}{EMS}$	Storck et al. (2016)
Overall experimental mean	*m*	$\frac{\sum G_{ir}}{i \times r}$	Storck et al. (2016)
Phenotypic variance	Vphen	$\frac{GMS}{r}$	Cruz et al. (2012)
Genotypic variance	Vgen	$\frac{GMS-EMS}{r}$	Cruz et al. (2012)
Mean residual variance	Vres	$\frac{EMS}{r}$	Cruz et al. (2012)
Coefficient of genetic variation	CVg	$100\frac{\sqrt{Vgen}}{m}$	Resende & Duarte (2007)
Coefficient of experimental variation	CVe	$100\frac{\sqrt{EMS}}{m}$	Pimentel-Gomes (1990)
Coefficient of relative variation	CVr	$\frac{CVg}{CVe}$	Resende & Duarte (2007)
Variation index	VI	$\frac{CVe}{\sqrt{r}}$	Pimentel-Gomes (1991)
Selective accuracy	SA	$\left(\frac{1}{1-F}\right)^{0.5}$	Resende & Duarte (2007)

Heritability	H	SA^2	Resende & Duarte (2007)
Least significant difference at 5%	LSD5	$q_{\alpha(i;Edf)}\sqrt{\frac{EMS}{r}}$	Lúcio (1997)
Least significant difference as a percentage of the average at 5%	LSD5 %	$100\frac{LSD5}{m}$	Lúcio (1997)

(*) σ^2: experimental variance; σ_b^2: variance of the repetitions; i: number of genotypes, which is 26; r: number of repetitions; $q_{\alpha(i;\ Edf)}$: is the critical value for Tukey's test performed at 5% ; Edf: are the degrees of freedom of the error estimated $Edf = (r - 1)(n - 1)$.

Table 3. Fitting quality indicators and parameters of the shifted power model used to define optimal sample sizes to estimate experimental precision statistics for lettuce yield per plant (g plant^{-1}).

Precision statistic (*)	α (†)	β	*c*	R^2 (‡)	RMSE	d index	Sample size
BMS	49479.0756	0.0611	-0.5002	0.9998	104.5083	0.9999	15
GMS	4209.1137	0.2466	-0.5043	0.9999	7.4542	0.9999	14
EMS	3477.3272	0.1963	-0.5076	0.9998	6.1631	0.9999	14
F	4.4436	-2.4683	-0.5220	0.9992	0.0100	0.9998	21
m	22.7886	0.0178	-0.4982	0.9998	0.0419	0.9999	15
Vphen	2104.5569	0.2466	-0.5043	0.9999	3.7271	0.9999	14
Vgen	2604.6147	0.2242	-0.5014	0.9997	6.1135	0.9999	14
Vres	1738.6634	0.1963	-0.5076	0.9998	3.0815	0.9999	14
CVe	29.2647	-0.4427	-0.4994	0.9998	0.0468	0.9999	17
CVg	33.4087	-0.1317	-0.5369	0.9990	0.1333	0.9997	15
CVr	1.4718	-1.5295	-0.5218	0.9991	0.0040	0.9998	19
VI	20.6933	-0.4427	-0.4994	0.9998	0.0331	0.9999	17
LSD5	154.0846	-0.4054	-0.4985	0.9998	0.2625	0.9999	17
LSD5%	120.2631	-0.4427	-0.4994	0.9998	0.1925	0.9999	17
SA	0.7493	-0.4100	-0.5503	0.9980	0.0038	0.9995	16
H	1.0673	-1.4406	-0.5429	0.9982	0.0042	0.9995	19

(*) The codes of the precision statistics are shown in Table 2; (†) α: coefficient of interception; β and *c*: coefficients of second and third order; (‡) R^2: coefficient of determination; RMSE: root mean square error; d index: Willmott's agreement index.

Table 4. Fitting quality indicators and parameters of the modified power models used for forecasting precision statistics for lettuce yield per plant (g plant^{-1}).

Precision statistic $^{(*)}$	δ $^{(†)}$	α	β	$R^{2\,(‡)}$	RMSE	d index	Forecasting equation
BMS	1.0000	34564.5503	-0.5006	0.9997	106.5003	0.9999	$BMS = log_{1.0000}\left(\frac{CI_{95\%}}{34564.5503 \times n^{-0.5006}}\right)$
GMS	1.0001	2904.0600	-0.5022	0.9998	7.6547	0.9999	$GMS = log_{1.0001}\left(\frac{CI_{95\%}}{2904.0600 \times n^{-0.5022}}\right)$
SEM	1.0001	3034.4802	-0.5061	0.9998	6.0941	0.9999	$EMS = log_{1.0001}\left(\frac{CI_{95\%}}{3034.4802 \times n^{-0.5061}}\right)$
F	4.0629	0.2105	-0.5063	0.9995	0.0082	0.9999	$F = log_{4.0629}\left(\frac{CI_{95\%}}{0.2105 \times n^{-0.5063}}\right)$
M	0.9192	1196122.3082	-0.4986	0.9998	0.0402	0.9999	$m = log_{0.9192}\left(\frac{CI_{95\%}}{1196122.3082 \times n^{-0.4986}}\right)$
Vphen	1.0002	1452.0300	-0.5022	0.9998	3.8273	0.9999	$Vphen = log_{1.0002}\left(\frac{CI_{95\%}}{1452.0300 \times n^{-0.5022}}\right)$
Vgen	0.9968	104929.3648	-0.5262	0.9986	14.4969	0.9996	$Vgen = log_{0.9968}\left(\frac{CI_{95\%}}{104929.3648 \times n^{-0.5262}}\right)$
Vres	1.0001	1517.2400	-0.5061	0.9998	3.0470	0.9999	$Vres = log_{1.0001}\left(\frac{CI_{95\%}}{1517.2400 \times n^{-0.5061}}\right)$
CVe	0.9859	47.7690	-0.4986	0.9998	0.0465	0.9999	$CVe = log_{0.9859}\left(\frac{CI_{95\%}}{47.7690 \times n^{-0.4986}}\right)$
CVg	0.8600	1513.8293	-0.5090	0.9998	0.0643	0.9999	$CVg = log_{0.8600}\left(\frac{CI_{95\%}}{1513.8293 \times n^{-0.5090}}\right)$
CVr	16.9134	0.1797	-0.5267	0.9984	0.0056	0.9996	$CVr = log_{16.9134}\left(\frac{CI_{95\%}}{0.1797 \times n^{-0.5267}}\right)$
VI	0.9801	33.7778	-0.4986	0.9998	0.0329	0.9999	$VI = log_{0.9801}\left(\frac{CI_{95\%}}{33.7778 \times n^{-0.4986}}\right)$
LSD5	0.9975	243.8286	-0.4980	0.9998	0.2632	0.9999	$LSD5 = log_{0.9975}\left(\frac{CI_{95\%}}{243.8286 \times n^{-0.4980}}\right)$

LSD5%	0.9965	196.3062	-0.4986	0.9998	0.1910	0.9999	$LSD5\% = log_{0.9965}\left(\frac{CI_{95\%}}{196.3062\times n^{-0.4986}}\right)$
AS	4.3731	0.2587	-0.5523	0.9972	0.0045	0.9993	$SA = log_{4.3731}\left(\frac{CI_{95\%}}{0.2587\times n^{-0.5523}}\right)$
H	31.1741	0.1784	-0.5510	0.9967	0.0057	0.9992	$H = log_{31.1741}\left(\frac{CI_{95\%}}{0.1784\times n^{-0.5510}}\right)$

(*) The codes of the precision statistics are shown in Table 2. (†) δ: adjustment factor of the coefficient of interception; α: coefficient of interception; β: exponential rate of decay; (‡) R^2: coefficient of determination; RMSE: root mean square error; d index: Willmott's agreement index.

Table 5. Upper and lower limits of the parameters of the modified power models used to forecast precision statistics for lettuce yield per plant (g plant^{-1}).

Precision statistic (*)	Lower limits			Modified power model			Upper limits		
	δ (†)	α	β	δ	α	β	δ	α	β
BMS	1.0000	29840.2628	-0.5033	1	34564.5503	-0.5006	1.0000	39288.8377	-0.4980
GMS	1.0001	2822.2513	-0.5044	1.0001	2904.0600	-0.5022	1.0001	2985.8686	-0.4999
SEM	1.0001	2984.0063	-0.5084	1.0001	3034.4802	-0.5061	1.0001	3084.9541	-0.5039
F	3.9069	0.1957	-0.5097	4.0629	0.2105	-0.5063	4.2190	0.2253	-0.5028
M	0.8755	-6134878.1345	-0.5000	0.9192	1196122.3082	-0.4986	0.9629	8527122.7508	-0.4972
Vphen	1.0002	1411.1249	-0.5044	1.0002	1452.0300	-0.5022	1.0002	1492.9352	-0.4999
Vgen	0.9951	-92776.3124	-0.5300	0.9968	104929.3648	-0.5262	0.9985	302635.0419	-0.5225
Vres	1.0001	1492.0028	-0.5084	1.0001	1517.2400	-0.5061	1.0001	1542.4772	-0.5039

CVe	0.9851	46.2018	-0.5008	0.9859	47.7690	-0.4986	0.9866	49.3361	-0.4964
CVg	0.8484	988.7712	-0.5108	0.86	1513.8293	-0.509	0.8717	2038.8874	-0.5073
CVr	13.8250	0.1595	-0.5340	16.9134	0.1797	-0.5267	20.0017	0.2000	-0.5194
VI	0.9790	32.6696	-0.5008	0.9801	33.7778	-0.4986	0.9811	34.8859	-0.4964
LSD5	0.9973	235.2356	-0.5003	0.9975	243.8286	-0.498	0.9976	252.4216	-0.4956
LSD5%	0.9964	189.8661	-0.5008	0.9965	196.3062	-0.4986	0.9967	202.7463	-0.4964
AS	2.8564	0.2029	-0.5635	4.3731	0.2587	-0.5523	5.8898	0.3145	-0.5410
H	21.0312	0.1544	-0.5622	31.1741	0.1784	-0.551	41.3170	0.2025	-0.5398

(*) The codes of the precision statistics are shown in Table 2. (†) δ: adjustment factor of the coefficient of interception; α: coefficient of interception; β: exponential rate of decay.

8.9 FIGURE CAPTIONS

Fig. 1. Minimum, 2.5 percentile, mean, 97.5 percentile, and maximum values obtained from the planned sample sizes of 1 to 100 lettuce plants per experimental unit for the experimental precision statistics block mean square (a), genotype mean square (b), error mean square (c), F-test value (d), mean (e), phenotypic variance (f), genotypic variance (g), mean residual variance (h), coefficient of experimental variation (i), coefficient of genetic variation (j), coefficient of relative variation (k), variation index (l), least significant difference at 5% (m), least significant difference as a percentage of the average at 5% (n), selective accuracy (o), and heritability (p) for lettuce yield per plant (g plant^{-1}).

(*) Dashed lines represent minimum and maximum values, colored straight lines represent 2.5 and 97.5 percentiles, and black straight lines represent mean values.

Fig. 2. Optimal sample sizes defined via shifted power model and maximum curvature point to estimate the precision statistics block mean square (a), genotype mean square (b), error mean square (c), F-test value (d), mean (e), phenotypic variance (f), genotypic variance (g), mean residual variance (h), coefficient of experimental variation (i), coefficient of genetic variation (j), coefficient of relative variation (k), variation index (l), least significant difference at 5% (m), least significant difference as a percentage of the average at 5% (n), selective accuracy (o), and heritability (p) for lettuce yield per plant (g plant^{-1}).

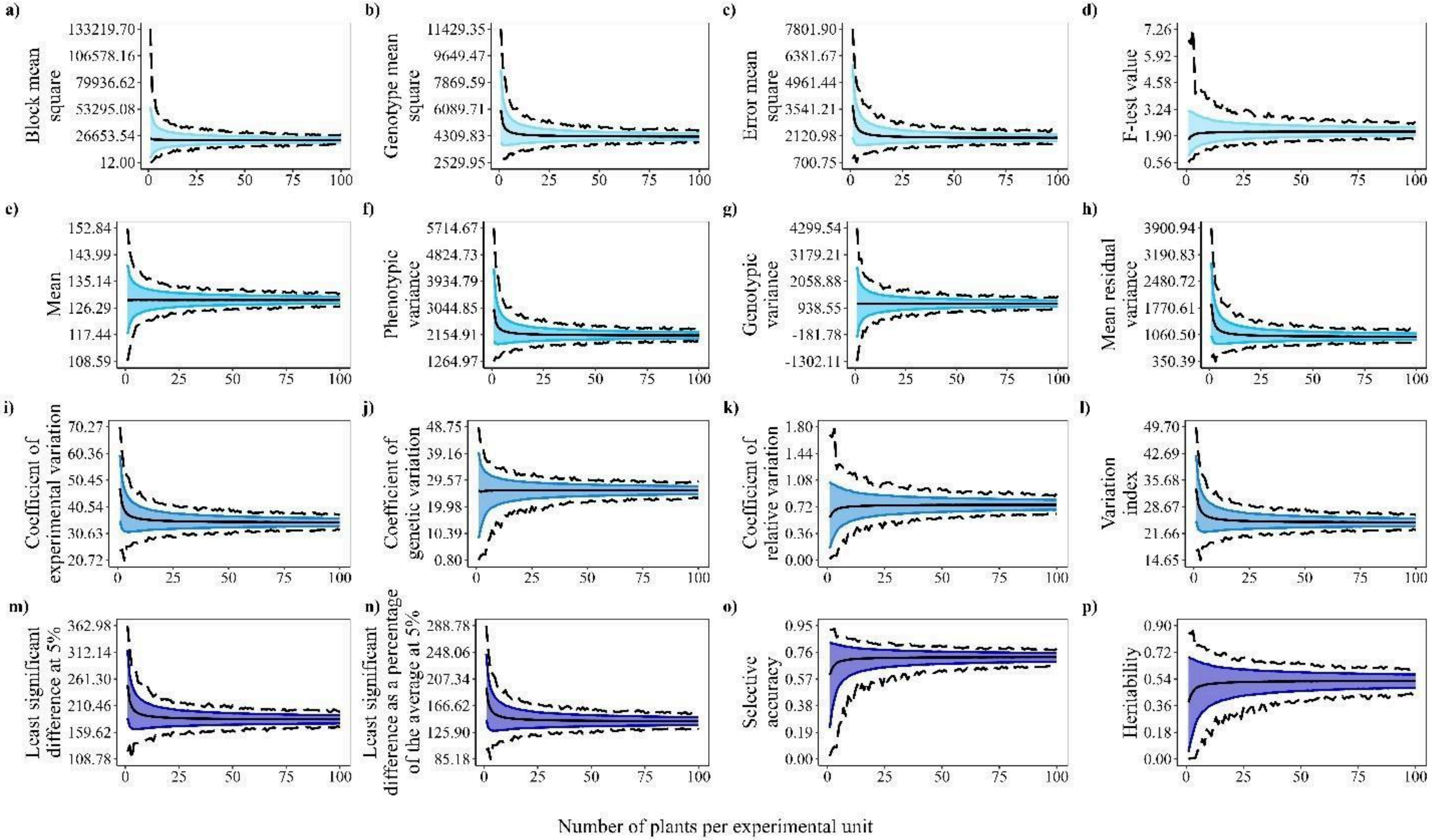
Fig. 3. Principal component analysis of the definition of the optimal sample size per experimental unit to estimate precision statistics for lettuce yield per plant (g plant^{-1}) and contribution percentage of the precision statistics to the total variation retained in the first principal component.

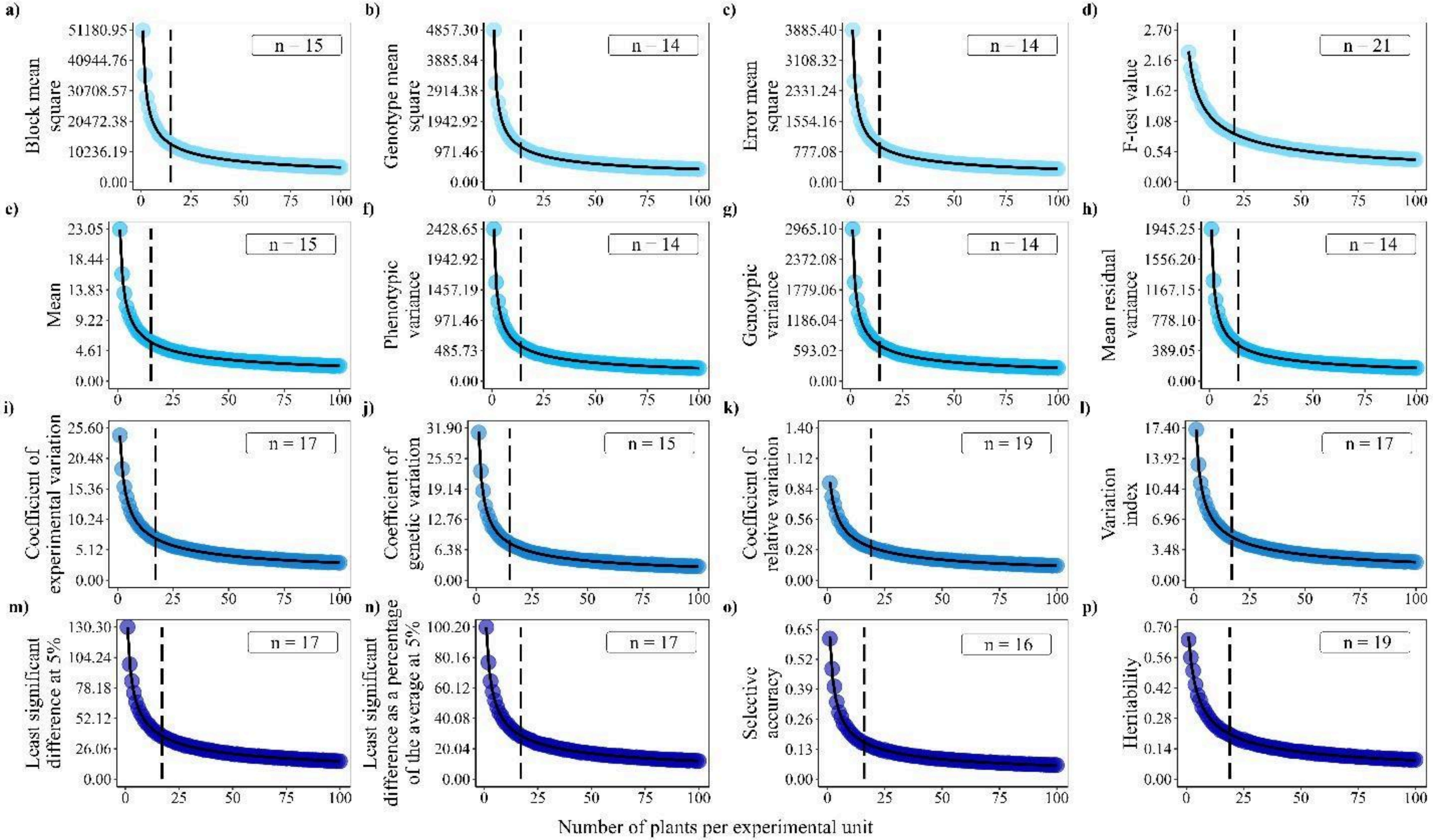
(*) PC1: first principal component; PC2: second principal component.

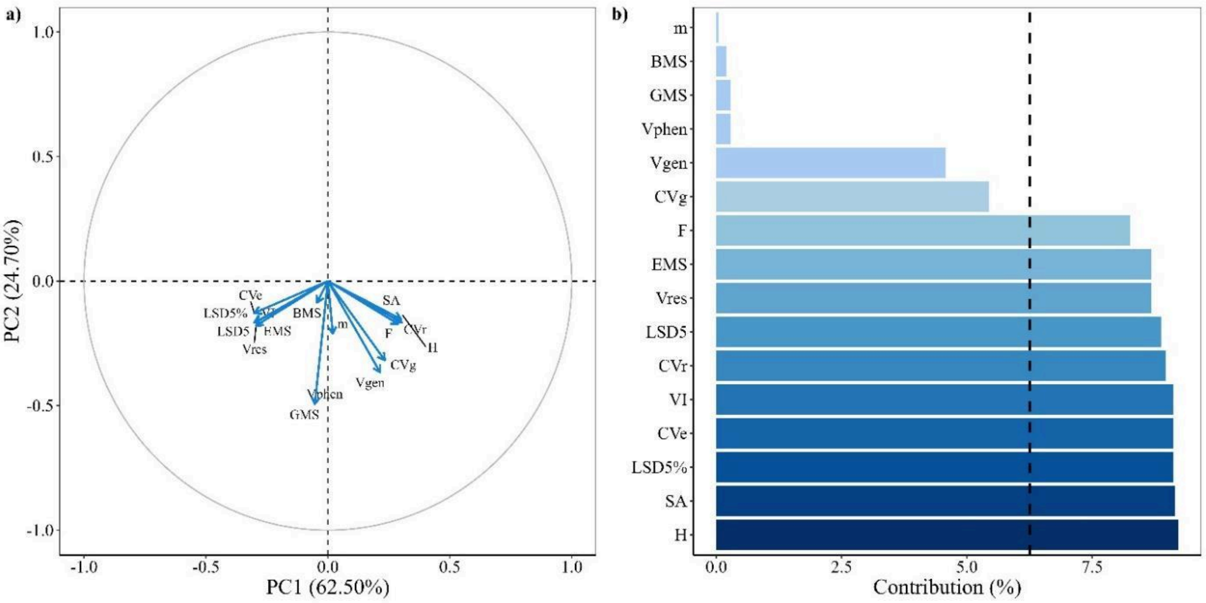
(†) The codes of the precision statistics are shown in Table 2.

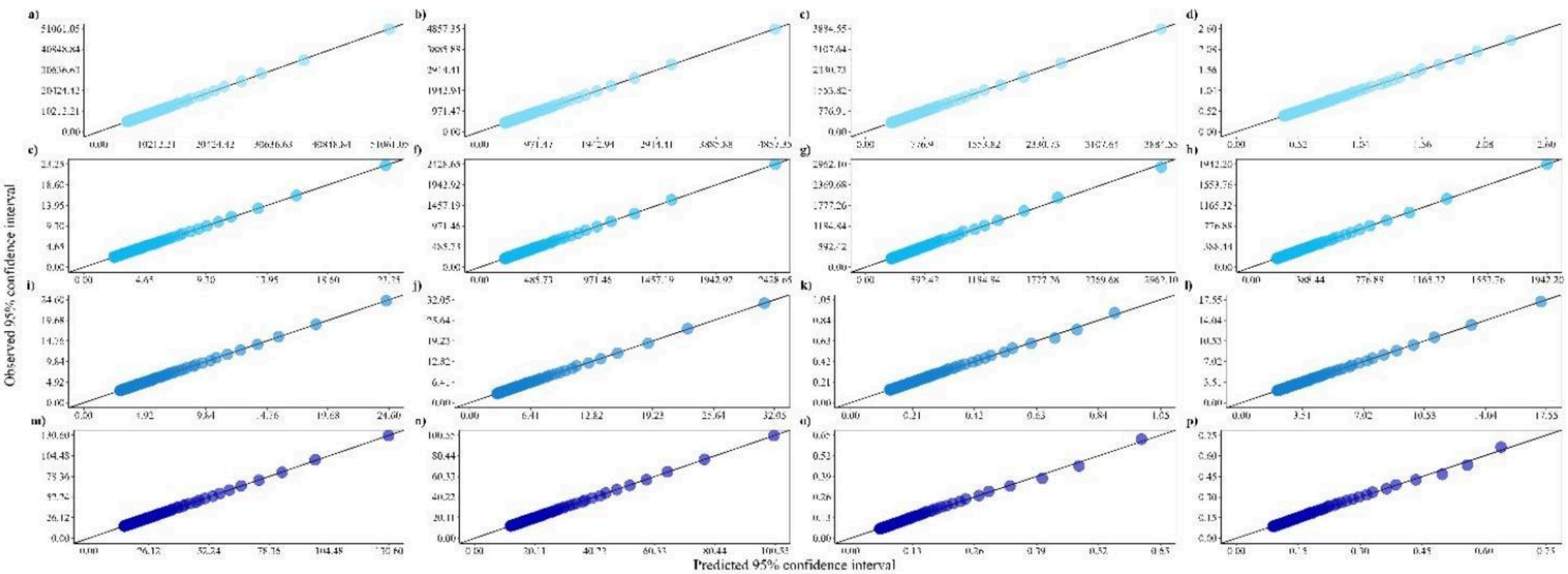
Fig. 4. Observed and predicted 95% confidence interval width of the precision statistics block mean square (a), genotype mean square (b), error mean square (c), F-test value (d), mean (e), phenotypic variance (f), genotypic variance (g), mean residual variance (h), coefficient of experimental variation (i), coefficient of genetic variation (j), coefficient of relative variation (k), variation index (l), least significant difference at 5% (m), least significant difference as a percentage of the average at 5% (n), selective accuracy (o), and heritability (p) for lettuce yield per plant (g plant^{-1}) obtained through the validation process of the modified power models.

8.10 FIGURES


a)
Block mean square
133219.70
106578.16
79936.62
53295.08
26653.54
12.00
b)
Genotype mean square
11429.35
9649.47
7869.59
6089.71
4309.83
2529.95
c)
Error mean square
7801.90
6381.67
4961.44
3541.21
2120.98
700.75
d)
F-test value
7.26
5.92
4.58
3.24
1.90
0.56
e)
Mean
152.84
143.99
135.14
126.29
117.44
108.59
f)
Phenotypic variance
5714.67
4824.73
3934.79
3044.85
2154.91
1264.97
g)
Genotypic variance
4299.54
3179.21
2058.88
938.55
-181.78
-1302.11
h)
Mean residual variance
3900.94
3190.83
2480.72
1770.61
1060.50
350.39
i)
Coefficient of experimental variation
70.27
60.36
50.45
40.54
30.63
20.72
j)
Coefficient of genetic variation
48.75
39.16
29.57
19.98
10.39
0.80
k)
Coefficient of relative variation
1.80
1.44
1.08
0.72
0.36
0.00
l)
Variation index
49.70
42.69
35.68
28.67
21.66
14.65
m)
Least significant difference at 5%
362.98
312.14
261.30
210.46
159.62
108.78
n)
Least significant difference as a percentage of the average at 5%
288.78
248.06
207.34
166.62
125.90
85.18
o)
Selective accuracy
0.95
0.76
0.57
0.38
0.19
0.00
p)
Heritability
0.90
0.72
0.54
0.36
0.18
0.00
0
25
50
75
100
Number of plants per experimental unit


a)
Block mean square
51180.95
40944.76
30708.57
20472.38
10236.19
0.00
n = 15
b)
Genotype mean square
4857.30
3885.84
2914.38
1942.92
971.46
0.00
n = 14
c)
Error mean square
3885.40
3108.32
2331.24
1554.16
777.08
0.00
n = 14
d)
F-test value
2.70
2.16
1.62
1.08
0.54
0.00
n = 21
e)
Mean
23.05
18.44
13.83
9.22
4.61
0.00
n = 15
f)
Phenotypic variance
2428.65
1942.92
1457.19
971.46
485.73
0.00
n = 14
g)
Genotypic variance
2965.10
2372.08
1779.06
1186.04
593.02
0.00
n = 14
h)
Mean residual variance
1945.25
1556.20
1167.15
778.10
389.05
0.00
n = 14
i)
Coefficient of experimental variation
25.60
20.48
15.36
10.24
5.12
0.00
n = 17
j)
Coefficient of genetic variation
31.90
25.52
19.14
12.76
6.38
0.00
n = 15
k)
Coefficient of relative variation
1.40
1.12
0.84
0.56
0.28
0.00
n = 19
l)
Variation index
17.40
13.92
10.44
6.96
3.48
0.00
n = 17
m)
Least significant difference at 5%
130.30
104.24
78.18
52.12
26.06
0.00
n = 17
n)
Least significant difference as a percentage of the average at 5%
100.20
80.16
60.12
40.08
20.04
0.00
n = 17
o)
Selective accuracy
0.65
0.52
0.39
0.26
0.13
0.00
n = 16
p)
Heritability
0.70
0.56
0.42
0.28
0.14
0.00
n = 19
0
25
50
75
100
Number of plants per experimental unit


a)
1.0
0.5
0.0
-0.5
-1.0
PC2 (24.70%)
-1.0
-0.5
0.0
0.5
1.0
PC1 (62.50%)
CVe
LSD5%
VI
LSD5
EMS
Vres
BMS
m
SA
F
CVr
H
CVg
Vgen
Vphen
GMS
b)
m
BMS
GMS
Vphen
Vgen
CVg
F
EMS
Vres
LSD5
CVr
VI
CVe
LSD5%
SA
H
0.0
2.5
5.0
7.5
Contribution (%)


a)
b)
c)
d)
e)
f)
g)
h)
i)
j)
k)
l)
m)
n)
o)
p)
Observed 95% confidence interval
Predicted 95% confidence interval

8.11 SUPPLEMENTARY MATERIAL

Supplementary material for this article is available.

9 ARTIGO 5 – PLANNING LETTUCE EXPERIMENTS: SAMPLE SIZE DEFINITION TO MEASURE THE PRECISION OF BIOMETRIC TRAITS

(Formatação da revista Scientia Horticulturae)

Submetido ao periódico: Scientia Horticulturae
Situação: Submetido

9.1 ABSTRACT

The aims of this study were (i) to define the optimal sample size per experimental unit to estimate precision statistics for lettuce biometric traits and (ii) to propose forecasting equations for precision statistics from the selection of a sample size per experimental unit. An experiment with 26 lettuce genotypes was performed and the plant height, number of leaves, neck diameter, and mean head diameter were measured. Then, a bootstrap resampling procedure was applied, creating sampling scenarios from 1 to 100 plants per experimental unit, and five precision statistics were calculated for each trait, fitting their 95% confidence interval width to the sampling scenarios via shifted power models. Sample size was defined by finding the maximum curvature point, using the perpendicular distances method, and considering the contribution of the statistics to the total variation retained in the first principal component. Also, forecasting formulas for precision statistics based on sample size were adapted using an inverted modified power model. The 95% confidence interval width of the statistics reduced with the increase in sample size, gaining precision. A greater variation was observed in the sample sizes between statistics than between traits. All traits and statistics can be reliably estimated with 24 lettuce plants per experimental unit. The SA and F statistics contributed the most to the total variation. The precision statistics here estimated can be accurately predicted using the forecasting formulas, as well as their confidence interval, allowing the optimization of the planning of lettuce experiments.

Keywords: *Lactuca sativa L.*, number of plants, precision statistics.

9.2 INTRODUCTION

Experimental precision can be measured through the calculation of several precision statistics (Cargnelutti Filho et al., 2009; Górdon-Mendoza and Camargo-Buitrago, 2015;

Souza et al., 2022). Some of the most common precision statistics are the coefficient of experimental variation (CVe) (Pimentel-Gomes, 1990), the variation index (VI) (Pimentel-Gomes, 1991), and the F-test value (F) (Resende and Duarte, 2007). In genetic studies, other indexes related to precision are also relevant, such as the coefficient of genetic variation (CVg) (Burton, 1952), which is widely used to know the existent genetic variation in experiments (Ziegler and Tambarussi, 2022). Another less-known but equally important indicator is selective accuracy (SA), as highlighted by Resende and Duarte (2007) and Resende and Alves (2022). This statistic establishes a correlation between the real and predicted genotypic values.

Several studies have shown how such precision statistics are affected by sample size (Cargnelutti Filho et al., 2009; Bittencourt et al., 2023; Souza et al., 2022; Souza et al., 2023a). A representative sample size must be selected in order to ensure precise and reliable results. However, other problems may derive from the use of excessively large sample sizes, such as the need for a higher amount of time and resources, besides leading to biased measurements in some cases (Toebe et al., 2018). Those aspects become extremely relevant in the field of agricultural sciences, where living beings such as plants are normally the study object, and considering these experiments are known for requiring great labor and large areas, especially in plant breeding programs where a great number of treatments (genotypes) are tested. In this sense, defining an ideal sample size can help optimize the planning and conduct of experiments (Confalonieri et al., 2009; Alvez-Silva et al., 2018). Nonetheless, this value may vary according to the crop, trait, and statistical parameter (Toebe et al., 2015; Souza et al., 2022).

Lettuce (*Lactuca sativa* L.) is considered one of the most popular vegetable crops in the world, being widely consumed in salads and sandwiches, for which it has great economic importance in the field of horticulture (Hassan et al., 2021). This is reflected in research, with numerous publications focusing on the crop every year (Zhang et al., 2020; Wei et al., 2021; Muscolo et al., 2022; Hasan and Jho, 2023). However, studies on sample size are still scarce for lettuce, making the decision on this number empirical, which may later affect experimental precision. Importantly, several traits are commonly measured in lettuce experiments, such as the number of leaves, plant height, and neck and mean head diameter, which may require specific sample size recommendations. Another point worth mentioning is the large number of lettuce genotypes available in the market. Therefore, sample-size studies should include as many genotypes as possible in order to be representative.

Moreover, recent work on the subject has focused on forecasting precision according to a selected sample size (Souza et al., 2022; Souza et al., 2023a; Souza et al., 2023b; Bittencourt et al., 2023), which provides a better understanding of the response of statistics to sample size variation and helps in the making of more efficient choices on the number of plants to be sampled per experimental unit. Therefore, the aims of this study were (i) to define the optimal sample size per experimental unit to estimate precision statistics for lettuce biometric traits and (ii) to propose forecasting equations for precision statistics from the selection of a sample size per experimental unit.

9.3 MATERIAL AND METHODS

1.1.1. Experimental conduction of the reference experiment

A lettuce experiment was performed in the experimental area of the Federal University of Santa Maria (UFSM), located in the municipality of Frederico Westphalen, Rio Grande do Sul State, Brazil (27º23’50” S latitude, 53º25’37” W longitude, and 522 m of altitude). Twenty-six lettuce cultivars were used in the experiment, which are described in

Supplementary Table 1. In Frederico Westphalen, the climate is classified as cfa, humid subtropical, with no dry season defined (Wrege et al., 2012), and the soil is of the Dystrophic Red Latosol type (Santos et al., 2018).

Initially, lettuce seedlings were grown in a greenhouse, under controlled conditions of temperature (15-20ºC) and humidity (60-70%). The sowing was carried out on November 7th, 2022, using expanded polystyrene trays with 128 cells and a vermiculite-based commercial substrate. The seedling production period lasted 72 days, after which the seedlings were transplanted to the field. A completely randomized block experimental design with two repetitions was used, resulting in 54 experimental units, with 24 plants each. The plants were spaced 0.30 m apart within each row and 0.30 m apart between rows. Base fertilization was applied based on soil analysis and the recommendations for the crop (CQFS - Comissão de Química e Fertilidade do Solo, 2016), followed by cover fertilization, 34 days after transplant. Finally, fifteen plants were randomly taken from each experimental unit, and the following traits were measured: number of leaves (NL), in units, plant height (PH), in cm, neck diameter (ND), in cm, and mean head diameter (MHD), in cm.

1.1.1. Bootstrap resampling procedure

The reference experiment was used to perform a bootstrap resampling in order to simulate experiments with different sampling scenarios. Hence, 100 different sample sizes were planned for each trait, which varied from 1 to 100 plants per experimental unit, with an interval of 1 plant between each sample size. The same methodology described by Bittencourt et al. (2023) and Souza et al. (2022) was applied.

Subsequently, the database was divided into experimental units and an analysis of variance was performed for each sample size, thus obtaining the mean value of each unit in each scenario for each trait. For this, the following mathematical model was used:

$$Y_{ij} = m + G_i + \beta_j + \varepsilon_{ij}$$

where Y_{ij} is the value observed in the response trait in the *ij* plot, m is the overall mean, G_i is the fixed effect of the i^{th} level (i = 1, 2, …, 26) of the genotype factor, β_j is the random effect of the j^{th} level (*j* = 1 and 2) of the block, and ε_{ij} is the experimental error effect, supposedly normal and independently distributed with a mean of zero and a common variance σ^2 (Storck et al., 2016).

The procedure was conducted with replacement and 10,000 resamples (Efron, 1979). This resulted in 1,000,000 experiments, considering the 100 sample sizes and 10,000 resamples for each size. Finally, five precision statistics (Table 1) were estimated for each resampling and each trait, obtaining the respective descriptive statistics (Supplementary Tables 2, 3, 4, and 5). The statistical analyses were performed with R software (R Development Core Team, 2023) and Microsoft Office Excel. Specific routines were used, which included the *sample*() and *aov*() functions.

1.1.1. Finding the ideal sample size per experimental unit

To define sample size per experimental unit, first, the 95% confidence interval width ($CI_{95\%}$) of each statistic was calculated for each trait. For this, the minimum values, 2.5 percentiles, means, 97.5 percentiles, and maximum values of the statistics were obtained for each resample of each sampling scenario, and the following equation was applied:

$$CI_{95\%} = UP - LP$$

where *UP* is the 97.5 percentile (upper percentile) and *LP* is the 2.5 percentile (lower percentile).

Next, the $CI_{95\%}$ values were adjusted to the sample sizes of 1 to 100 plants per experimental unit through a shifted power model parameterized using the *nls*() function, as follows:

$$CI_{95\%} = \alpha \times (n - \beta)^{c} + \varepsilon$$

where α is the coefficient of interception, *n* is the sample size, β is the coefficient of second order, *c* is the coefficient of third order, and ε is the random-effect error. The upper and lower limits of the parameters of the shifted power model were also obtained (Supplementary Table 6).

Afterward, the maximum curvature point was defined for each statistic and trait, using the perpendicular distances method, described by Silva and Lima (2017) and following the recommendations of Bittencourt et al. (2022) and Souza et al. (2022). For this, the *maxcurv* function was used, and the maximum curvature point was considered the optimal sample size for each case. Moreover, a principal component analysis was carried out, setting a mean of zero and a unitary variance to all statistics. This was done using the *svd*() function. Through this analysis, the contribution of each statistic to the total variation in the sampling scenarios was verified, which allowed making the final decision on the optimal sample size considering the statistics that had the greatest contributions.

1.1.1. Forecasting precision statistics based on sample size

The methodology of Souza et al. (2022) was followed and adapted to propose forecasting formulas for precision statistics based on sample size. Thus, a modified power model proposed by Olivoto et al. (2018) was applied, so that the following model was used for each statistic:

$$CI_{95\%} = \delta^{PS} \times \alpha \times n^{\beta} + \varepsilon$$

where δ is the adjustment factor of the coefficient of interception (Olivoto et al., 2018), α is the coefficient of interception, n is the sample size, β is the exponential rate of decay, PS is the precision statistic mean value, and ε is the random-effect error. A total of 700,000 values, representing 70% of the resamples for each statistic, were used to parametrize the model, and the remaining 30% were used in a validation process.

Validation consisted of simple linear regressions between the observed and predicted $CI_{95\%}$ of the precision statistics. The *hydrogof* package was used for the analyses, and the fitting quality of the models was evaluated through the coefficient of determination (R^2), Willmott's agreement index (d index), and root mean square error (RMSE). Lastly, the modified power models were inverted to generate the forecasting formulas, as demonstrated below:

$$CI_{95\%} = \delta^{PS} \times \alpha \times n^{\beta} + \varepsilon \quad (I)$$

$$\delta^{PS} = \frac{CI_{95\%}}{\alpha \times n^{\beta}} \quad (II)$$

$$PS = log_{\delta}\left(\frac{CI_{95\%}}{\alpha \times n^{\beta}}\right) \quad (III)$$

9.4 RESULTS

1.1.1. Experimental precision achieved in the reference experiment

The reference experiment obtained high experimental precision as verified through the CV, CVg, F, VI, and SA. In general, NL was the trait with the highest precision, obtaining the highest F, CVg, and SA values, followed by PH, which obtained the lowest CVe and VI values, and MHD was the less precise evaluated trait, followed by ND. Medium CVe values were obtained, which varied from 16.04% to 18.14%, and lower VI values were found, which varied from 11.34% to 12.83%, corresponding to PH and NL, respectively. F values varied from 3.69 for NL to 2.33 for MHD. Regarding the other genetic indicators, CVg varied from 21.08% for NL to 14.17% for MHD, while SA values were close to one, varying from 0.85 for NL to 0.76 for MHD. Furthermore, significant differences were found between genotypes for all traits, with p-values of 0.000876, 0.002530, 0.004210, and 0.019300, respectively for NL, PH, ND, and MHD.

1.1.1. Confidence interval width of the precision statistics in different sampling scenarios

Figs. 1, 2, 3, and 4 (a, b, c, d, and e) show the response of the $CI_{95\%}$ of the precision statistics to the variation of the sample size per experimental unit. As expected, for all statistics and traits, the $CI_{95\%}$ was reduced as the number of sampled plants per experimental unit increased up to a point where a certain stabilization was reached. Thus, higher $CI_{95\%}$ values were obtained in scenarios of small sample sizes in comparison to greater samples. For example, considering the CVe of the number of leaves, a $CI_{95\%}$ of 11.74 was obtained when only one plant was sampled per experimental unit, whereas a value of 1.77 was observed when sampling 50 plants per experimental unit. Moreover, the mean property of some statistics was underestimated (F, CVg, and SA) at small sample sizes while others were overestimated (CV and VI) in those scenarios, eventually stabilizing. This can be illustrated by the mean value of the VI obtained for PH, which was overestimated when only 5 plants per experimental unit were sampled, resulting in 12.23% and reducing to 11.57% with the sampling of 20 plants. On the other hand, the value of SA, for example, which was underestimated, resulted in 0.80 when 5 plants were sampled and increased to 0.82 with 20 plants per experimental unit.

1.1.1. Optimal sample sizes defined per experimental unit for lettuce traits

The indicators shown in Table 2 verified the fitting quality of the shifted powers model used in the first part of the sample size definition process. As observed, the models presented good fitting quality, which can be concluded once R^2 and d index values were close to 1 and low RMSE values were obtained. The R^2 values obtained were higher than 0.9993, 0.9993, 0.9981, and 0.9994, for PH, NL, ND, and MHD, respectively, while the d index values were higher than 0.9998, 0.9998, 0.9995, and 0.9999 for PH, NL, ND, and MHD, respectively. Regarding the RMSE, the values varied from 0.0007 to 0.0221 for PH, 0.0006 to 0.0216 for NL, 0.0188 to 0.0540 for ND, and 0.0013 to 0.0280 for MHD.

The optimal sample sizes are shown in Figs. 1, 2, 3, and 4 (g, h, i, j, and k). Such sizes varied among statistics and slightly among traits, with 24 plants per experimental unit being the maximum optimal sample size found, corresponding to the estimate of F for ND, whereas 13 plants per experimental unit was the minimum optimal sample size found, which corresponded to the estimate of SA for PH, NL, and ND. In this sense, the principal component analysis (Fig. 5) indicated that the statistics that contributed the most to the total variation retained in the first principal component were SA and F for all traits, although VI and CVe also presented high contributions for PH, NL, and ND. For this reason, the sampling of 24 plants per experimental unit is considered enough to measure the precision of all lettuce biometrics traits here evaluated reliably through all precision statistics here used.

1.1.1. Models to forecast precision statistics for lettuce traits

The forecasting models used to predict precision statistics for lettuce traits also presented good fitting quality. This was evidenced by the fitting quality indicators shown in Table 3. The R^2 values obtained were higher than 0.9996, 0.9995, 0.9987, and 0.9995 for PH, NL, ND, and MHD, respectively, while the d index values were all higher than 0.9997. Moreover, the RMSE values varied from 0.0006 to 0.0242 for PH, 0.0005 to 0.0216 for NL, 0.0023 to 0.0303 for ND, and 0.0012 to 0.0254 for MHD. In order to allow applying equations in confidence intervals, the upper and lower limits of the parameters of the models were obtained and are shown in Table 4. Finally, the validation of the forecasting models is shown in Supplementary Figs. 1 and 2, and it is possible to observe that the predicted $CI_{95\%}$ values were extremely close to the observed $CI_{95\%}$ values.

9.5 DISCUSSION

Through this study, the planning of field experiments with lettuce can be enhanced, once the selection of optimal sample size is essential to achieve reliable results (Cargnelutti Filho et al., 2013; Toebe et al., 2018), especially taking into account that statistics used to measure experimental precision are affected by sample size variation (Souza et al., 2022). Also, defining this number per experimental unit becomes even more useful considering most experiments use experimental designs, requiring the application of experimental restrictions (Storck et al., 2016). Therefore, the sample size recommendations here can be useful to researchers performing experiments with lettuce where traits such as the plant height, number of leaves, neck diameter, and mean head diameter are evaluated. Importantly, such plant growth parameters are associated with lettuce yield (Lou et al., 2022) and play an important role in plant breeding studies (Peixoto et al., 2021). Furthermore, the forecasting formulas proposed allow for knowing how precise the experiment will be based on the chosen sample size (Souza et al., 2022; Bittencourt et al., 2023). This contributes to making a decision on such a number considering the specific goals and requirements of the research, which is namely useful in experiments with a high number of treatments, such as plant breeding programs, where time and labor may be a limiting factor.

When evaluating experimental precision, taking into account various statistics is crucial for drawing conclusions on this matter, once each statistic is influenced by specific factors, such as the components of their calculation structures (Table 1). An example of this is the influence of the overall experimental mean on the CVe (Doring and Reckling, 2018), which is not as outstanding for the VI once the latter includes the number of repetitions, which causes VI values to be lower than CVe values. Another important point is the inclusion of the genotype mean square (GMS) in the calculation of F, and therefore in SA, for which these statistics are strongly associated with genetic variances, differently than CVe and VI, which are more associated with residual variances, as they use only the error mean square (EMS). Hence, CVg, F, and SA are extremely relevant in studies that assess genotypes and should be prioritized when measuring the experimental precision of those (Resende and Duarte, 2007; Resende and Alves, 2022). This can be perceived in the reference experiment, where, based on the CVe and VI, the experimental precision is considered medium (Pimentel-Gomes, 1990; Pimentel-Gomes, 1991), as these values are between 10 and 20 %, however, according to the scale by Resende and Duarte (2007) for F and SA, the experimental precision is high, once F values were between 1.96 and 5.26, and SA values were between 0.70 and 0.90.

As observed in previous studies on sample size definition based on the $CI_{95\%}$ (Cargnelutti Filho et al., 2013; Toebe et al., 2015; Toebe et al., 2018; Souza et al., 2023b), the maximum and minimum values of the statistics approached their real values as sample size increased, and stabilized once the optimal sample size was reached, which indicates a gain in the precision for estimating the value of the statistics with the increase in the number of sampled plants, reducing the chances of over and underestimation of results. Furthermore, although there were slight differences in the optimal sample sizes between traits, the precision statistics had a more prominent effect since a greater variation was observed in the sample sizes found between precision statistics. This was also observed by Bittencourt et al. (2023) and Souza et al. (2023a), when defining sample size for different traits of cauliflower seedlings and soybean genotypes, respectively, which highlights that statistics respond differently to sample size variation, making sample size definition for specific indicators extremely relevant.

Also, the F statistic was the one that required the highest number of plants for all traits, which also occurred in the work of Bittencourt et al. (2023), Souza et al. (2022), and Souza et al. (2023a), suggesting this statistic may not be an appropriate precision indicator in studies with small sample sizes, which is common in horticultural research (Bittencourt et al., 2022). Importantly, in this study, F contributed significantly to the total variation retained in the first principal component, presenting the second greatest contribution after SA and, at the same time, obtained the maximum optimal sample size of 24 plants per experimental unit to estimate ND, which was reduced to 21 for PH and NL, and 20 for MHD. Therefore, in order to estimate all traits and statistics reliably, 24 plants per experimental unit are required, however, specific recommendations can be followed for each trait and statistic as needed.

Researchers can also use the forecasting formulas proposed to know how much precision could be gained or lost in the case of selecting a slightly higher or lower sample size than the optimal one (Table 3). From such equations, a precision interval can be defined, that is, a range for the value of each statistic, by using the lower and upper limits of the parameters of both the shifted power and modified power models presented in Supplementary Table 6 and Table 4, respectively. In order to apply this methodology, the following steps should be followed: 1) Select a sample size (n) and statistic of interest; 2) Use that sample size in the shifted power model to find the $CI_{95\%}$ of the statistic [$CI_{95\%} = \alpha\times(n - \beta)^{c} + \varepsilon$]; 3) With the $CI_{95\%}$ value, use the forecasting formula for the precision statistic in question to find its value $\left[PS = log_{\delta}\left(\frac{CI_{95\%}}{\alpha\times n^{\beta}}\right)\right]$. This procedure will result in a predicted mean value of the statistic when using the selected sample size, however, the same steps should be followed again replacing the parameters of the models by their upper and lower limits to obtain a confidence interval for such value. Importantly, the equations here presented should be only applied by researchers performing experiments in similar environmental conditions to the ones of this study, and assessing the same traits here measured. In different scenarios, such formulas must be adapted and properly calibrated to obtain accurate results.

9.6 CONCLUSIONS

A greater variation was observed in the optimal sample sizes found between precision statistics than between traits. All precision statistics and traits can be reliably estimated with the sampling of 24 lettuce plants per experimental unit. The precision statistics SA and F contributed the most to the total variation retained in the first principal component, considering all traits. The forecasting models demonstrated excellent fitting quality and allowed predicting precision statistics for lettuce biometric traits. The recommendations and equations proposed are valuable for optimizing the planning of experiments with lettuce that measure the plant height, number of leaves, neck diameter, and mean head diameter.

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9.8 TABLES

Table 1. Codes, equations, and references of the precision statistics.

Precision statistic	Code	Equation(*)	Reference
F-test value	F	$\frac{GMS}{EMS}$	Storck et al. (2016)
Coefficient of genetic variation	CVg	$100\frac{\sqrt{Vgen}}{m}$	Resende & Duarte (2007)
Coefficient of experimental variation	CVe	$100\frac{\sqrt{EMS}}{m}$	Pimentel-Gomes (1990)
Variation index	VI	$\frac{CVe}{\sqrt{r}}$	Pimentel-Gomes (1991)
Selective accuracy	SA	$\left(\frac{1}{1-F}\right)^{0.5}$	Resende & Duarte (2007)

(*) GMS: genotype mean square, given by $\sigma^2 + \frac{r}{i-1}\sum_i G_i^2$; EMS: error mean square, given by σ^2; *Vgen*: genetic variance, given by $\frac{GMS-EMS}{r}$; *m*: overall experimental mean, given by $\frac{\Sigma G_{ir}}{i\times r}$; σ^2: experimental variance; *i*: number of genotypes; *r*: number of repetitions.

Table 2. Fitting quality indicators and parameters of the shifted power model used to define optimal sample sizes to estimate experimental precision statistics for the plant height (PH), number of leaves (NL), neck diameter (ND), and mean head diameter (MHD) in experiments with lettuce.

Trait	Precision statistic (*)	α (†)	β	*c*	R^2 (‡)	RMSE	d index
PH	F	6.0961	-2.8499	-0.5241	0.9993	0.0123	0.9998
	CVe	11.2211	-0.1383	-0.4898	0.9998	0.0221	0.9999
	CVg	11.5335	0.2852	-0.4995	0.9999	0.0183	0.9999
	VI	7.9345	-0.1383	-0.4898	0.9998	0.0156	0.9999
	AS	0.3381	0.3572	-0.5117	0.9998	0.0007	0.9999
NL	F	6.0870	-2.5868	-0.5197	0.9993	0.0134	0.9998
	CVe	12.3670	-0.1128	-0.4948	0.9998	0.0216	0.9999
	CVg	10.8926	0.2680	-0.4992	0.9998	0.0196	0.9999
	VI	8.7448	-0.1128	-0.4948	0.9998	0.0153	1.0000
	SA	0.2483	0.3808	-0.5093	0.9998	0.0006	0.9999
ND	F	6.8906	-4.9969	-0.5454	0.9981	0.0188	0.9995
	CVe	13.2389	0.1004	-0.4886	0.9997	0.0293	0.9999
	CVg	14.4669	0.1914	-0.5180	0.9993	0.0540	0.9998
	VI	9.3613	0.1004	-0.4886	0.9997	0.0207	0.9999
	SA	0.4534	0.2451	-0.5400	0.9984	0.0027	0.9996
MHD	F	3.5603	-1.5897	-0.5155	0.9994	0.0079	0.9999
	CVe	10.7004	-0.2822	-0.5015	0.9998	0.0178	0.9999
	CVg	11.1993	0.1605	-0.5098	0.9997	0.0280	0.9999
	VI	7.5663	-0.2822	-0.5015	0.9998	0.0126	0.9999
	SA	0.4288	0.1705	-0.5152	0.9996	0.0013	0.9999

(*) The codes of the precision statistics are shown in Table 1 (†) α: coefficient of interception; β: coefficient of second order; *c*: coefficient of third order; (‡) R^2: coefficient of determination; RMSE: root mean square error; d index: Willmott's agreement index.

Table 3. Fitting quality indicators and parameters of the modified power models for forecasting precision statistics and forecasting equations for the plant height (PH), number of leaves (NL), neck diameter (ND), and medium head diameter (MHD) of lettuce plants.

Trait	Precision statistic [(*)]	δ [(†)]	α	β	R^2 [(‡)]	RMSE	d index	Forecasting equation
PH	F	2.1154	0.5228	-0.5111	0.9996	0.0099	0.9999	$F = log_{2.1154}\left(\frac{CI_{95\%}}{0.5228 \times n^{-0.5111}}\right)$
	CVe	0.9884	13.5743	-0.4905	0.9998	0.0218	0.9999	$CVe = log_{0.9884}\left(\frac{CI_{95\%}}{13.5743 \times n^{-0.4905}}\right)$
	CVg	0.6186	36939.5167	-0.4986	0.9998	0.0242	0.9999	$CVg = log_{0.6186}\left(\frac{CI_{95\%}}{36939.5167 \times n^{-0.4986}}\right)$
	VI	0.9836	9.5985	-0.4905	0.9998	0.0154	0.9999	$VI = log_{0.9836}\left(\frac{CI_{95\%}}{9.5985 \times n^{-0.4905}}\right)$
	AS	0.0912	2.3659	-0.5015	0.9999	0.0006	0.9999	$SA = log_{0.0912}\left(\frac{CI_{95\%}}{2.3659 \times n^{-0.5015}}\right)$
NL	F	1.7999	0.6587	-0.5079	0.9995	0.0115	0.9999	$F = log_{1.7999}\left(\frac{CI_{95\%}}{0.6587 \times n^{-0.5079}}\right)$
	CVe	0.9910	14.6059	-0.4952	0.9998	0.0216	0.9999	$CVe = log_{0.9910}\left(\frac{CI_{95\%}}{14.6059 \times n^{-0.4952}}\right)$
	CVg	0.6384	139918.7163	-0.5033	0.9998	0.0209	0.9999	$CVg = log_{0.6384}\left(\frac{CI_{95\%}}{139918.7163 \times n^{-0.5033}}\right)$
	VI	0.9872	10.3279	-0.4952	0.9998	0.0153	0.9999	$VI = log_{0.9872}\left(\frac{CI_{95\%}}{10.3279 \times n^{-0.4952}}\right)$
	AS	0.0451	3.3765	-0.4999	0.9998	0.0005	0.9999	$SA = log_{0.0451}\left(\frac{CI_{95\%}}{3.3765 \times n^{-0.4999}}\right)$
ND	F	2.4632	0.4300	-0.5261	0.9987	0.0155	0.9997	$F = log_{2.4632}\left(\frac{CI_{95\%}}{0.4300 \times n^{-0.5261}}\right)$
	CVe	1.0067	11.8345	-0.4877	0.9997	0.0303	0.9999	$CVe = log_{1.0067}\left(\frac{CI_{95\%}}{11.8345 \times n^{-0.4877}}\right)$
	CVg	0.6413	16811.2358	-0.5045	0.9998	0.0305	0.9999	$CVg = log_{0.6413}\left(\frac{CI_{95\%}}{16811.2358 \times n^{-0.5045}}\right)$
	VI	1.0096	8.3682	-0.4877	0.9997	0.0215	0.9999	$VI = log_{1.0096}\left(\frac{CI_{95\%}}{8.3682 \times n^{-0.4877}}\right)$
	AS	0.2625	1.2786	-0.5258	0.9988	0.0023	0.9997	$SA = log_{0.2625}\left(\frac{CI_{95\%}}{1.2786 \times n^{-0.5258}}\right)$

MHD	F	3.3596	0.2041	-0.5087	0.9995	0.0072	0.9999	$F = log_{3.3596}\left(\frac{CI_{95\%}}{0.2041 \times n^{-0.5087}}\right)$
	CVe	0.9714	17.6780	-0.5013	0.9998	0.0177	0.9999	$CVe = log_{0.9714}\left(\frac{CI_{95\%}}{17.6780 \times n^{-0.5013}}\right)$
	CVg	0.6895	2145.0384	-0.5064	0.9997	0.0254	0.9999	$CVg = log_{0.6895}\left(\frac{CI_{95\%}}{2145.0384 \times n^{-0.5064}}\right)$
	VI	0.9598	12.5002	-0.5013	0.9998	0.0125	0.9999	$VI = log_{0.9598}\left(\frac{CI_{95\%}}{12.5002 \times n^{-0.5013}}\right)$
	AS	0.3129	1.0171	-0.5114	0.9996	0.0012	0.9999	$SA = log_{0.3129}\left(\frac{CI_{95\%}}{0.5114 \times n^{-0.9996}}\right)$

(*) The codes of the precision statistics are shown in Table 1. (†) δ: adjustment factor of the coefficient of interception; α: coefficient of interception; β: exponential rate of decay; (‡) R²: coefficient of determination; RMSE: root mean square error; d index: Willmott’s agreement index.

Table 4. Upper and lower limits of the parameters of the modified power models used to forecast precision statistics for the plant height (PH), number of leaves (NL), neck diameter (ND), and mean head diameter (MHD) of lettuce plants.

Trait	Precision statistic (*)	Lower limits			Modified power model			Upper limits		
		δ (†)	α	β	δ	α	β	δ	α	β
PH	F	2.0777	0.4988	-0.5144	2.1154	0.5228	-0.5111	2.1530	0.5468	-0.5078
	CVe	0.9865	13.0470	-0.4931	0.9884	13.5743	-0.4905	0.9903	14.1016	-0.4879
	CVg	0.6018	20335.8361	-0.5011	0.6186	36939.5167	-0.4986	0.6354	53543.1974	-0.4961
	VI	0.9809	9.2256	-0.4931	0.9836	9.5985	-0.4905	0.9862	9.9713	-0.4879
	AS	0.0842	2.2313	-0.5038	0.0912	2.3659	-0.5015	0.0982	2.5005	-0.4993
NL	F	1.7705	0.6270	-0.5115	1.7999	0.6587	-0.5079	1.8292	0.6904	-0.5042
	CVe	0.9894	14.0762	-0.4976	0.9910	14.6059	-0.4952	0.9925	15.1356	-0.4929
	CVg	0.6226	67474.0655	-0.5053	0.6384	139918.7163	-0.5033	0.6543	212363.3671	-0.5013
	VI	0.9850	9.9533	-0.4976	0.9872	10.3279	-0.4952	0.9895	10.7025	-0.4929
	SA	0.0403	3.1010	-0.5024	0.0451	3.3765	-0.4999	0.0498	3.6519	-0.4974
ND	F	2.3926	0.4022	-0.5320	2.4632	0.4300	-0.5261	2.5337	0.4578	-0.5202
	CVe	1.0052	11.4317	-0.4907	1.0067	11.8345	-0.4877	1.0083	12.2372	-0.4847
	CVg	0.6198	7967.9118	-0.5077	0.6413	16811.2358	-0.5045	0.6628	25654.5599	-0.5013
	VI	1.0074	8.0834	-0.4907	1.0096	8.3682	-0.4877	1.0117	8.6530	-0.4847
	SA	0.2140	1.1165	-0.5334	0.2625	1.2786	-0.5258	0.3111	1.4408	-0.5182

MHD	F	3.2217	0.1869	-0.5122	3.3596	0.2041	-0.5087	3.4975	0.2214	-0.5052
	CVe	0.9693	16.8782	-0.5036	0.9714	17.6780	-0.5013	0.9736	18.4778	-0.4991
	CVg	0.6595	844.8452	-0.5095	0.6895	2145.0384	-0.5064	0.7194	3445.2316	-0.5034
	VI	0.9569	11.9347	-0.5036	0.9598	12.5002	-0.5013	0.9628	13.0658	-0.4991
	SA	0.2652	0.9116	-0.5151	0.3129	1.0171	-0.5114	0.3607	1.1225	-0.5076

(*) The codes of the precision statistics are shown in Table 1. (†) δ: adjustment factor of the coefficient of interception; α: coefficient of interception; β: exponential rate of decay.

9.9 FIGURE CAPTIONS

Fig. 1. Minimum, 2.5 percentile, mean, 97.5 percentile, and maximum values obtained from the planned sample sizes of 1 to 100 lettuce plants per experimental unit for the experimental precision statistics F-test value (a), coefficient of experimental variation (b), coefficient of genetic variation (c), variation index (d), and selective accuracy (e), and optimal sample sizes defined via shifted power model and maximum curvature point to estimate the precision statistics F-test value (f), coefficient of experimental variation (g), coefficient of genetic variation (h), variation index (i), and selective accuracy (j) for the plant height of lettuce plants.

(*) Dashed lines represent minimum and maximum values, colored straight lines represent 2.5 and 97.5 percentiles, and black straight lines represent mean values.

Fig. 2. Minimum, 2.5 percentile, mean, 97.5 percentile, and maximum values obtained from the planned sample sizes of 1 to 100 lettuce plants per experimental unit for the experimental precision statistics F-test value (a), coefficient of experimental variation (b), coefficient of genetic variation (c), variation index (d), and selective accuracy (e), and optimal sample sizes defined via shifted power model and maximum curvature point to estimate the precision statistics F-test value (f), coefficient of experimental variation (g), coefficient of genetic variation (h), variation index (i), and selective accuracy (j) for the number of leaves of lettuce plants.

Fig. 3. Minimum, 2.5 percentile, mean, 97.5 percentile, and maximum values obtained from the planned sample sizes of 1 to 100 lettuce plants per experimental unit for the experimental precision statistics F-test value (a), coefficient of experimental variation (b), coefficient of genetic variation (c), variation index (d), and selective accuracy (e), and optimal sample sizes

defined via shifted power model and maximum curvature point to estimate the precision statistics F-test value (f), coefficient of experimental variation (g), coefficient of genetic variation (h), variation index (i), and selective accuracy (j) for the neck diameter of lettuce plants.

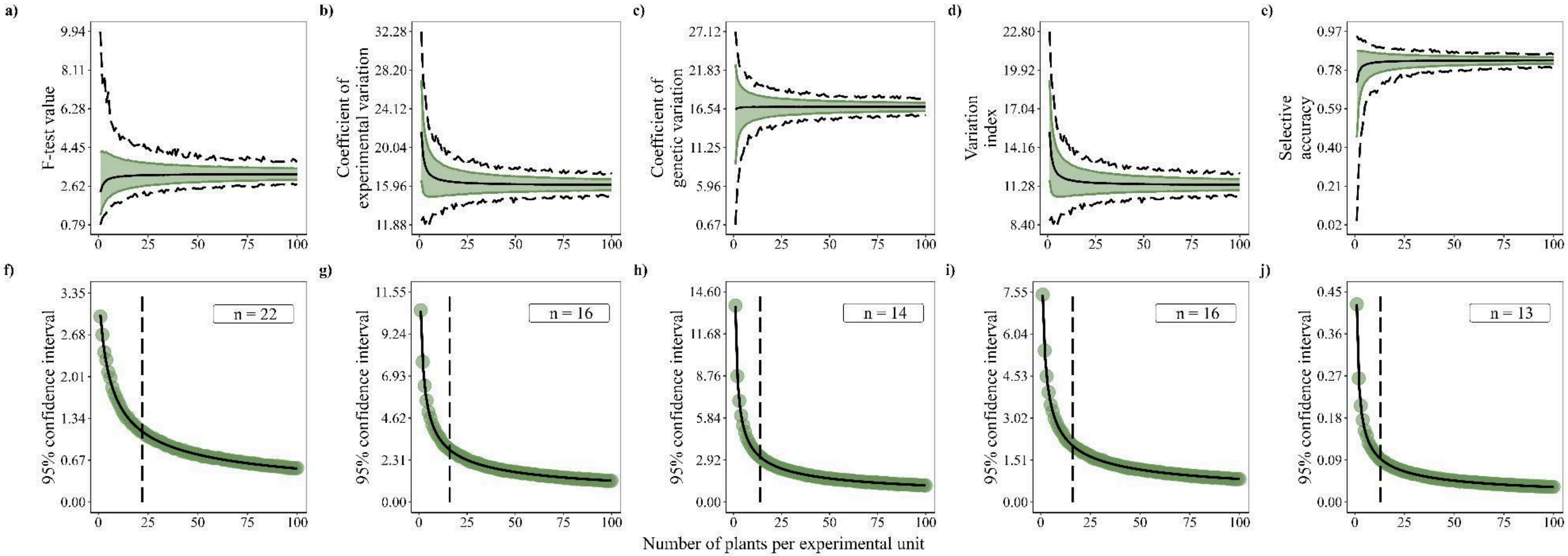
Fig. 4. Minimum, 2.5 percentile, mean, 97.5 percentile, and maximum values obtained from the planned sample sizes of 1 to 100 lettuce plants per experimental unit for the experimental precision statistics F-test value (a), coefficient of experimental variation (b), coefficient of genetic variation (c), variation index (d), and selective accuracy (e), and optimal sample sizes defined via shifted power model and maximum curvature point to estimate the precision statistics F-test value (f), coefficient of experimental variation (g), coefficient of genetic variation (h), variation index (i), and selective accuracy (j) for the mean head diameter of lettuce plants.

Fig. 5. Principal component analysis of the definition of the optimal sample size per experimental unit to estimate precision statistics for the plant height (a), number of leaves (b), neck diameter (c), and mean head diameter (d) of lettuce plants, and contribution percentage of the precision statistics to the total variation retained in the first principal component for the plant height (e), number of leaves (f), neck diameter (g), and mean head diameter (h) of lettuce plants.

(*) PC1: first principal component; PC2: second principal component.

(†) The codes of the precision statistics are shown in Table 1.

9.10 FIGURES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F-test value
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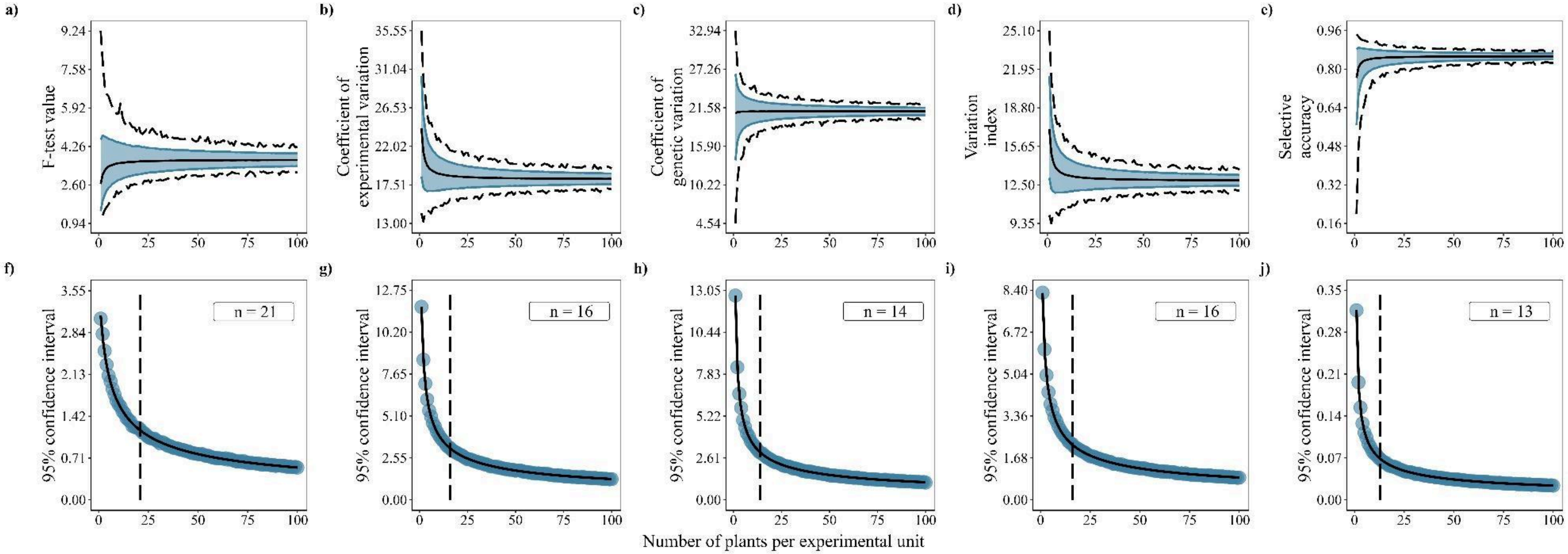
8.11
6.28
4.45
2.62
0.79
b)
Coefficient of experimental variation
32.28
28.20
24.12
20.04
15.96
11.88
c)
Coefficient of genetic variation
27.12
21.83
16.54
11.25
5.96
0.67
d)
Variation index
22.80
19.92
17.04
14.16
11.28
8.40
c)
Selective accuracy
0.97
0.78
0.59
0.40
0.21
0.02
f)
95% confidence interval
3.35
2.68
2.01
1.34
0.67
0.00
n = 22
g)
95% confidence interval
11.55
9.24
6.93
4.62
2.31
0.00
n = 16
h)
95% confidence interval
14.60
11.68
8.76
5.84
2.92
0.00
n = 14
i)
95% confidence interval
7.55
6.04
4.53
3.02
1.51
0.00
n = 16
j)
95% confidence interval
0.45
0.36
0.27
0.18
0.09
0.00
n = 13
0
25
50
75
100
Number of plants per experimental unit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F-test value
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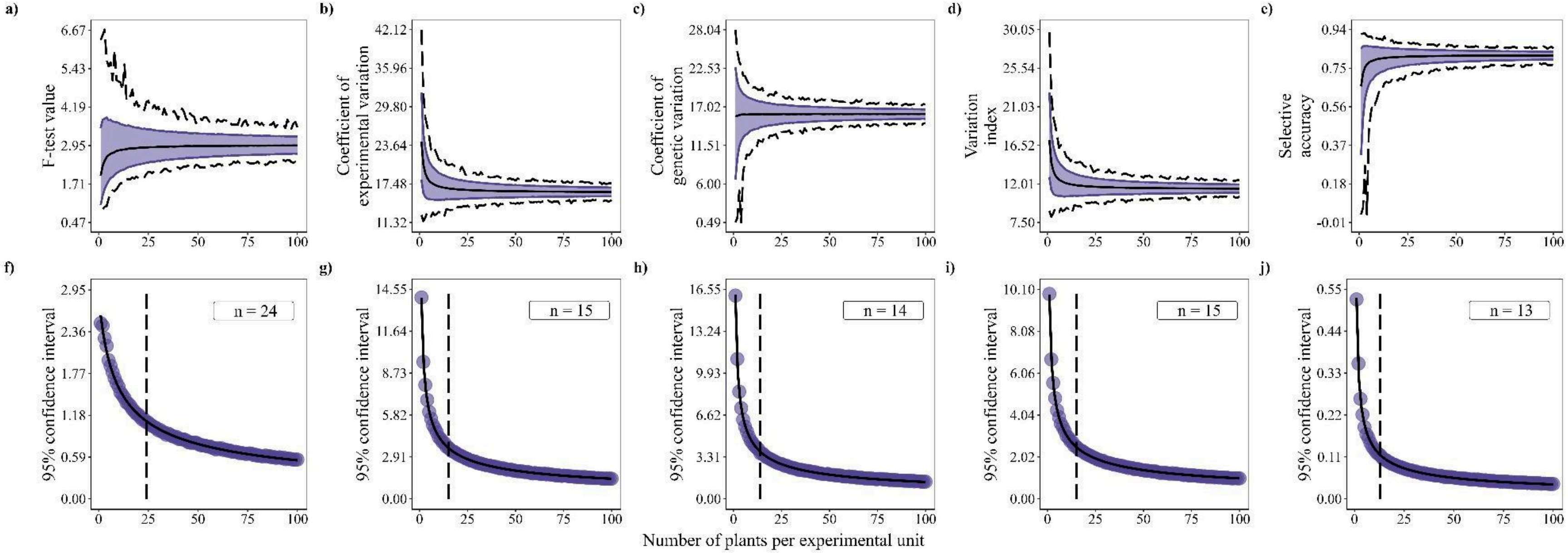
7.58
5.92
4.26
2.60
0.94
b)
Coefficient of experimental variation
35.55
31.04
26.53
22.02
17.51
13.00
c)
Coefficient of genetic variation
32.94
27.26
21.58
15.90
10.22
4.54
d)
Variation index
25.10
21.95
18.80
15.65
12.50
9.35
e)
Selective accuracy
0.96
0.80
0.64
0.48
0.32
0.16
0
25
50
75
100
f)
95% confidence interval
3.55
2.84
2.13
1.42
0.71
0.00
n = 21
g)
12.75
10.20
7.65
5.10
2.55
0.00
n = 16
h)
13.05
10.44
7.83
5.22
2.61
0.00
n = 14
i)
8.40
6.72
5.04
3.36
1.68
0.00
n = 16
j)
0.35
0.28
0.21
0.14
0.07
0.00
n = 13
Number of plants per experimental unit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F-test value
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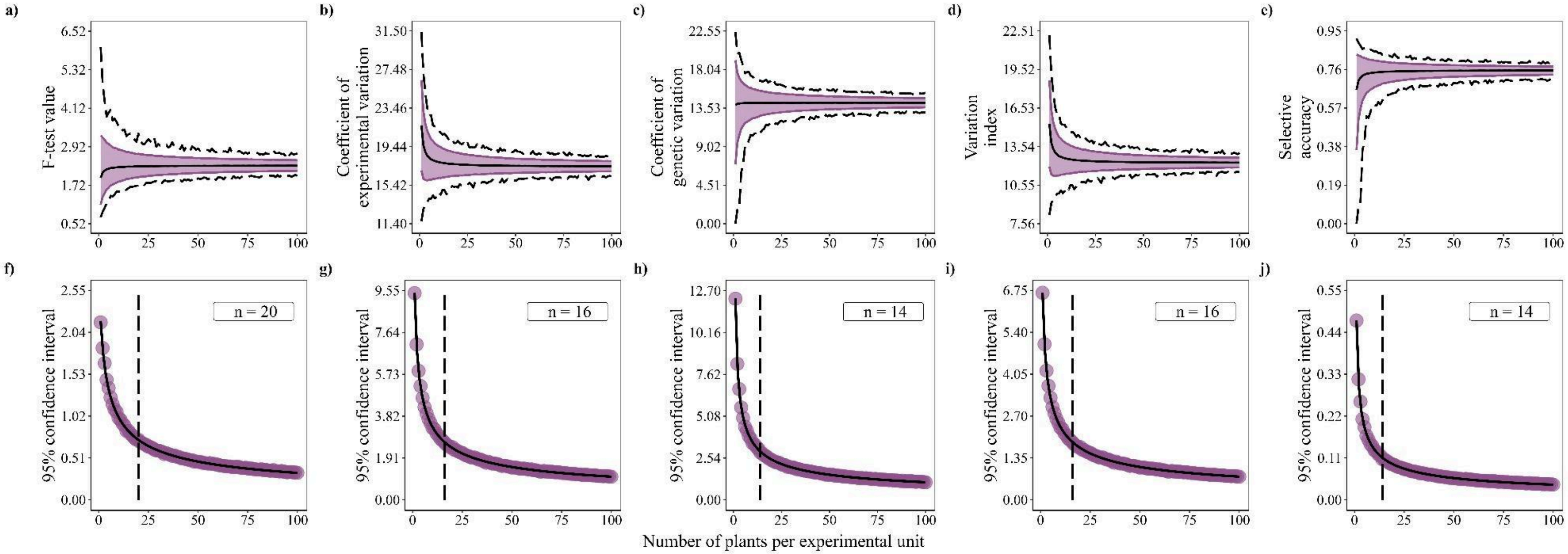
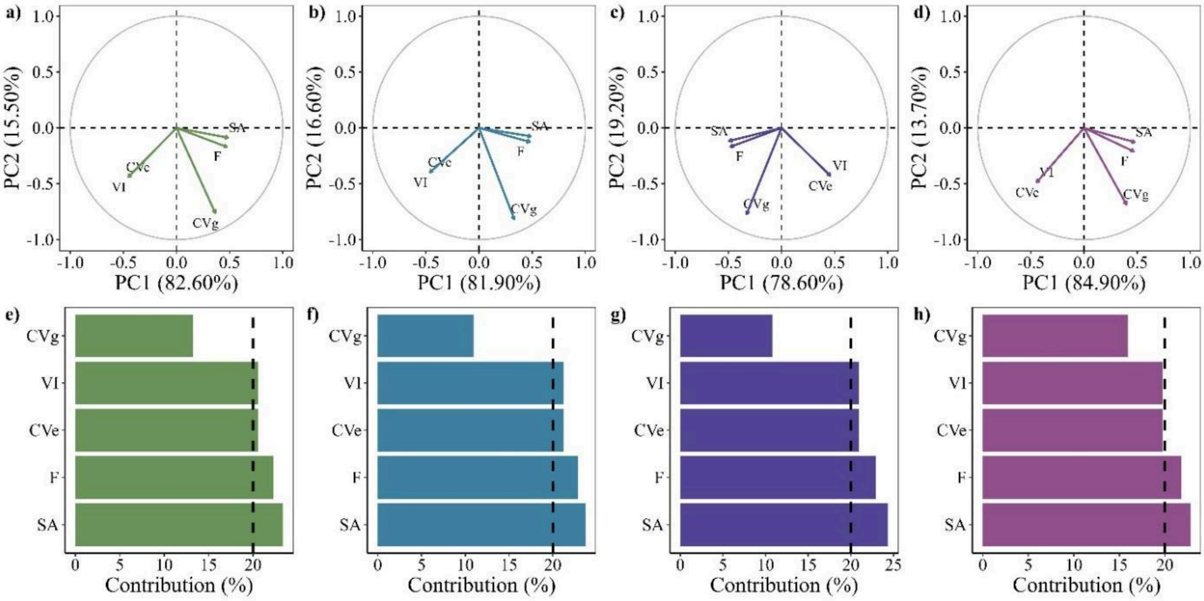
4.19
2.95
1.71
0.47
b)
Coefficient of experimental variation
42.12
35.96
29.80
23.64
17.48
11.32
c)
Coefficient of genetic variation
28.04
22.53
17.02
11.51
6.00
0.49
d)
Variation index
30.05
25.54
21.03
16.52
12.01
7.50
c)
Selective accuracy
0.94
0.75
0.56
0.37
0.18
-0.01
f)
95% confidence interval
2.95
2.36
1.77
1.18
0.59
0.00
n = 24
g)
95% confidence interval
14.55
11.64
8.73
5.82
2.91
0.00
n = 15
h)
95% confidence interval
16.55
13.24
9.93
6.62
3.31
0.00
n = 14
i)
95% confidence interval
10.10
8.08
6.06
4.04
2.02
0.00
n = 15
j)
95% confidence interval
0.55
0.44
0.33
0.22
0.11
0.00
n = 13
0
25
50
75
100
Number of plants per experimental unit


a)
F-test value
6.52
5.32
4.12
2.92
1.72
0.52
0
25
50
75
100
b)
Coefficient of experimental variation
31.50
27.48
23.46
19.44
15.42
11.40
c)
Coefficient of genetic variation
22.55
18.04
13.53
9.02
4.51
0.00
d)
Variation index
22.51
19.52
16.53
13.54
10.55
7.56
c)
Selective accuracy
0.95
0.76
0.57
0.38
0.19
0.00
f)
95% confidence interval
2.55
2.04
1.53
1.02
0.51
0.00
n = 20
g)
95% confidence interval
9.55
7.64
5.73
3.82
1.91
0.00
n = 16
h)
95% confidence interval
12.70
10.16
7.62
5.08
2.54
0.00
n = 14
i)
95% confidence interval
6.75
5.40
4.05
2.70
1.35
0.00
n = 16
j)
95% confidence interval
0.55
0.44
0.33
0.22
0.11
0.00
n = 14
Number of plants per experimental unit



9.11 SUPPLEMENTARY MATERIAL

Supplementary material for this article is available.

10 DISCUSSÃO

A partir dos estudos realizados, tamanhos amostrais ótimos por unidade experimental foram propostos para mudas de couve-flor e plantas de alface, considerando a estimativa de diferentes estatísticas de precisão. Além disso, a comparação de métodos de definição de um ponto de máxima curvatura permitiu a seleção de metodologias precisas e eficientes para a determinação do tamanho ótimo de amostra para mudas de couve-flor. Ainda, as equações preditivas propostas permitem conhecer o valor de estatísticas de precisão por meio da seleção de um determinado tamanho amostral, sendo ferramentas úteis para facilitar a decisão de pesquisadores quanto ao número de plantas a amostrar por unidade experimental com base na precisão desejada.

Quatro metodologias para a definição do tamanho amostral de mudas de couve-flor, considerando a estimativa da média geral experimental, a partir da determinação de um ponto de máxima curvatura, foram comparadas: o método geral, o método de distâncias perpendiculares, o método de resposta linear platô e o método *spline*. Assim, os métodos testados apresentaram divergências, corroborando os resultados obtidos por Souza et al. (2023b, 2023c e 2023d), onde os mesmos métodos foram testados para a definição do tamanho amostral para a cultura da soja. Nesses estudos, assim como no presente, o método geral e o *spline* levaram ao dimensionamento de tamanhos amostrais inadequados, sugerindo que estes apresentam uma tendência de sub ou superestimativa do tamanho de amostra. Dessa forma, os métodos de distâncias perpendiculares e de resposta linear platô proporcionaram resultados que corresponderam a menores amplitudes dos intervalos de confiança da média, o que confere a estes maior precisão para o dimensionamento amostral. Ainda, é importante ressaltar que o método de distâncias perpendiculares apresentou maior eficiência por apresentar valores menores, porém suficientemente precisos, pelo qual o mesmo foi utilizado nos seguintes estudos de dimensionamento amostral aqui apresentados.

De forma geral, observou-se, em todos os estudos expostos, a redução da amplitude do intervalo de confiança em resposta ao aumento do tamanho amostral por unidade experimental, até sua eventual estabilização. Isto permitiu a determinação de tamanhos amostrais ótimos a serem coletados dentro de cada unidade experimental para as culturas da couve-flor e da alface. Esta abordagem considera as restrições experimentais impostas pelos delineamentos experimentais (STORCK et al., 2016), isto é, a separação da área em unidades experimentais, tornando as recomendações de amostragem mais práticas (SOUZA et al., 2022). Nesse sentido, os tamanhos amostrais ótimos variaram conforme as diferentes estatísticas e características avaliadas. Em experimentos com mudas de couve-flor, ao mensurar o número de folhas, comprimento de raiz, altura e comprimento total, os tamanhos amostrais ótimos oscilaram de 15 mudas por unidade experimental para a estimativa confiável da média geral experimental, a 16 mudas para os quadrados médios da análise de variância, o coeficiente de variação experimental, o índice de variação e as diferenças mínimas significativas, e 20 mudas por unidade experimental para a estatística F.

Em alface, ao avaliar a produtividade por planta (massa fresca), observou-se a necessidade de amostrar pelo menos 19 plantas por unidade experimental para estimar a maioria das estatísticas de precisão confiavelmente, considerando aquelas que contribuíram mais para a variação total retida no primeiro componente principal, com exceção da estatística F, para a qual foi necessária a amostragem de 21 plantas por unidade experimental. Por outro lado, para medir a altura de planta, o número de folhas, o diâmetro do colo e o diâmetro médio da cabeça, 24 plantas por unidade experimental foram necessárias para estimar estatísticas de precisão, sendo este número o maior tamanho de amostra encontrado, atribuído também à estatística F. Tal variação evidencia as diferentes respostas das estatísticas e caracteres à variação do tamanho amostral, ressaltando a necessidade de considerar condições específicas ao realizar o dimensionamento amostral de culturas. Entretanto, notou-se que a variação existente entre estatísticas foi mais significativa do que a variação entre caracteres, o qual também foi evidenciado no estudo de Souza et al. (2023b). Nesse sentido, a estatística F destacou-se por ser a que requereu maiores tamanhos amostrais em todas as condições avaliadas, sugerindo que este indicador deve ser utilizado com cautela em estudos onde tamanhos amostrais pequenos são coletados, o qual inclui grande parte das pesquisas com as culturas da alface e couve-flor (TEMPESTA et al., 2019; COSTA et al., 2020; MUSTAFA et al., 2023; QIAO et al., 2023). Apesar disso, é importante ressaltar que esta estatística se torna especialmente relevante para a avaliação da precisão de estudos que comparam genótipos, por considerar variâncias genéticas em sua estrutura de cálculo, assim como para a estimativa da acurácia seletiva (RESENDE e DUARTE, 2007).

Portanto, os resultados obtidos a partir destas pesquisas poderão servir como um guia para a padronização do tamanho de amostra em estudos com couve-flor e alface, aumentando a eficiência durante a coleta de dados, assim como a confiabilidade de seus resultados. Além disso, a predição da precisão experimental a partir da seleção do tamanho amostral, obtida pelas equações propostas, constitui uma ferramenta útil para adequar a tomada de decisão com relação ao número de plantas a serem amostradas por unidade experimental aos objetivos específicos da pesquisa, podendo considerar os diversos fatores que influenciam na amostragem. Assim, em casos em que é inviável utilizar os tamanhos amostrais ótimos recomendados, ainda é possível prever qual será o intervalo de confiança das estatísticas utilizando tamanhos amostrais menores, ou inclusive avaliar o ganho de precisão ao utilizar tamanhos amostrais maiores.

11 CONCLUSÕES GERAIS

Os estudos realizados contribuem para a otimização do planejamento experimental para experimentos realizados com as culturas da couve-flor e da alface, por meio do dimensionamento amostral, considerando restrições experimentais, para técnicas e características específicas. Os métodos de distâncias perpendiculares e resposta linear platô foram considerados adequados para a definição do tamanho amostral a partir de um ponto de máxima curvatura para mudas de couve-flor. A estimativa confiável da média geral experimental em experimentos com mudas de couve-flor requer pelo menos 15 mudas por unidade experimental, enquanto para estimar estatísticas de precisão, 16 mudas são necessárias, à exceção da estatística F, para a qual 20 mudas por unidade experimental devem ser amostradas. Em experimentos com alface, estatísticas de precisão podem ser estimadas confiavelmente a partir da amostragem de 19 plantas por unidade experimental para a produtividade por planta e 24 plantas por unidade experimental para a altura de planta, número de folhas, diâmetro do colo e diâmetro médio da cabeça. Finalmente, os modelos de potência modificados adaptados para prever estatísticas de precisão para mudas de couve-flor e plantas de alface permitiram a formulação de equações preditivas, as quais podem ser utilizadas por outros pesquisadores e calibradas para outras culturas e características.

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