

# Repeatability and the optimal number of measurements for screening of soybean cultivars under water deficit<sup>1</sup>

Repetibilidade e número ótimo de medições para screening de cultivares de soja sob déficit hídrico

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**ABSTRACT** - This study aimed at determining the optimal number of replications and measurements necessary for the screening of soybean cultivars subjected to three conditions of water availability during the initial stage of development. Two experiments were carried out in a greenhouse. A randomized block design was used, in a factorial scheme with two factors (cultivar and water availability), six replications, ten cultivars and three conditions of water availability (control condition, in which the sand was moistened with water; stress control in the water potential of -0.2 MPa and stress condition in the water potential of -0.4 MPa). At 20 days after cultivation, measurements were taken, subsequently estimating the repeatability coefficient, of the number of necessary measurements and of the determination coefficient. The repeatability coefficient was estimated by distinct statistical procedures, such as method of analysis of variance, principal components with basis on the matrices of phenotypic variances and covariances; principal components with basis on the matrices of correlation and structural analysis. For the determination of the number of replications, the repeatability coefficients ranged from low to high in experiment I, except for the root variables, which presented high values in both experiments. In experiment II, in turn, the magnitudes of the determination coefficients were above 90%. For plant height, number of leaves, root length and dry matter of the aerial part, only two replications would be necessary to obtain 80% confidence. Regarding the number of measurements per parcel, only one plant could be evaluated in each parcel, with 85% confidence for both experiments. Only two replications and one measurement per parcel are enough for high estimates of repeatability. This way, adequate efficiency is obtained in the selection of superior materials for tolerance to water deficit, during the initial stages of soybean development.

**Key words:** *Glycine max.* Number of repetitions. Drought stress. Polyethylene glycol. Early selection.

**RESUMO** - Este estudo objetivou determinar o número ótimo de repetições e medições necessárias para screening de cultivares de soja submetidas a três condições de disponibilidade hídrica durante o estágio inicial de desenvolvimento. Dois experimentos foram conduzidos em casa de vegetação. Utilizou-se delineamento de blocos ao acaso, em esquema fatorial com dois fatores, seis repetições, dez cultivares e três condições de disponibilidade hídrica (condição controle, na qual a areia foi umedecida em água; condição estresse no potencial hídrico de -0.2 MPa e condição estresse no potencial hídrico de -0.4 Mpa). Aos 20 dias após o plantio, procedeu-se as mensurações, sendo posteriormente estimado do coeficiente de repetibilidade, do número de medições necessárias e do coeficiente de determinação. O coeficiente de repetibilidade foi estimado por distintos procedimentos estatísticos, tais como o método da análise de variância, componentes principais baseada na matriz de variâncias e covariâncias fenotípicas, componentes principais baseada na matriz de correlação e análise estrutural. Para determinação do número de repetições, os coeficientes de repetibilidade variaram de baixo a alto no experimento I, exceto para as variáveis radiculares. Estas, apresentaram altos valores nos dois experimentos. No experimento II, por outro lado, as magnitudes dos coeficientes de determinação foram superiores a 90%. Para altura da planta, número de folhas, comprimento de raiz e matéria seca da parte aérea, seriam necessárias apenas duas repetições para obter confiabilidade de 80%. Em relação ao número de medições por parcela, apenas uma planta poderia ser avaliada em cada parcela, com confiabilidade de 85%, para os dois experimentos. Apenas duas repetições e uma medição por parcela são suficientes para altas estimativas de repetibilidade. Desse modo, têm-se adequada eficiência na seleção de materiais superiores para tolerância ao déficit hídrico, durante os estádios iniciais de desenvolvimento da soja.

**Palavras-chave:** *Glycine max.* Número de repetições. Estresse por seca. Polietilenoglicol. Seleção precoce.

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## INTRODUCTION

Soybean is one of the main cultures cultivated all over the world, and it plays an important role in agricultural production, human food safety and global trade. Even though it is a very versatile culture, such an oilseed is sensitive to water deficit (HARTMAN; WEST; HERMAN, 2011), especially in the initial stages of development, contributing to delays in the establishment and development of the crop. Water deficit causes drastic effects on the growth, development and productivity of plants (SALLAM *et al.*, 2019). According to Mohammadi *et al.* (2012), water deficit inhibits soybean growth, both in the seedling stage and in the initial stage of development (WANG; KOMATSU, 2017). According to Bolat *et al.* (2014), these stages of soybean development are the most sensitive ones to water deficit.

Soybean genetic breeding for tolerance to water deficit requires the evaluation of a great number of genetic materials and multiple morphological traits, in a limited period of time. Robust and fast phenotypic evaluation is still a great challenge and it requires a lot of resources and labor. For this purpose, breeders have statistical methodologies at their disposal, like, for instance, the estimate of the repeatability coefficient, to help them. It is a widely used parameter to quantify if the repeated measurements in a feature are the same, expressing the total variation in terms of the contribution of the genotype and of the environment (CRUZ; REGAZZI; CARNEIRO, 2012; FERREIRA *et al.*, 2010). Repeated measurements consist of phenotyping the same genotype repeatedly over time and space (RESENDE, 2015).

Repeatability expresses the maximum value that heritability can reach, since it expresses the proportion of phenotypic variance, which is assigned to the genetic differences confused with the permanent effects that act on the individual (CRUZ; CARNEIRO; REGAZZI, 2004). Hence, high values of the repeatability estimate of the character indicate that it is possible to predict the real value of the individual with a small number of measurements. This way, the knowledge of this coefficient allows the optimization of time and labor in experiments, in addition to increasing the efficiency of selection.

The repeatability coefficient can be estimated through different methods, such as, for instance, the method of analysis of variance, the method of principal components, and the method of structural analysis (GUILHEN *et al.*, 2019; RODRIGUES *et al.*, 2020). These methods have been applied in the most diverse cultures (DIEL *et al.*, 2020; FERREIRA *et al.*, 2020; PEDROZO *et al.*, 2011). In soybean, these methods were used in the evaluation of morphological descriptors (MATSUO *et al.*, 2012), evaluation of the content of oil

and protein (JIANG, 2020), associations among methods of analysis of adaptability, stability and productivity (WOYANN *et al.*, 2018), and others.

Up to now, studies using this coefficient were not carried out during the induction process of water deficit applied in soybean genotypes in the initial stage of development, aiming at the selection of those tolerant. The use of measurements of repeatability favors the best definition of the amount of plants to be measured, to obtain the real value of the individual, with reliability and optimization of the evaluation time. Therefore, this study aims at determining the optimal number of replications and measurements necessary for the screening of soybean cultivars subjected to different conditions of water availability during the initial stage of development.

## MATERIAL AND METHODS

### Data collection

Two experiments were carried out at the Universidade Federal de Viçosa, in a greenhouse. Experiment I was installed in September 2019 and experiment II in February 2020. Both experiments were carried out under a randomized block design, in a factorial scheme with 2 factors with six replications. The factors were defined by 10 cultivars and 3 conditions of water availability. Each parcel was formed by 10 plants, totaling 180 experimental units. The following commercial soybean cultivars were used: CD 2737 RR, CD 208 RR, P 98Y11, BRS 282, CD 248 RR, FT Guafra, BRS 232, M 7211 RR, Fundacep 57 RR and Conquista. The seeds were treated with carbendazim + thiram in the dosage recommended by the manufacturer (200 mL of commercial product/100 kg of seeds) and sown in trays containing 15 kg of sand, being subjected to three conditions of water availability at sowing time. The conditions of water availability were: a control condition, in which the sand was moistened with water until it reached field capacity; a stress condition at the water potential of -0.2 MPa and a stress condition at the water potential of -0.4 MPa. For the stress conditions, a polyethylenoglicol solution (PEG 6000) was prepared according to the respective water potential, as recommended by Villela, Doni Filho and Sequeira *et al.* (1991), and it was added to the sand. PEG 6000 has been widely used with the aim of simulating water deficit (DANTAS *et al.*, 2017; FLORES; PÉREZ-SÁNCHEZ; JURADO, 2017). It is an inert product whose main function is to make the water of the medium unavailable for the plants.

After sowing, the trays were properly weighed, and the initial weight of each tray was registered on the first day. Irrigation was carried out daily from 10 a.m to 4

p.m, as appropriate, according to the difference of weight of the trays. Water potential was checked at 15 days after sowing by means of a pressure pump (scholander) (SCHOLANDER *et al.*, 1965). At 20 days after sowing, the plants were evaluated with regard to germination (%). Afterwards, the aerial part was separated from the root, and the following evaluations were carried out: height (cm), number of leaves, root length in depth (cm) and dry matter of the aerial part and of the root (g). Because these variables are measured manually, they were measured in the 10 plants of each parcel. After that, the roots were evaluated with the help of the WinRHIZO software, through which the following variables were obtained: total length (cm), projected area (cm<sup>2</sup>), surface area (cm<sup>2</sup>), average diameter (mm), length/volume (cm/m<sup>3</sup>) and volume (cm<sup>3</sup>). These variables were measured in only four replications for each treatment.

### Repeatability analyses

Initially, the analysis of variance was carried out, aiming at identifying the existence of genetic variability among treatments, for both experiments. The study of repeatability was carried out only for the characters in which significant differences ( $P < 0,05$ ) were found.

For the determination of the optimal number of replications, the average of the 10 plants of each parcel was carried out, for all the variables evaluated. On the other hand, for the best optimization of resources, the determination of the number of measurements per parcel was performed only for variables height, root length in depth and number of leaves. Thus, the repeatability coefficient was estimated by distinct statistical procedures, according to Cruz, Regazzi and Carneiro (2012): method of analysis of variance, principal components with basis on the matrices of phenotypic variances and covariances; principal components with basis on the matrices of correlation and structural analysis, as described next.

### Method of Analysis of Variance (ANOVA) with two factors of variation

When evaluating  $p$  cultivars and  $\eta$  repeated measurements, the repeatability coefficient can be estimated through the intra-class correlation obtained from the analysis of variance. This model makes it possible to remove effects of temporary environment that might be confused with the variation within the genotypes, contributing to an underestimation of the repeatability coefficient (CRUZ; REGAZZI; CARNEIRO, 2012; RODRIGUES *et al.*, 2020).

Statistical model employed:

$$Y_{ijk} = \mu + g_i + a_j + ga_{ij} + b_{jk} + \varepsilon_{ijk}$$

Where:

$Y_{ijk}$  = observation regarding the  $i$ -th cultivar in the  $j$ -th

environment, in  $k$ -th repetition;

$\mu$  = general average;

$g_i$  = random effect of the  $i$ -th cultivar under the influence of the permanent environment ( $i = 1, 2, \dots, p$ );

$a_j$  = fixed effect of the temporary environment on the  $j$ -th measurement ( $j = 1, 2, \dots, \eta$ );

$ga_{ij}$ : effect of genotype x environment interaction

$b_{jk}$ : effect of  $k$ -th bloc ( $k = 1, 2, \dots, k$ ) within of  $j$ -th environment, and

$\varepsilon_{ij}$  = experimental error established by the temporary effects of the environment on the  $j$ -th measurement of the  $i$ -th cultivar.

The repeatability coefficient is given according to equation 1:

$$r = \frac{\hat{\sigma}^2_g}{\hat{\sigma}^2 + \hat{\sigma}^2_g} \quad (1)$$

Where:

$\hat{\sigma}^2$  : variance estimator

$\hat{\sigma}^2_g$  : genotypic variance estimator

### Methods of principal components

This method is proposed by Abeywardena, 1972 and widely used by several authors (GUILHEN *et al.*, 2019, MARÇAL *et al.*, 2016; SILVA *et al.*, 2018). The estimates of the repeatability coefficients were obtained with basis on the matrix of correlations among cultivars in each pair of measurements through which the eigenvalues and the normalized eigenvectors are determined. The eigenvector, whose elements present the same sign and close magnitudes, is the one that expresses the tendency of cultivars to keep their relative positions in the several time intervals. The proportion of the eigenvalue, associated with the eigenvector, and the estimator of the repeatability coefficient, are given according to what is shown in equation 2:

$$r = \frac{\lambda_k}{\sum_j \lambda_j} \quad (2)$$

Where:

$\lambda_k$  = eigenvalue associated with the eigenvector, whose elements have the same sign and similar magnitude;

The repeatability coefficient can be estimated alternatively, through the technique of principal components applied in the matrix of phenotypic variances and covariances. In this case, the estimator of the repeatability coefficient is given according to equation 3:

$$r = \hat{\rho} = \frac{\lambda_1 - \hat{\sigma}_y^2}{\hat{\sigma}_y^2 (n - 1)} \quad (3)$$

Where:

$\lambda_1$  = the eigenvalue of  $\hat{\Gamma}$  associated with the eigenvector whose elements have the same sign and similar magnitude.

**Method of structural analysis**

This method was proposed by Mansour, Nordheim and Rutledge (1981). Several studies used this method to estimate the repeatability coefficient (RODRIGUES *et al.*, 2020; SILVA *et al.*, 2018) and presents some conceptual differences in relation to the method of principal components. In this method, R is considered the parametric matrix of correlations among the genotypes in each pair of evaluation and  $\hat{R}$  is its estimator. An estimator of the repeatability coefficient based on the structural analysis is given according to equation 4:

$$r = \frac{\alpha' \hat{R} \alpha - 1}{n - 1} \tag{4}$$

Where  $\alpha'$  is the eigenvector with parametric elements associated with the greatest R vector.

Similarly, the repeatability coefficient can be obtained by using the parametric eigenvector ( $\alpha$ ) and the matrix of  $\hat{\Gamma}$  covariance, and it is given according to equation 5:

$$r = \frac{\alpha \hat{\Gamma} \alpha - \sigma^2_y}{\sigma^2_y(n - 1)} \tag{5}$$

After the repeatability coefficient (r) was estimated, the estimate of the number of measurements ( $\eta_0$ ) necessary to predict the real value of the individuals with the desired

value of genotypic determination ( $R_2$ ) was obtained according to equation 6:

$$n_0 = \frac{R^2(1 - R)}{(1 - R^2)r} \tag{6}$$

The coefficient of genotypic determination ( $R^2$ ), which represents the percentage of certainty of the prediction of the real value of the selected individuals based on  $\eta$  measurements was obtained according to equation 7:

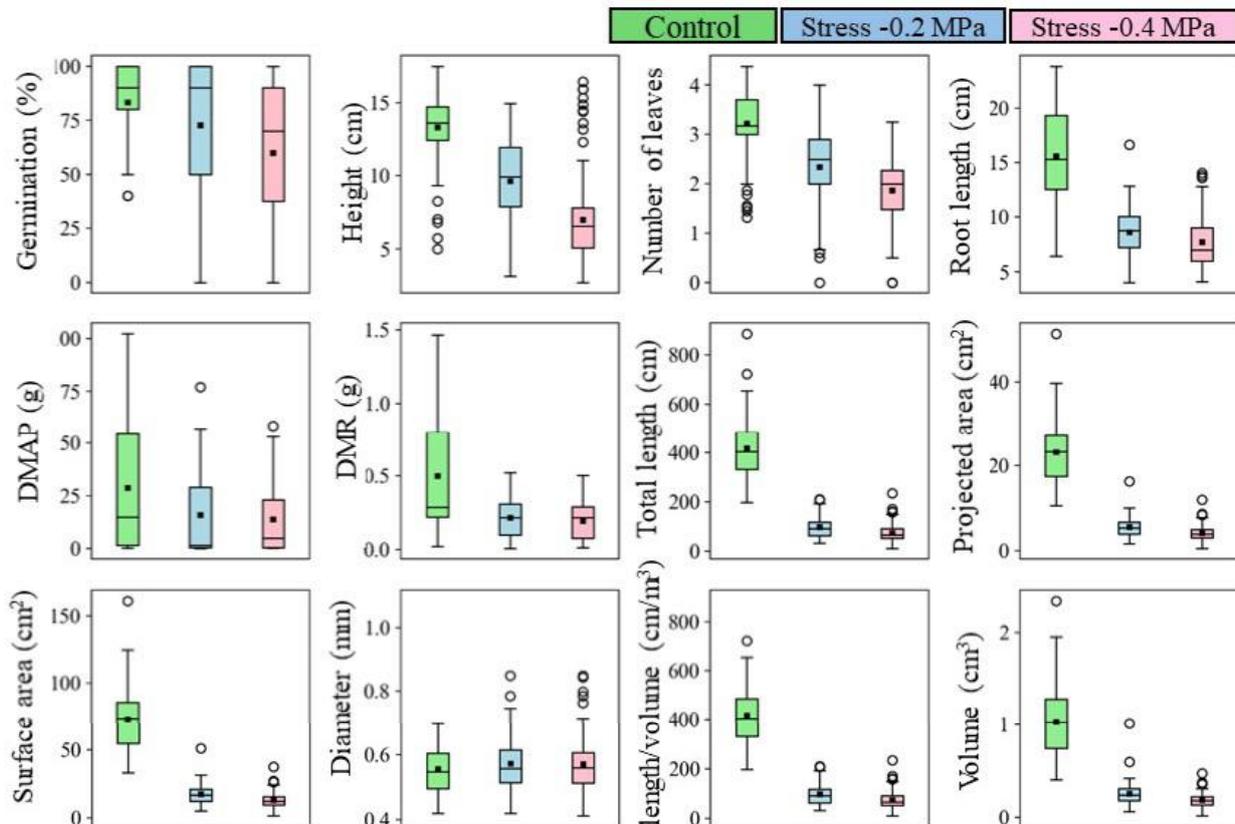
$$R^2 = \frac{nP}{1 + P(n - 1)} \tag{7}$$

The estimates of the repeatability coefficient, number of necessary measurements and determination coefficient were obtained by means of the Genes program (CRUZ, 2013).

**RESULTS AND DISCUSSION**

Significant differences were noticed, indicating the presence of genetic variability among all treatments, for all the variables analyzed in both experiments (Table 1). The behavior of the variables used in this study indicates the efficiency of the methodology of imposition of water deficit by using PEG 6000, and confirms the existence of genetic variability among treatments (Figure 1).

**Figure 1** - Boxplots of the variables used in the study. DMAP: dry matter of the aerial part; DMR: dry matter and of the root



**Determination of the number of replications**

*Estimates of the repeatability coefficients and determination*

The analysis of variance demonstrated significant differences among the cultivars studied, pointing out that the component of genetic variance mixed with the permanent effects of the environments was significant. This assumes great relevance in breeding programs, aiming at the optimization of the number of measurements. The estimates of the coefficients of repeatability and of determination associated with the minimum number of replications for the evaluations carried out are shown in Table 1.

The repeatability coefficients ranged from low to high in experiment I. Resende (2002) classifies this coefficient as low when  $r \leq 0,30$ , medium when  $0,30 < r < 0,60$  and high when  $r \geq 0,60$ . The highest estimates were obtained through the method of principal components based on the matrices of phenotypic variances and covariances. The highest values were observed for variable height, being 0.73 and 94 for the repeatability coefficients and determination, respectively. On the other hand, the lowest value was verified in variable germination (0.17) through the ANOVA methods, whose  $R^2$  was 55.69.

Generally speaking, this method presented the lowest estimates, if compared to the other ones. Low values in the estimates of the repeatability coefficients, below 0.4, result in difficulties for the identification of the best genotypic values from the analysis of the phenotypic averages (FERREIRA *et al.*, 1999).

Contrary to what was observed in experiment I, the estimates of the repeatability coefficients were high in experiment II. For all the methods, the magnitudes of the determination coefficients were higher than 90%, indicating greater precision in the prediction of the real value of the variables analyzed. Only variable number of leaves presented medium repeatability coefficients, being 0.56, 0.58, 0.58, 0.56 for the ANOVA, PCcor, AEcor and AEcov methods, respectively. Again, the method of principal components stood out in relation to the other methods, presenting the highest repeatability coefficients for all the variables.

These results reinforce the importance of analyses of repeatability in soybean genetic breeding for tolerance to water deficit. The repeatability coefficients are influenced by the nature of the character, with the genetic properties of the population and with the conditions in which the individuals are developed (CRUZ; CARNEIRO;

**Table 1** - Estimates of the coefficients of repeatability (r) and of determination (R<sup>2</sup>) for experiment I and experiment II

| Variable     | Experiment I  |                |       |                |       |                |       |                |       |                |
|--------------|---------------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|
|              | ANOVA         |                | PCcov |                | PCcor |                | EAcor |                | EAcov |                |
|              | r             | R <sup>2</sup> | r     | R <sup>2</sup> | r     | R <sup>2</sup> | r     | R <sup>2</sup> | r     | R <sup>2</sup> |
| Germination  | 0.17          | 55.69          | 0.39  | 79.52          | 0.30  | 71.88          | 0.19  | 58.17          | 0.17  | 55.85          |
| Height       | 0.42          | 81.12          | 0.73  | 94.17          | 0.74  | 94.47          | 0.43  | 82.13          | 0.42  | 81.20          |
| N° of leaves | 0.30          | 71.54          | 0.66  | 92.18          | 0.70  | 93.20          | 0.37  | 77.56          | 0.30  | 71.71          |
| DMAP         | 0.44          | 82.58          | 0.60  | 90.01          | 0.50  | 85.58          | 0.43  | 82.18          | 0.47  | 84.13          |
| Root length  | 0.50          | 85.85          | 0.72  | 93.90          | 0.65  | 91.62          | 0.46  | 83.90          | 0.52  | 86.67          |
| DMR          | 0.18          | 57.47          | 0.28  | 69.83          | 0.22  | 63.34          | 0.20  | 60.34          | 0.20  | 60.18          |
| Variable     | Experiment II |                |       |                |       |                |       |                |       |                |
|              | ANOVA         |                | PCcov |                | PCcor |                | EAcor |                | EAcov |                |
|              | r             | R <sup>2</sup> | r     | R <sup>2</sup> | r     | R <sup>2</sup> | r     | R <sup>2</sup> | r     | R <sup>2</sup> |
| Germination  | 0.64          | 91.50          | 0.66  | 92.07          | 0.65  | 91.69          | 0.64  | 91.59          | 0.64  | 91.50          |
| Height       | 0.72          | 93.83          | 0.75  | 94.64          | 0.73  | 94.29          | 0.73  | 94.26          | 0.72  | 93.83          |
| N° of leaves | 0.56          | 88.52          | 0.61  | 90.21          | 0.58  | 89.27          | 0.58  | 89.22          | 0.56  | 88.52          |
| DMAP         | 0.73          | 94.18          | 0.73  | 94.28          | 0.74  | 94.44          | 0.74  | 94.41          | 0.73  | 94.18          |
| Root length  | 0.80          | 96.10          | 0.83  | 96.65          | 0.81  | 96.27          | 0.81  | 96.22          | 0.80  | 96.10          |
| DMR          | 0.76          | 94.90          | 0.76  | 95.11          | 0.78  | 95.39          | 0.77  | 95.35          | 0.76  | 94.90          |

DMAP: dry matter of the aerial part; DMR: dry matter and of the root. PCcov: principal components based on matrices of phenotypic variances and covariance; PCcor: principal components based on the correlation matrices; EAcor: structural analysis based on intraclass correlation matrices; and EAcov: structural analysis based on the variance and covariance matrices

REGAZZI, 2004). This effect is observed between the two experiments since, because they were carried out in distinct seasons (winter and summer), they suffered different environmental influences. Moreover, when there are low or medium values of repeatability, there is greater variability in a certain trait, which can be caused by climate variations (MARTUSCELLO *et al.*, 2015).

With regard to the root variables measured by the Winrhizo, the repeatability coefficients were high ( $> 0.60$ ) for all the variables, except for variable diameter, in all methods used, in both experiments (Table 2). Variable diameter presented low repeatability coefficients. The lowest value observed in experiment II was for the ANOVA method and the structural analysis, whose estimates of repeatability for both methods was 0.05 and associated  $R^2$  of 16%. The diameter of the roots assumes great importance in the root architecture of soybean cultivars tolerant to water deficit (OYA *et al.*, 2004). However, the number of replications used in this study does not seem to have been enough for a better estimate of the repeatability coefficient, when making use of the Winrhizo software to carry out the measurement.

#### Determination of the real value of the variables

Based on the repeatability coefficient, the number of measurements necessary for the desired precision can be estimated. Thus, the lower the repeatability coefficient is, the higher the number of measurements necessary for high precision, and vice-versa (CRUZ; REGAZZI; CARNEIRO, 2012). To predict the real value of the variables, with certain levels of confidence ( $R^2$  with values 0.80, 0.85, 0.90, 0.95 or 0.99), in general, the method of principal components presented the lowest minimum values of replications necessary in experiment I (Figure 2).

For plant height, number of leaves, root length and dry matter of the aerial part, only two replications would be necessary to obtain 80% confidence, through the method of principal components with basis on the matrices of phenotypic variances and covariances. For these same variables, the more the level of reliability is increased, the higher is the number of replications, too. The other methods evaluated required an optimal number of replications greater than four in all the variables.

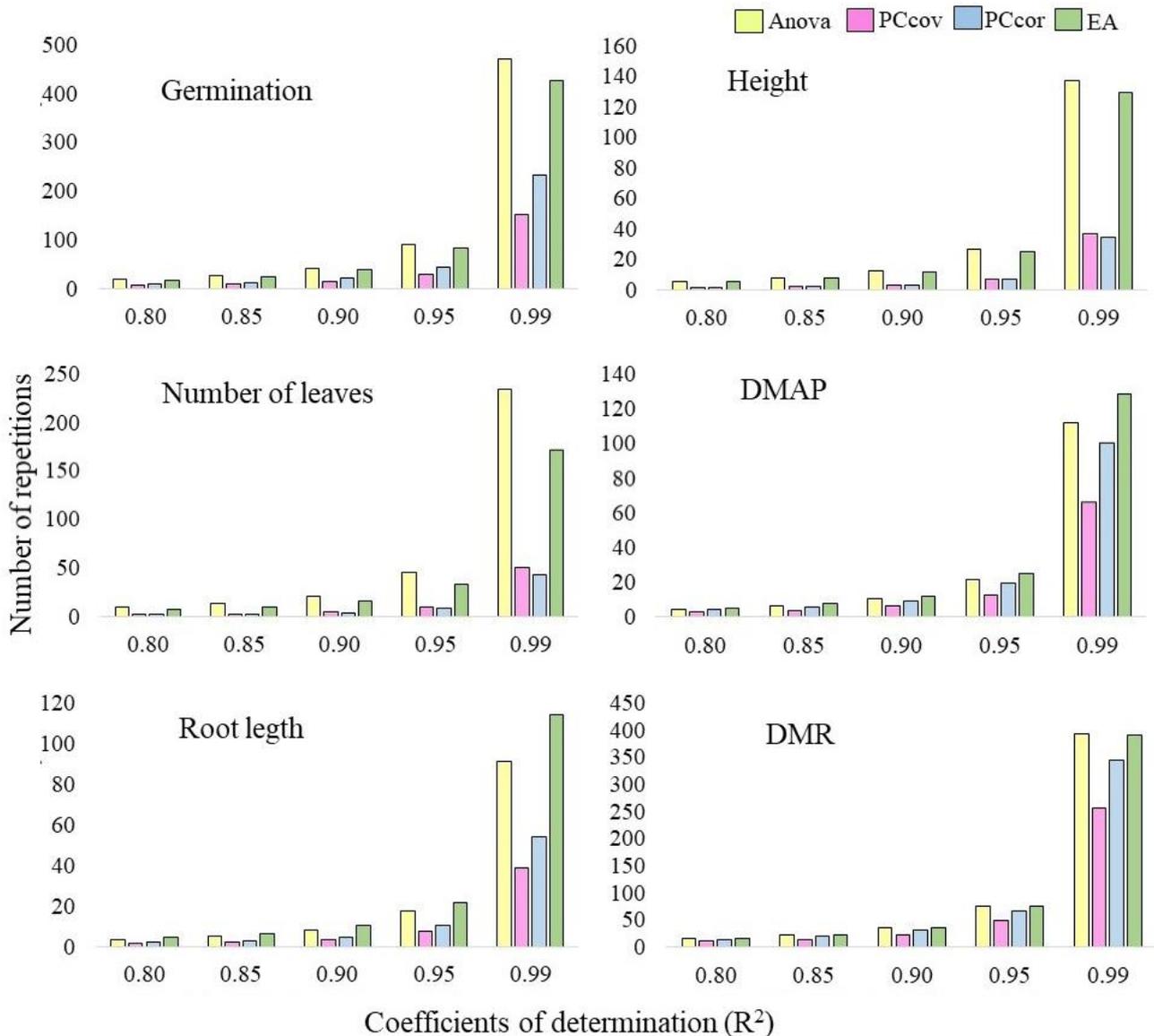
Similar to what was observed in experiment I, in experiment II, as a whole, the method of principal components presented the smallest minimum values of

**Table 2** - Estimates of the coefficients of repeatability ( $r$ ) and of determination ( $R^2$ ) for the variables obtained using the Winrhizo software in the experiment I and experiment II

| Variable         | Experiment I  |       |       |       |       |       |       |       |       |       |
|------------------|---------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                  | ANOVA         |       | PCcov |       | PCcor |       | EAcor |       | EAcov |       |
|                  | $r$           | $R^2$ | $r$   | $R^2$ | $r$   | $R^2$ | $r$   | $R^2$ | $r$   | $R^2$ |
| Total length     | 0.87          | 96.3  | 0.87  | 96.3  | 0.87  | 96.3  | 0.87  | 96.3  | 0.87  | 96.28 |
| Projected area   | 0.89          | 96.9  | 0.89  | 96.9  | 0.89  | 96.89 | 0.89  | 96.88 | 0.89  | 96.86 |
| Surface area     | 0.89          | 96.9  | 0.89  | 96.9  | 0.89  | 96.89 | 0.89  | 96.88 | 0.89  | 96.86 |
| Average diameter | 0.27          | 59.9  | 0.35  | 68.3  | 0.29  | 61.56 | 0.27  | 59.13 | 0.27  | 59.87 |
| Length/volume    | 0.87          | 96.3  | 0.87  | 96.3  | 0.87  | 96.3  | 0.87  | 96.3  | 0.87  | 96.28 |
| Volume           | 0.87          | 96.3  | 0.87  | 96.5  | 0.87  | 96.42 | 0.87  | 96.41 | 0.87  | 96.34 |
| Variable         | Experiment II |       |       |       |       |       |       |       |       |       |
|                  | ANOVA         |       | PCcov |       | PCcor |       | EAcor |       | EAcov |       |
|                  | $r$           | $R^2$ | $r$   | $R^2$ | $r$   | $R^2$ | $r$   | $R^2$ | $r$   | $R^2$ |
| Total length     | 0.8           | 94    | 0.81  | 94.4  | 0.81  | 94.3  | 0.8   | 94.26 | 0.8   | 93.96 |
| Projected area   | 0.78          | 93.5  | 0.8   | 94.2  | 0.8   | 94    | 0.79  | 93.89 | 0.78  | 93.53 |
| Surface area     | 0.78          | 93.5  | 0.8   | 94.2  | 0.8   | 94    | 0.79  | 93.89 | 0.78  | 93.53 |
| Average diameter | 0.05          | 16.2  | 0.3   | 63    | 0.19  | 49.11 | 0.1   | 30.68 | 0.05  | 16.22 |
| Length/volume    | 0.8           | 94    | 0.81  | 94.4  | 0.81  | 94.3  | 0.8   | s     | 0.8   | 93.96 |
| Volume           | 0.71          | 90.9  | 0.74  | 91.9  | 0.72  | 91.09 | 0.71  | 90.91 | 0.71  | 90.91 |

PCcov: principal components based on matrices of phenotypic variances and covariance; PCcor: principal components based on the correlation matrices; EAcor: structural analysis based on intraclass correlation matrices; and EAcov: structural analysis based on the variance and covariance matrices

**Figure 2** - Number of repetitions required associated with different coefficients of determination of the experiment I

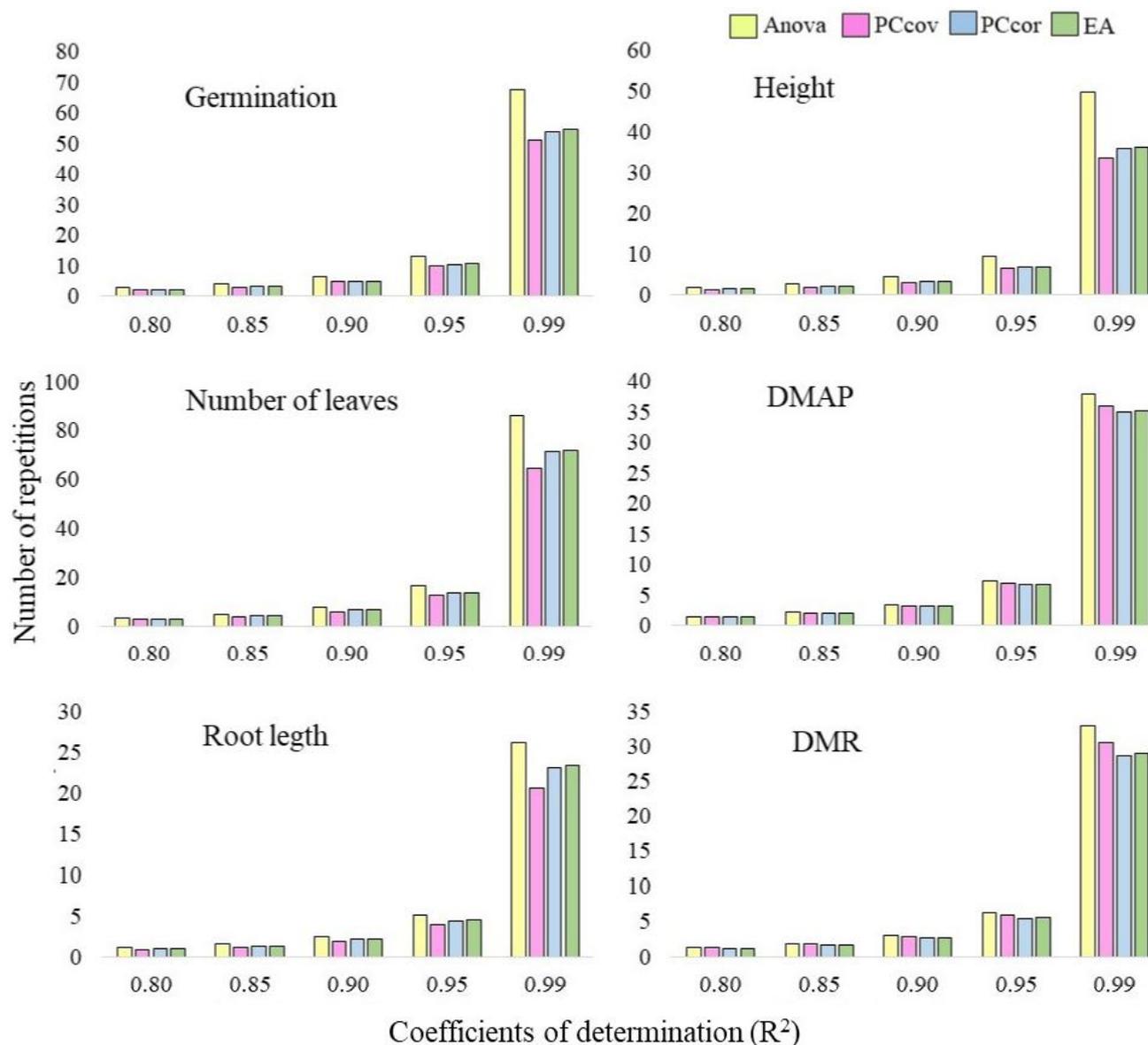


necessary replications (Figure 3). On the other hand, the ANOVA method presented the highest values for all the variables. Through this method, for 80% confidence, 3, 2, 3, 1, 1 replications would be necessary for variables germination, height, number of leaves, root length, dry matter of the aerial part, and dry matter of the root, respectively. However, for 90% confidence, 6, 4, 7, 2, 3, 3 replications would be necessary for the same variables through the ANOVA method. The method of structural analysis was similar to the method of principal components.

It was observed that, to predict the real value of the variables measured by the winrhizo, with certain

levels of reliability, the variables presented a similar behavior in both experiments. (Figure 4 and Figure 5).

On the whole, both in experiment I (figure 4) and in experiment II (figure 5), for the root variables measured by the Winrhizo software, only one replication would be enough to estimate the real value of the variable with 80% confidence for all the variables used in this study, except for the diameter. Nonetheless, two and four replications would be necessary for accuracy of 90% and 95%, respectively. Concerning the methods employed, they presented similar results for all the variables. Only the ANOVA method presented slightly superior results if compared to the other methods in experiment II, for 99% confidence.

**Figure 3** - Number of repetitions required associated with different coefficients of determination in the experiment II

### Determination of the number of measurements per parcel

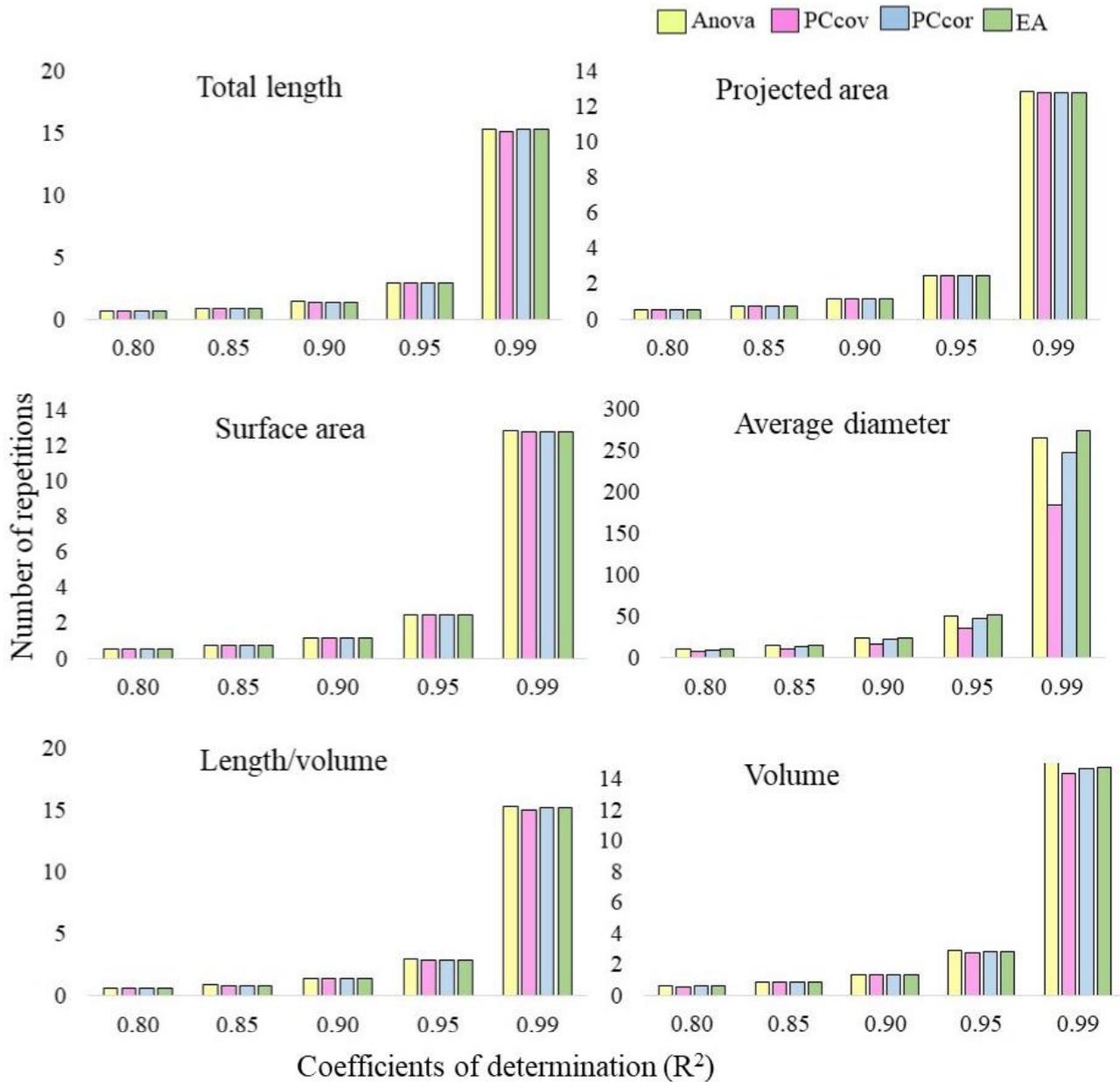
#### *Estimates of the coefficients of repeatability and determination, and of the real value of the variables*

The estimates of the coefficients of repeatability and determination evaluated in the 10 plants per parcel are shown in Table 3, regarding all the treatments of experiment I and of experiment II. High repeatability coefficients were observed for variables height, number of leaves and root length, for both experiment I and experiment II. In experiment I, all the methods presented the same estimates for all the variables. Regardless of the method employed,

in both experiments the repeatability coefficients were higher than 0.90, with  $R^2$  of 99%. This result suggests that in studies like these, a smaller number of replications within the parcel is enough for appropriate precision in the selection of soybean cultivars tolerant to water deficit.

As for the prediction of the real value of the variables, the results indicate that only one plant could be evaluated in each parcel, with 85% confidence (Figure 6). Two measurements per parcel would guarantee 95% confidence. In the determination of the number of measurements, there were no differences in any of the experiments. The evaluated methods presented very similar behaviors. Only at the level of 99% confidence the

**Figure 4** - Number of repetitions required associated with different coefficients of determination, for variables measured by the Winrhizo software, in the experiment I

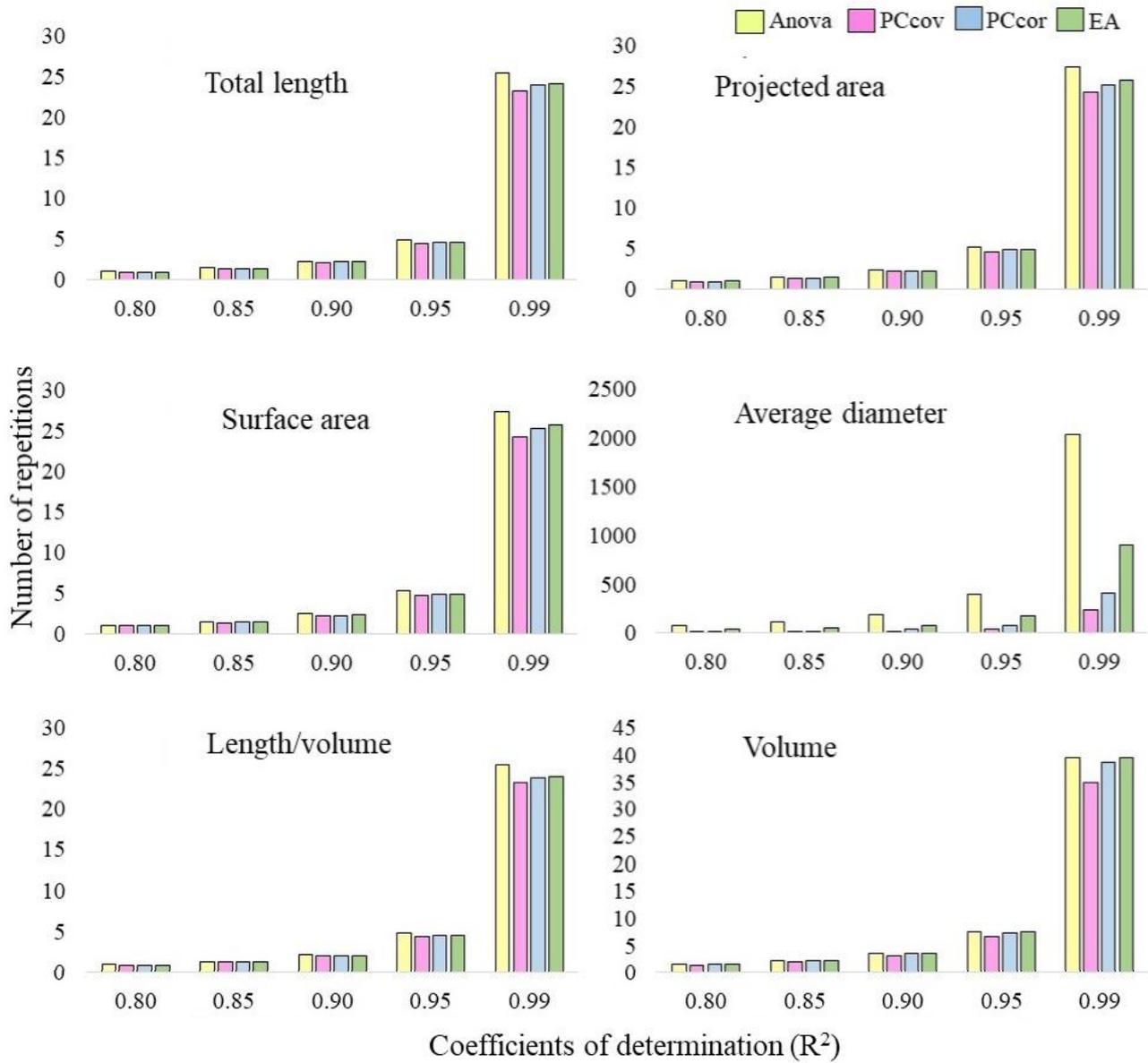


ANOVA method presented slightly superior results for some variables. The high estimates of the repeatability coefficients (Table 3) and the requirement of only one measurement per parcel (Figure 6) suggest regularity in the performance of the cultivars in both experiments. This way, indeed, few measurements are enough to evaluate the relative superiority of one cultivar over another (CRUZ; REGAZZI; CARNEIRO, 2012).

In the light of the above, it is highlighted that the knowledge of the repeatability coefficients of such

variables assumes great importance in soybean breeding programs for tolerance to water deficit. One of the greatest contributions of the use of this coefficient is the reduction of cost, time and labor to carry out experiments. Hence, breeders can proceed with the reduction of the number of replications, as well as the number of measurements per parcel; this way, they can concentrate efforts on the evaluation of a higher number of cultivars. In addition, this coefficient allows the accurate selection of cultivars that present tolerance potential to this kind of stress.

**Figure 5** - Number of repetitions required associated with different coefficients of determination, for variables measured by the Winrhizo software, in the experiment II



**Table 3** - Estimates of the coefficients of repeatability (r) and of determination ( $R^2$ ), within the parcel for experiment I and experiment II

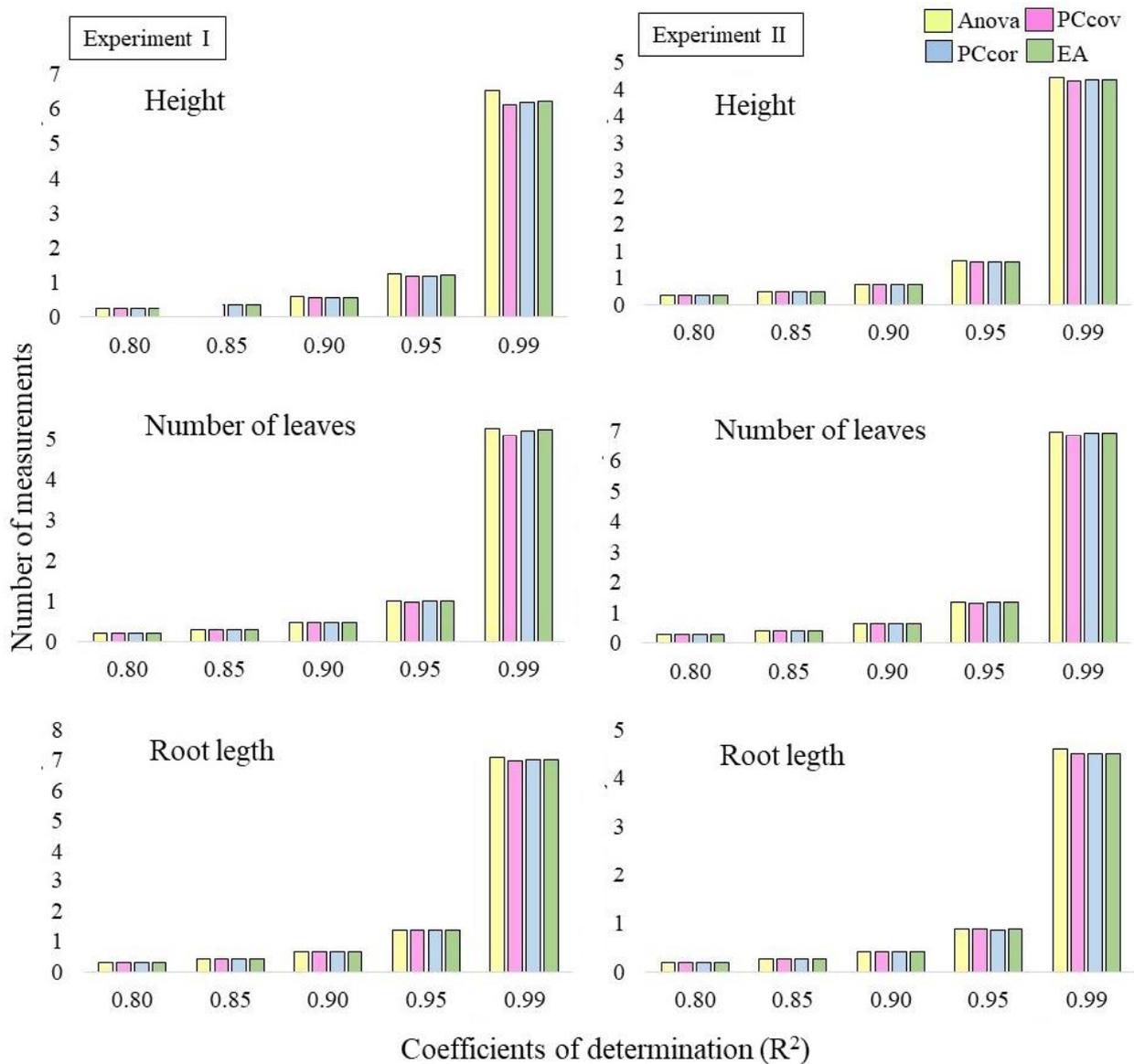
| Variable     | Experiment I |       |       |       |       |       |       |       |       |       |
|--------------|--------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|              | ANOVA        |       | PCcov |       | PCcor |       | EAcor |       | EAcov |       |
|              | r            | $R^2$ | r     | $R^2$ | r     | $R^2$ | r     | $R^2$ | r     | $R^2$ |
| Height       | 0.94         | 99.18 | 0.94  | 99.24 | 0.94  | 99.23 | 0.94  | 99.22 | 0.94  | 99.18 |
| N° of leaves | 0.95         | 99.34 | 0.95  | 99.36 | 0.95  | 99.35 | 0.95  | 99.34 | 0.95  | 99.34 |
| Root length  | 0.93         | 99.11 | 0.93  | 99.13 | 0.93  | 99.12 | 0.93  | 99.12 | 0.93  | 99.11 |

Continuation Table 3

| Experiment II |       |                |       |                |       |                |       |                |       |                |
|---------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|
| Variable      | ANOVA |                | PCcov |                | PCcor |                | EAcor |                | EAcov |                |
|               | r     | R <sup>2</sup> |
| Height        | 0.96  | 99.47          | 0.96  | 99.48          | 0.96  | 99.48          | 0.96  | 99.48          | 0.96  | 99.47          |
| N° of leaves  | 0.93  | 99.13          | 0.94  | 99.14          | 0.93  | 99.14          | 0.93  | 99.14          | 0.93  | 99.13          |
| Root length   | 0.96  | 99.42          | 0.96  | 99.43          | 0.96  | 99.44          | 0.96  | 99.43          | 0.96  | 99.42          |

PCcov: principal components based on matrices of phenotypic variances and covariance; PCcor: principal components based on the correlation matrices; EAcor: structural analysis based on intraclass correlation matrices; and EAcov: structural analysis based on the variance and covariance matrices

Figure 6 - Number of measurements required associated with different determination coefficients within the parcel for the experiment I and the experiment II



## CONCLUSIONS

The use of repeatability coefficients suggests the performance of a reduced number of replications and measurements per parcel, for the traits evaluated in this study. Only two replications and one measurement per parcel are enough for high estimates of repeatability. This way, adequate efficiency is obtained in the selection of superior materials for tolerance to water deficit, during the initial stages of soybean development.

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