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Genetics/ Original Article

Repeatability coefficient for the determination of the optimal number of harvests for the selection of zucchini hybrids

Abstract – The objective of this work was to estimate the optimal number of harvests for the reliable selection of zucchini (*Cucurbita pepo*) hybrids through the repeatability coefficient. The experimental design was randomized complete blocks with 33 treatments (31 experimental hybrids and 2 commercial ones) and four replicates, with six plants per plot. Fifteen harvests were carried out. Seven morphoagronomic fruit characteristics were evaluated, and repeatability coefficients were estimated using four statistical methods. The repeatability coefficients ranged from low to moderate, regardless of the studied characteristic. For a high-precision selection ($R^2 \geq 90\%$), a high number of evaluated harvests was required, especially for traits related to fruit yield, as follows: 30 to 54 harvests for selection based on total yield; and 43 to 83 harvests for commercial yield, which varied according to the statistical estimation method. The principal component analysis based on the covariance matrix required the least number of harvests for a satisfactory selection precision. Fifteen harvests are sufficient for a satisfactory selection of all evaluated characteristics, with a precision above 70%.

Index terms: *Cucurbita pepo*, biometric, plant breeding.

Coeficiente de repetibilidade para a determinação do número ótimo de colheitas para seleção de abobrinha-italiana

Resumo – O objetivo deste trabalho foi estimar o número ótimo de colheitas para a seleção confiável de híbridos de abobrinha-italiana (*Cucurbita pepo*) por meio do coeficiente de repetibilidade. O delineamento experimental foi em blocos ao acaso, com 33 tratamentos (31 híbridos experimentais e 2 comerciais) e quatro repetições, com seis plantas por parcela. Realizaram-se 15 colheitas. Sete características morfoagronômicas dos frutos foram avaliadas, e os coeficientes de repetibilidade foram estimados por meio de quatro métodos estatísticos. Os coeficientes de repetibilidade variaram de baixos a moderados, independentemente da característica estudada. Para uma seleção de alta precisão ($R^2 \geq 90\%$), um elevado número de colheitas avaliadas foi necessário, especialmente para as características relacionadas à produtividade de frutos, conforme a seguir: 30 a 54 colheitas para a seleção baseada na produtividade total; e 43 a 83 colheitas para a produtividade comercial, que variou conforme o método estatístico de estimação. A análise de componentes principais baseada na matriz de covariância demandou a menor quantidade de colheitas para uma precisão satisfatória da seleção. São suficientes 15 colheitas para a seleção satisfatória de todas as características avaliadas, com precisão superior a 70%.

Termos para indexação: *Cucurbita pepo*, biometria, melhoramento genético.

Maria Elisângela Ferreira de Oliveira[1],
Valter Carvalho de Andrade Júnior[1 ✉],
Orlando Gonçalves Brito[1],
Sebastião Márcio Azevedo[1],
Jeferson Carlos de Oliveira Silva[1],
Eduardo Alves da Silva[1] and
Alcinei Mistico Azevedo[2]

[1] Universidade Federal de Lavras, Departamento de Agricultura, Caixa Postal 3037, CEP 37200-900 Lavras, MG, Brazil. E-mail: mariaelisangela94@hotmail.com, valter.andrade@ufla.br, orlandocefet@yahoo.com.br, sebastiao.azevedo@ufla.br, jefersonteng@gmail.com, easufsj@gmail.com

[2] Universidade Federal de Minas Gerais, Instituto de Ciências Agrárias, Avenida Universitária, nº 1.000, Bairro Universitário, CEP 39404-547 Montes Claros, MG, Brazil. E-mail: alcineimistico@hotmail.com

✉ Corresponding author

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Introduction

Zucchini (*Cucurbita pepo* L.) is an important vegetable produced in Brazil, which stands out among the ten species with the highest economic value (Armond et al., 2016). Furthermore, zucchini production has great nutritional and economic relevance in the country, especially for family farming (Souza et al., 2017; Tejada et al., 2020). Since the crop requires a high demand for labor, especially in the fruit harvesting (Lúcio et al., 2021), and genetic breeding research usually requires a high number of evaluations and harvests (Lúcio et al., 2021), research costs can be changed as needed (Cruz et al., 2012). Thus, in the selection of superior genotypes, it is necessary to define the ideal minimum number of harvests during the crop production cycle, to speed up the genetic progress and reduce research costs (Martuscello et al., 2015; Cavalcante et al., 2018). The main statistical approach used for this purpose is the estimation of the repeatability coefficient for evaluated characters (Cruz et al., 2012; Della Bruna et al., 2012; Malikouski et al., 2021).

The repeatability coefficient is the correlation between measurements in the same individual, whose evaluations are repeated in time and/or space, and it is widely used in the selection of superior individuals in cultures with multiple harvests (Lessa et al., 2014). This allows of the estimation of the ideal number of harvests and the reduction of experimental errors in the selection of individuals (Cardoso, 2006; Della Bruna et al., 2012). Moreover, the repeatability coefficient expresses the maximum value that heritability in the broad sense can reach, in other words, the maximum value of the total variation which can be effectively heritable (Jesus et al., 2021).

The repeatability coefficient has already been studied in zucchini by Feijó et al. (2005), but just a single cultivar was evaluated. However, in repeatability studies for purposes of plant breeding, several genotypes should be evaluated simultaneously, to allow of more comprehensive results. It is noteworthy that the repeatability coefficient varies according to the genetic structure of the population, and it may differ between half-sib or full-sib progenies (Cruz et al., 2012). This fact exposes the lack of this type of information in the literature, which is too important for zucchini breeding programs, as it guarantees the optimization of time and resources spent on research.

However, in several vegetables, the repeatability coefficient has already been studied, such as cucumber (*Cucumis sativus*) (Cardoso, 2006), tomato (*Solanum lycopersicum*) (Adewale & Adebo, 2018), and kale (*Brassica oleracea* L. var. *acephala*) (Azevedo et al., 2016; Brito et al., 2019). Thus, there is a need for research related to the evaluation of the minimum optimal number of measurements required for an accurate selection of superior individuals in zucchini, especially with high-potential yield and high-quality fruit.

The objective of this work was to estimate the optimal number of harvests for the reliable selection of zucchini hybrids through the repeatability coefficient.

Materials and Methods

The experiment was carried out from March to May 2020, at the Center for Development and Technology Transfer of Universidade Federal de Lavras (UFLA), located in the Fazenda Palmital, in the municipality of Ijaci (21º10'S, 44º55'W, at 832 m), in the southern region of the state of Minas Gerais, Brazil. According to the Köppen-Geiger's climate classification, the climatic pattern of the region is Cwb and Cwa, mesothermic humid, tropical of altitude, with mild summer. The annual mean for temperature is 19.4ºC, and for rainfall is 1,530 mm which is concentrated between November and February (Ijaci, 2018).

The experiment was performed in randomized complete blocks design, with 33 treatments, four replicates and six plants per plot, and 31 treatments of experimental hybrids – HT 19107, HT 19108, HT 19109, HT 19111, HT 19113, HT 19117, HT 19118, HT 19120, HT 19121, HT 19122, HT 19123, HT 19128, HT 19135, HT 19138, HT 19140, HT 19141, HT 19146, HT 19147, HT 19148, HT 19149, HT 19150, HT 19152, HT 19155, HT 19156, HT 19157, HT 19158, HT 19159, HT 19161, HT 19162, HT 19164 e HT 19165) – and two commercial cultivars, the HT 2000 – Adelle, and the HT 19166 – Clarisse).

The hybrids were seeded in styrofoam trays with 162 cells, in Carolina Soil substrate (Carolina Soil, Santa Cruz do Sul, RS, Brazil), with one seed for each cell. After the sowing, the trays were kept in the greenhouse for 15 days until the day of transplanting in the experimental area. Soil preparation was made from subsoiling, followed by raising beds of 1.1 m width by 33.0 m length each, spaced at 0.30 m. The experiment was carried out in a double row system, with 0.80 m between rows and 0.6 m between plants. Fertilization, based on the results of the previously performed soil analysis, was performed as recommended by Ribeiro et al. (1999). Then, beds were covered with mulching. Each plot was 1.1 m wide and 1.5 m long, totaling 1.65 m^2.

At 45 days after the seedlings were planted, the harvests were carried out every two days, totaling 15 harvests throughout the crop cycle. Evaluations were performed for the parameters described as follows. Fruit diameter (Diam, cm) and length (Leng, cm), measured with a measuring tape. Fruit shape (Shap), determined with a scale of notes from 1 to 5, as follows: 1, very bad (deformed, oval); 2, bad (a little long and oval); 3, medium (elongated with deformations); 4, good (close to the ideal commercial shape); and 5, ideal (elongated, straight, between 18 and 25 cm). The uniformity of the fruit was also evaluated by grade scales from 1 to 5, as follows: 1, very bad fruit (nonuniform); 2, bad fruit; 3, average uniformity; 4, good; and 5, excellent and very uniform fruit. The number of total fruit was determined from the count of all fruit harvested in the plot of each treatment (fruit per plot). The number of commercial fruit was determined by counting only the fruit with an adequate commercial standard in the plots (fruits per plot). Fruit with commercial standard were considered those of length between 18 and 25 cm, weight between 200 and 250 g, and with adequate and uniform shape. Total yield was determined from the total mass of all fruit in each plot (kg per plot). For the commercial yield, only the mass of commercial fruit of the plots (kg per plot) was used .

To meet the premises of the analysis of variance (normality, homogeneity. and independence of residues), data on the number of total and commercial fruit were transformed into square roots. In the repeatability study, the statistical model was used with the evaluation factors seasons and hybrids, as suggested by Cruz et al. (2012) for experiments like this one, as follows:

$$Y_{ij}= m+g_i+a_j+e_{ij},$$

where Y_{ij} are observations concerning the i^{th} hybrid in the j^{th} season (harvest); m is overall mean; g_i is the random effect of the i^{th} hybrid, under the influence of the permanent environment (i=1, 2, ..., 33 hybrids); a_j is the effect of the j^{th} season (j= 1, 2, ..., 15 harvests); and e_{ij} is the experimental error associated with the observation Y_{ij}.

For a greater reliability of results, repeatability was estimated by four methods: analysis of variance; principal component analysis (PCA) based on correlation and covariance matrices; and structural analysis based on the correlation matrix. The performed statistical analyses were obtained from the genetic-statistical software Genes (Cruz, 2013), and all the statistics used are detailed by Cruz et al. (2012).

Results and Discussion

There was a significant difference ($p\leq0.01$) in all variables evaluated for the number of harvests and treatments (Table 1), which shows variability between hybrids and harvests. This result indicates that the component of genetic variation, confounded with the permanent effects of the environment, is significant (Manfio et al., 2011). The parameters total fruit yield, commercial fruit yield, number of commercial fruit, and fruit uniformity showed coefficients of variation (CV) between 26.97 and 55.07%, which are values considered high. When a variable has a high CV, there is an indication that it is more affected by the environment (Azevedo et al., 2016) and, consequently, it shows a lower experimental precision in its evaluation (Lopes et al., 2021).

Although showing high values, the found CV corroborates those commonly found in the literature for some evaluated characteristics in Cucurbitaceae, such as fruit yield, the number of fruit per plant, and mean fruit mass (Akter et al., 2013). The production of zucchini fruit is strongly affected by temporary environmental changes, such as daily temperature fluctuations and the presence of pollinators, which can contribute to significant variations among the harvests (Oluoch, 2012). This fact suggests that a greater number of harvests should be carried out to attain more reliable results in the selection.

The obtained CV values of diameter, length, and shape of fruit were considered low, between 4.98 and 11.11%, which reflects the lesser influence of the environment and greater genetic effect on these characteristics (Azevedo et al., 2016). In a selection of promising hybrids, traits with greater genetic

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influence favor the genetic improvement of crops in multiple harvests (Santin et al., 2019). This occurs because, generally, fewer evaluations are required to attain more accurate results.

Except for fruit shape and commercial fruit yield, the estimates of repeatability coefficients were similar among the other variables, regardless of the adopted statistical procedure (Table 2), with values between 0.14 and 0.29, which are considered low. Resende (2015) states that repeatability values greater than 0.60 are considered high and promote high reliability in the measurements, while coefficients between 0.30 and 0.60 are considered moderate, and those below 0.30 are low. For fruit shape and commercial fruit yield, the repeatability coefficients were also low, but with greater variation, with values between 0.19 and 0.34 for shape, and 0.09 and 0.17 for commercial fruit yield.

The similarity observed between the repeatability coefficients for the different variables in the crop, was also reported by Feijó et al. (2005) for the optimal number of harvests in zucchini, using the same statistical procedures applied in the present study. However, as observed by Azevedo et al. (2016) for the optimal number of harvests in kale, the repeatability coefficients by the analysis of variance were always equal to or lower than those observed in the multivariate methods (structural analysis and PCA). These findings were also reported by Feijó et al. (2005) and Manfio et al. (2011), who obtained similar results when they were estimating repeatability coefficients in zucchini and macaúba palm (*Acrocomia aculeata*), respectively.

The repeatability coefficients estimated in both statistical methods ranged from low to moderate, regardless of the variables studied. This is possibly due to environmental variations, which requires increasing the number of harvests in order to obtain significant selection gains and verify the genetic differences between the hybrids (Andrade Júnior et al., 2020). Low values of the repeatability coefficient are related to the fact that the genotypic variance used to estimate repeatability does not show a purely genetic composition, as there is interference from the permanent effects of the environment that are confused with the genotypic variance (Santin et al., 2019).

When low repeatability coefficients are verified, increases of the number of harvests are recommended to increase the experimental precision. This is suggested because many genes are often related to the expression of a particular character, which can result in significant variations between crops, making it difficult to identify genetic effects (Cruz et al., 2012). As fruit yield is a trait with this profile, controlled by many genes, it is common to obtain low repeatability coefficients. This observation has already been reported for other fruit crops, such as guava (Quintal et al., 2017) and Tahiti lime (Malikouski et al., 2021).

The fruit yield of zucchini shows a strong response to environmental conditions because of the floral biology and ecophysiology of the crop. Thus, substantial changes in climatic factors directly affect the proportion of male and female flowers and, consequently, its fruit production (Lima et al., 2022). This fact explains the marked variations between this species cultivations, as environmental factors can favor or compromise the pollination rate. Furthermore, its cycle can be influenced by various environmental conditions such as temperature, solar radiation, and precipitation (Bannayan et al., 2011). Another factor that can result in strong variations between cultivations and reduce the repeatability coefficients is the rapid growth and development of

Table 1. Summary of the analysis of variance of 15 harvests of 33 zucchini (*Cucurbita pepo*) hybrids, for the following parameters: fruit diameter, fruit length, fruit shape, uniformity, number of total fruit (NTfruit), number of commercial fruit (NCfruit), total yield (TYield), and commercial yield (CYield).

Source of variation	Diameter (cm)	Length (cm)	Shape (score)	Uniformity (score)	NTfruit (fruit per plot)	NCfruit (fruit per plot)	TYield (kg per plot)	CYield (kg per plot)
Season	166.54**	81.49**	1.02**	22.97**	22.97**	48.05**	3.57**	3.34**
Hybrids	3.22**	4.25**	0.73**	2.20**	2.20**	1.59**	0.28**	0.12**
Error	0.73	0.95	0.17	0.69	0.69	0.53	0.09	0.13
CV (%)	4.98	5.01	11.11	26.97	26.97	33.68	30.81	55.07
General average(1)	17.16	19.45	3.71	3.08	3.08	2.16	0.97	0.65

(1)Referring to the 15 harvests carried out. **Significant by F-test, at 1% probability.

zucchini fruit in a short period. This is a characteristic of the culture and it makes responses to environmental effects more prominent.

According to the four statistical methods studied, the coefficients of determination (R^2) were lower than 86.26% (Table 2); these values are considered below the ideal (≥90%) (Manfio et al., 2011), which may be circumvented by increasing the number of crops. However, Resende (2002) and Souza Sobrinho et al. (2010) consider that an R^2 above 80% provides a reasonable precision already for the reliable selection of genotypes. This is especially true for populations at an early stage of genetic improvement, as in the present study. In this case, it is possible to consider that R^2 values greater than 70% already allow of a satisfactory primary distinction between good and bad genotypes. However, in more advanced phases of genetic improvement programs, this minimum precision should be increased to obtain a more consistent information.

Table 2. Estimates of repeatability, by different methods, for fruit diameter, fruit length, fruit shape, uniformity, number of total fruit (NTfruit), number of commercial fruit (NCfruit), total yield (Tyield), and commercial yield (CYield), in 15 harvests of 33 zucchini (*Cucurbita pepo*) hybrids.

Variable		Analysis of variance	PCA		Structural analysis
			Covariance	Correlation	
Diameter	Coef.	0.22	0.28	0.26	0.24
	R^2 (%)	77.30	82.59	80.74	79.20
Length	Coef.	0.22	0.29	0.25	0.22
	R^2 (%)	77.61	83.13	80.66	77.55
Shape	Coef.	0.21	0.34	0.28	0.19
	R^2 (%)	76.29	86.27	82.52	74.98
Uniformity	Coef.	0.15	0.20	0.18	0.14
	R^2 (%)	68.31	74.64	70.65	67.09
NTfruit	Coef.	0.15	0.19	0.17	0.14
	R^2 (%)	68.31	74.64	70.65	67.09
NCfruit	Coef.	0.14	0.23	0.17	0.13
	R^2 (%)	66.71	77.96	71.74	66.12
TYield	Coef.	0.14	0.23	0.19	0.17
	R^2 (%)	66.64	78.47	74.58	71.38
CYield	Coef.	0.10	0.17	0.13	0.09
	R^2 (%)	58.03	71.56	63.83	56.64

PCA, principal component analysis; Coef., repeatability coefficient; and R^2, coefficient of determination.

For fruit diameter and shape, the performance of the 15 harvests promoted reliability of about 80% (R^2) by the analysis of variance and the structural analysis, and 85% by the methods of the PCA based on in the covariance and correlation matrices (Figure 1). As for fruit uniformity, the 15 harvests provided the minimum reliability of 70%, regardless of the statistical method of estimation. The PCA method based on the covariance matrix estimated the smallest number of harvests for a precision greater than 90% when evaluating the diameter, length, shape, and uniformity of fruit, requiring at least 23, 22, 17, and 37 harvests, for these characteristics, respectively.

It is important to consider that the zucchini production cycle can extend up to 90 days, with multiple harvests at regular intervals of two to three days (Lúcio et al., 2021). That is, it is possible to carry out between 20 and 30 harvests, depending on the cultivar and environmental conditions. Therefore, it is often unfeasible to carry out a high number of harvests to obtain a high precision in the selection (≥90%), due to physiological and agronomic limitations inherent to the culture. Thus, it is up to the breeder to verify the satisfactory precision thresholds for a feasible number of harvests.

For the number of total and commercial fruit (Figure 2), 15 harvests were considered to provide the minimum reliability of 70% by the analysis of variance and the structural analysis, while the methods based on covariance and correlations showed, in general, the minimum reliability of 75%. Concerning the total yield, the 15 harvests provided at least 70% reliability in both studied methods. However, the 15 harvests provided the minimum reliability of 70% for commercial yield only when the PCA method based on the covariance matrix was used, as in the other methods this reliability varied between 60 and 65%.

We observed that for a precision greater than 90%, between 30 and 54 harvests are necessary for total yield, while, for commercial yield, from 43 to 83 harvests are required; these values may vary according to the adopted statistical method (Figure 2). Similar results were also verified for the number of total and commercial fruit. Furthermore, we found that the PCA based on the covariance matrix required the least number of harvests to estimate the optimal number of evaluations with high precision, for both evaluated

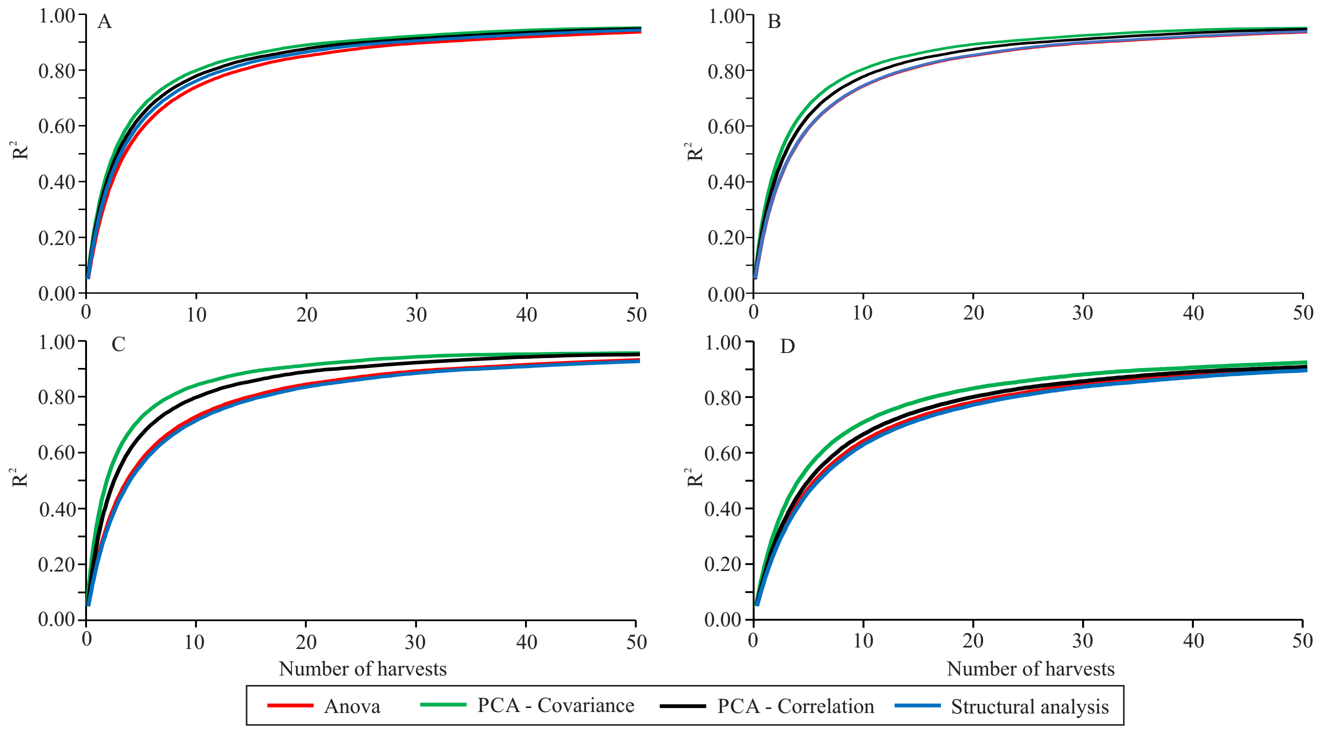

Figure 1. Coefficient of determination (R^2) calculated by different methods as a function of the number of harvests, in 15 harvests of 33 zucchini (*Cucurbita pepo*) hybrids, for the following parameters: A, fruit diameter; B, fruit length; C, fruit shape; and D, fruit uniformity. ANOVA, analysis of variance; and PCA, principal component analysis.

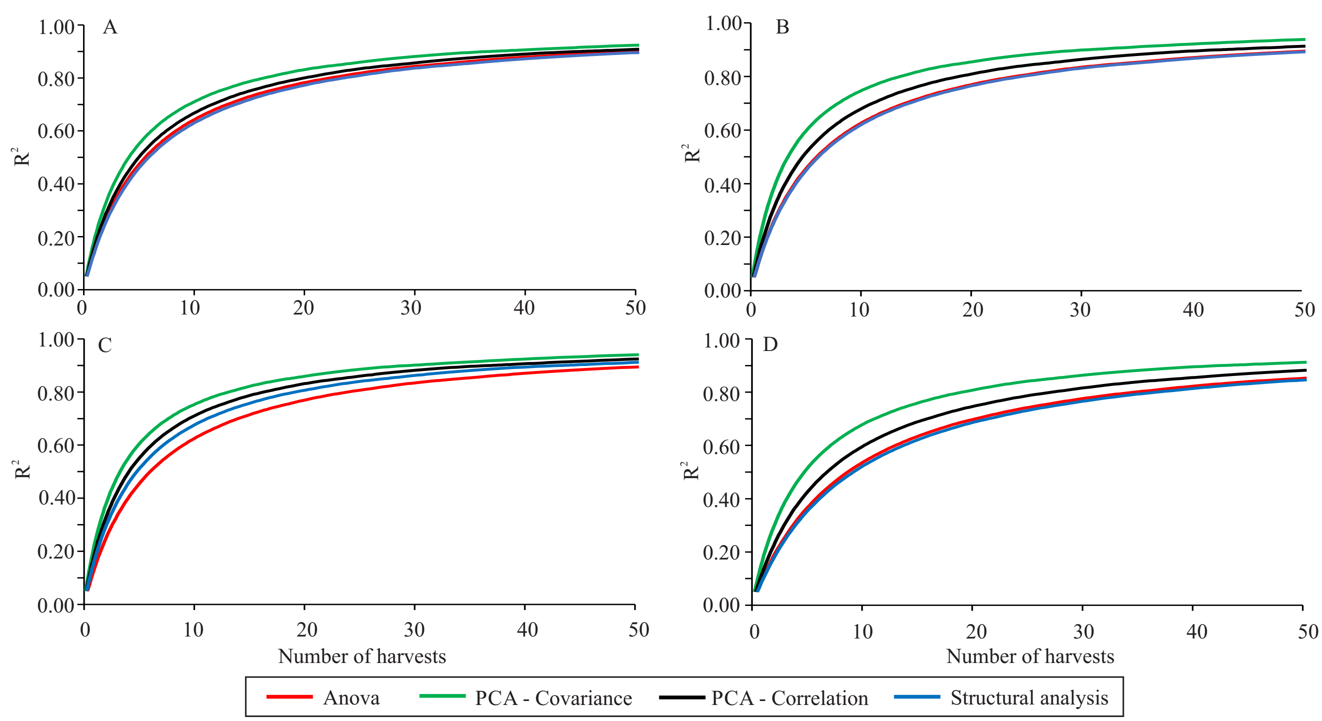

Figure 2. Coefficient of determination (R^2) calculated by different methods as a function of the number of harvests, in 15 harvests of 33 zuchini (*Cucurbita pepo*) hybrids, for the following parameters: A, number of total fruit; B, number of commercial fruit; C, total production; and D, commercial production. Anova, analysis of variance; and PCA, principal component analysis.

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characteristics. In general, the number of harvests necessary for high precision was considered very high however. This observation has already been reported by Feijó et al. (2005), who verified the need for at least 27 harvests for a reliable selection of zucchini. This large number of required harvests is related to the significant production fluctuations that occur between harvests throughout the zucchini cycle, as already reported by Lúcio et al. (2008).

The ideal number of harvests varies according to the culture, the level of improvement of the studied population, environmental conditions, and desired characteristics (Santin et al., 2019). Brito et al. (2019) found that, for half-sib kale progenies, four harvests are required to obtain 80% reliability for the number of leaves. Azevedo et al. (2016), when evaluating clones of the same crop, found that only three harvests were needed for a reliable selection of the same variable, a difference that can be explained by the fact that clones are a more genetically uniform population than half-sib progenies. For multiple fruit harvests of passion fruit, Neves et al. (2010) identified the need for ten measurements, to attain 80% reliability in the results, which indicates that crops which produce fruit require a greater number of evaluations for the selection of superior genotypes. These results reinforce the need for a deep understanding of repeatability coefficients in plant breeding programs, especially to contribute to the accurate selection of genotypes and to promote a greater genetic progress in the culture of zucchini.

Conclusions

1. Fifteen harvests are sufficient for a reliable selection of morphoagronomic characteristics of fruit of zucchini (*Cucurbita pepo*) hybrids, with precision greater than 70%.
2. The principal component analysis based on the covariance matrix requires the smallest number of harvests for a reliable selection of zucchini hybrids.
3. The repeatability coefficients for yield characteristics and fruit quality of zucchini are considered low.

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