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Repeatability of agronomic and morphological characteristics in ornamental pepper

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ABSTRACT

Breeding programs seek to reduce the time spent assessing characteristics more times than necessary. However, this reduction should in no way undermine the representativeness and reliability of the information. Thus, an important success factor in breeding programs is the knowledge of the ideal number of assessments needed to evaluate commercially important characteristics in each species or population. In this context, this study aimed to determine the repeatability coefficient of eight biometric traits in four populations of ornamental pepper, seeking to increase the efficiency of evaluation and selection processes. There was no significant influence of the calculation method used over the estimate of the repeatability coefficient. The results indicated that five plants are necessary to assess plant height; leaf number, length and width; and fruit number, length and width in the ornamental pepper material we used with a determination coefficient of 90%.

Keywords: *Capsicum*, ornamental plants, statistical analysis, breeding, germplasm.

RESUMO

Repetibilidade em características morfoagronômicas de pimenteiras ornamentais

Nos programas de melhoramento é importante reduzir o tempo consumido realizando-se repetições de avaliações além do necessário. Entretanto, esta redução não deve comprometer a representatividade e confiabilidade das informações. Assim, um importante fator de sucesso em programas de melhoramento é o conhecimento do número ideal de repetições para avaliação de características de importância comercial em cada espécie ou população. Nesse contexto, este trabalho teve como objetivo determinar o coeficiente de repetibilidade de oito características biométricas em quatro populações de pimenteiras ornamentais, buscando aumentar a eficiência do processo de avaliação e seleção. Não houve influência significativa do método de cálculo utilizado sobre a estimativa do coeficiente de repetibilidade. Os resultados indicaram que cinco plantas são suficientes para avaliar altura de planta; número, comprimento e largura de folha; e número, comprimento e largura de frutos de genótipos de pimenteira ornamental, com coeficiente de determinação de 90%.

Palavras chave: *Capsicum*, plantas ornamentais, análise estatística, melhoramento, germoplasma.

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In 2009, a breeding program was established to develop ornamental pepper cultivars to be grown in pots. In addition to the ornamental aspect, main objectives of this program are to develop cultivars with reduced plant size and high resistance to the effects of ethylene. So far, six families with 36 plants were obtained out of crosses between cultivars and accessions of *Capsicum annuum* from the germplasm banks of the Federal Universities of Viçosa (BGH-UFV) and Paraíba (UFPB). Among those 36 plants, 18 have promising ornamental qualities.

To identify *Capsicum* progenies with ornamental potential, one needs to assess the phenotypic behavior of the main characteristics to estimate the genotypic variability and the phenotype recurrence. However, a major challenge in breeding for ornamental purposes is to find the adequate number of plants that allow a reliable estimation of differences among genotypes. And it becomes even more complex when the divergence or genetic diversity among and within populations present either in natural conditions or in germplasm banks and breeding programs (Diniz Filho, 2000, *apud* Cruz *et al*., 2011) is taken into consideration.

For a particular cultivar to be granted protection, it must meet three basic criteria: it has to be distinct, homogenous and stable. A cultivar is distinct when it is indisputably unique in relation to any other whose existence, on the date of application for protection, is recognized (Ferraz & Campos, 2009). However, the literature has little information on the minimum number of *Capsicum* plants that should be assessed to provide a reliable value for a given characteristic. According to Cruz *et al*. (2004) and Ferreira *et al*. (2010), such a number can be estimated and tested by the repeatability coefficient. The repeatability coefficient, on its turn, can be estimated when a characteristic is assessed recurrently in a given genotype. That means, the repeatability coefficient comes from more than one measurement in the same genotype and sets the minimum number of assessments needed for reliably estimating the value of a given characteristic, as well as the degree of determination.

Repeatability corresponds to the maximum value that heritability can assume, since it expresses the share of the phenotypic variation which is due to effects confounded with those that permanently influence the progeny. Therefore, the repeatability coefficient is essential to guide breeding works (Ferreira *et al*., 1999; Cruz *et al*., 2004).

This research aimed to define the number of assessments needed to predict the value of ornamental characteristics in pepper and consequently to guarantee the efficiency of the evaluation and selection processes.

MATERIAL AND METHODS

The experiment was set in a completely randomized design, with ten replications, one-plant plots, and carried out in a greenhouse of the Flower Sector and Post-Harvest Laboratory, at the Federal University of Viçosa (UFV), from September, 2011, to April, 2012. We evaluated two accessions from the germplasm banks of the Agricultural Research Corporation of Minas Gerais, at the Regional Unit Zona da Mata, and from UFV (MG 7073 and BGH 1039, respectively), and two cultivars (Biquinho and Pirâmide Ornamental), from ISLA®.

Pepper seedlings grew in a protected environment, in polystyrene trays containing the commercial substrate Bioplant®. Seedlings with two to three pairs of leaves were transplanted to 900 mL pots (11 cm height x 9.5 cm basal diameter x 13.5 cm top diameter) also filled with Bioplant®. All cultural practices were carried out according to the technical recommendations for pepper.

When 50% of the plants in a population showed at least 30% of fully ripe fruits (typical shape for each material, color demanded by the market, and no wilting), we assessed the following traits, in all replications: Plant height (PH) (from ground to the top of the last fully expanded leaf); Average canopy diameter (CD) (longitudinal and transversal diameters, using a caliper); Number of leaves (NL); Size of leaves (length/width) (LL and LW) (length and width of 10 fully expanded leaves taken randomly); Number of ripe fruits per plant (NFP); Ripe fruit length (FL) (measured in 10 fully ripe fruits taken randomly) and; Average diameter of ripe fruits (FD) (average from three measurements per fruit, in 10 fruits taken randomly).

Estimates of the repeatability coefficients were obtained using analysis of variance (ANOVA); principal components based on the correlation (PCCo) and co-variance (PCCv) matrixes; and structural analysis (SA), based on the correlation matrix between families in each pair of evaluation (Mansour *et al*., 1981). According to Cruz *et al*. (2004), this estimator corresponds to the arithmetic average of the phenotypic correlation between genotypes considering each pair of measurements.

For ANOVA, we estimated the repeatability coefficient using the results of the analysis of variance, according to the model $\mathbf{Y_{ij}=\mu+g_i+e_{ij}}$, where:

$\mathbf{Y_{ij}}$**:** score regarding the ith subsample; **μ:** general average; $\mathbf{g_i}$**:** random effect of the ith subsample under the influence of the permanent environment (i = 1, 2, ..., p); $\mathbf{e_{ij}}$**:** effect of the temporary environment associated with the jth measurement in the ith subsample (j= 1, 2, ...).

We calculated the repeatability coefficient using the following equation:

$$r=\hat{\rho}=\frac{\hat{Cov}\left(Y_{ij},Y_{ij'}\right)}{\sqrt{\hat{V}\left(Y_{ij}\right)\hat{V}\left(Y_{ij'}\right)}}=\frac{\hat{\sigma}_g^2}{\hat{\sigma}_Y^2}=\frac{\hat{\sigma}_g^2}{\hat{\sigma}^2+\hat{\sigma}_g^2}$$

where:

Y_ij and Y_{ij}': different measurements carried out in the same individual.

Having the repeatability coefficient (r), we estimated the adequate number of measurements (0) necessary to predict the real value of individuals for a given value of genotypic determination (R^2) using the expression

$$\eta_0=\frac{R^2\left(1-\hat{r}\right)}{\left(1-R^2\right)\hat{r}}.$$

To obtain the genotypic determination coefficient (R^2), which represents the percentage of certainty of predicting the real value of selected individuals based on n measurements, we used the equation

$$R^2=\frac{\eta r}{1+r(\eta-1)}.$$

We calculated all estimates using the repeatability procedure of the software GENES (Cruz, 2006ab).

RESULTS AND DISCUSSION

We observed significant differences among genotypes in all traits, but in average canopy diameter (Table 1), which hampers the application of the repeatability coefficient (r) to this characteristic. These results

Table 1. Analysis of variance of the traits assessed in the genetic materials BGH 1039, BGH 7073, Biquinho, and Pirâmide Ornamental of ornamental pepper (análise de variância para as características analisadas nos materiais genéticos BGH 1039, BGH 7073, Biquinho e Pirâmide Ornamental de pimenteira ornamental). Viçosa, UFV, 2012.

Characteristics	QM values	QM residues	Average	CV (%)
Plant height	116.9069*	6.6084	30.5975	8.4016
Average canopy diameter	0.0027	0.0070	1.0415	8.0341
Number of leaves	78515.8917*	145.7620	178.5250	6.7627
Leaf length	14.8617*	0.1913	7.4250	5.8906
Leaf width	9.9329*	0.0744	3.5825	7.6137
Number of fruits/plant	1799.6667*	27.8148	35.9000	14.6907
Fruit lenght	4.4740*	0.0786	3.8700	7.2457
Fruit width	18.5680*	0.0243	18.3650	84.9030

* Significant by F test, p>0.05 (significativo pelo teste F , p>0,05).

Table 2. Estimates of the coefficients of repeatability (r) and determination (R^2) and of the minimum number of assessments using the methods of analysis of variance (ANOVA), principal components based on correlation (PCCo) or in the covariance (PCCv) matrixes; and the structural analysis based on the correlation matrix (SA) for plant height; leaf number, length and width; ripe fruit number, length and average diameter in four ornamental pepper genetic materials (estimativa dos coeficientes de repetibilidade (*r*) e de determinação (R^2) e do número mínimo de mensurações utilizando os métodos de análise de variância (ANOVA), componentes principais baseado na matrizes de correlação (PCCo) e de covariância (PCCv) e análise estrutural baseado na matriz de correlação (SA) para as características altura da planta; número, comprimento e largura da folha; número, comprimento e diâmetro médio de frutos maduros em quatro acessos de pimenta). Viçosa, UFV, 2012.

Trait	Average value obtained after 10 assessments			R^2	Number of assessments necessary for different determination coefficients							
					ANOVA		PCCv		CPC		AS	
					η_0	-1	η_0	-1	η_0	-1	η_0	-1
		$\hat{r}$	R^2	0.80	2.397	(2)	2.238	(2)	1.923	(2)	2.056	(2)
	ANOVA	0.6253	94.347	0.85	3.395	(3)	3.171	(3)	2.724	(3)	2.912	(3)
PH	PCCo	0.6412	94.701	0.90	5.392	(5)	5.036	(5)	4.326	(4)	4.625	(5)
	PCCv	0.6754	95.413	0.95	11.384	(11)	10.632	(11)	9.133	(9)	9.764	(10)
	SA	0.6605	95.112	0.99	59.315	(59)	55.396	(55)	47.585	(48)	50.876	(51)
		$\hat{r}$	R^2	0.80	0.074	(1)	0.016	(1)	0.018	(1)	0.018	(1)
	ANOVA	0.9817	99.814	0.85	0.105	(1)	0.022	(1)	0.025	(1)	0.025	(1)
NL	PCCo	0.9961	99.960	0.90	0.167	(1)	0.035	(1)	0.040	(1)	0.040	(1)
	PCCv	0.9955	99.955	0.95	0.353	(1)	0.075	(1)	0.085	(1)	0.085	(1)
	SA	0.9955	99.955	0.99	1.841	(2)	0.388	(1)	0.445	(1)	0.445	(1)
		$\hat{r}$	R^2	0.80	0.522	(1)	0.254	(1)	0.305	(1)	0.310	(1)
	ANOVA	0.8846	98.712	0.85	0.739	(1)	0.360	(1)	0.432	(1)	0.439	(1)
LL	PCCo	0.9403	99.368	0.90	1.174	(1)	0.572	(1)	0.686	(1)	0.697	(1)
	PCCv	0.9291	99.243	0.95	2.478	(2)	1.207	(1)	1.449	(1)	1.471	(1)
	SA	0.9281	99.231	0.99	12.909	(13)	6.288	(6)	7.551	(8)	7.665	(8)
		$\hat{r}$	R^2	0.80	0.302	(1)	0.231	(1)	0.242	(1)	0.244	(1)
	ANOVA	0.9298	99.251	0.85	0.428	(1)	0.327	(1)	0.342	(1)	0.346	(1)
LW	PCCo	0.9455	99.426	0.90	0.679	(1)	0.519	(1)	0.544	(1)	0.550	(1)
	PCCv	0.9430	99.399	0.95	1.434	(1)	1.096	(1)	1.148	(1)	1.161	(1)
	SA	0.9424	99.392	0.99	7.471	(7)	5.710	(6)	5.983	(6)	6.050	(6)
		$\hat{r}$	R^2	0.80	0.628	(1)	0.466	(1)	0.549	(1)	0.579	(1)
	ANOVA	0.8643	98.454	0.85	0.890	(1)	0.660	(1)	0.778	(1)	0.821	(1)
NFP	PCCo	0.8956	98.848	0.90	1.413	(1)	1.049	(1)	1.235	(1)	1.304	(1)
	PCCv	0.8793	98.646	0.95	2.983	(3)	2.214	(2)	2.608	(3)	2.752	(3)
	SA	0.8735	98.572	0.99	15.541	(16)	11.537	(12)	13.587	(14)	14.339	(14)
		$\hat{r}$	R^2	0.80	0.716	(1)	0.493	(1)	0.602	(1)	0.611	(1)
	ANOVA	0.8483	98.242	0.85	1.014	(1)	0.699	(1)	0.853	(1)	0.866	(1)
FL	PCCo	0.8902	98.781	0.90	1.610	(2)	1.110	(1)	1.354	(1)	1.375	(1)
	PCCv	0.8692	98.517	0.95	3.399	(3)	2.343	(2)	2.859	(3)	2.902	(3)
	SA	0.8675	98.495	0.99	17.710	(18)	12.210	(12)	14.899	(15)	15.123	(15)
		$\hat{r}$	R^2	0.80	0.53	(1)	0.39	(1)	0.46	(1)	0.47	(1)
	ANOVA	0.8829	98.690	0.85	0.75	(1)	0.56	(1)	0.66	(1)	0.67	(1)
FD	PCCo	0.9093	99.012	0.90	1.19	(1)	0.89	(1)	1.05	(1)	1.07	(1)
	PCCv	0.8953	98.844	0.95	2.52	(3)	1.89	(2)	2.22	(2)	2.27	(2)
	SA	0.8933	98.819	0.99	13.13	(13)	9.87	(10)	11.57	(12)	11.87	(12)

[1]Approximated number; calculated number (número aproximado; número calculado); PH= plant height (altura de planta); NL= number of leaves (número de folhas); LL= leaf length (comprimento de folha); LW= leaf width (largura de folha); NFP= number of ripe fruits per plant (número de frutos maduros); FL= fruit lenght (comprimento de fruto); FD= fruit average diameter (diâmetro médio de frutos).

confirm the presence of variability among the genotypes we used, opening the door for success during breeding and strengthening the importance of repeatability studies to determine the minimum number of plants needed to predict the real value of a given trait (Matsuo *et al*., 2012).

The magnitude of the r values for each trait obtained through different methods agreed to a large extent, raising the reliability of r estimates (Table 2). Independent of the method, r estimates were high, since r values are classified as high when $r \geq 0.6$ (Resende, 2002). Considering all methods, r values ranged from 0.6253 (plant height) to 0.9961 (number of leaves). Cruz *et al*. (2004), Carganelutti Filho & Gonçalves (2011), and Matsuo *et al*. (2012) state that when the repeatability is high, increases in the number of assessments result in little gain in accuracy if compared to a single evaluation of a single individual.

We observed that r estimates using ANOVA were always lower or equal to those obtained by other methods. On the other hand, r estimates by PCCo were always higher than the others, except for plant height. ANOVA does not isolate the periodicity factor, but drives it to the experimental error, increasing its value and consequently underestimating the repeatability. Thus, r values are more efficiently estimated by PCCo, which considers the cyclical component of the character (Abeywardena, 1972).

The estimates of determination coefficients obtained out of the 10 replications for all traits in all methods were higher than 94% (Table 2), indicating these characteristics can be assessed very consistently. Therefore, it is very likely that the number of replications might be reduced, saving resources and time.

The minimum number of measurements necessary to predict the real value of the genotypes with determination coefficients of 95% for plant height; leaf number, length, and width; and ripe fruit number, length, and average diameter are 10, 1, 1, 1, 3, 3, and 2, respectively, for any of the methods studied. For a determination coefficient of 90%, it is possible to reduce the number of records need for the same traits to 5, 1, 1, 1, 1, 1, 1 and 1, respectively. Thus, these estimates show that with five individual plants it is possible to assess these traits with a rather good determination coefficient. There were no differences among repeatability coefficients as function of the method used to estimate them. Five plants are sufficient to reliably assess the traits of ornamental pepper we studied with a determination coefficient of 90%.

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