academicJournals

Vol. 12(30), pp. 2442-2451, 27 July, 2017
DOI: 10.5897/AJAR2017.12372
Article Number: 129F46F65338
ISSN 1991-637X

http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Repeatability analysis on morphological descriptors in the early stages of development

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Received 11 April, 2017; Accepted 20 June, 2017

The insufficiency of morphological descriptors reveals the importance of studies related to this topic for the genetic improvement of soybean, with attention to the possible descriptors measured in early stages of plant development which allows obtaining fast results. The aim of this work was to estimate the coefficient of repeatability of some morphological descriptors in the early stages of development of soybean and the minimum number of evaluations necessary to predict the real value of genotypes. Five (5) experiments were carried out with 124, 93, 90, 16 and 16 genotypes of soybean for the first, second, third, fourth, and fifth experiment, respectively, with characteristics combined at the stage V3. It was concluded that the length of the first internode requires fewer measurements compared to other measured characteristics. With six measurements, it was possible to obtain 95% and 90% reliability for plant height in V3 and first internode length, respectively, by the methods ANOVA, CP (correl), CP(cov) and AE(correl). With seven measurements, it was obtained 90% reliability for the epicotyl length for all methods used; 85% for the petiole length of unifoliate leaf by method of CP(cov) and 90% by methods ANOVA, CP(correl), AE(correl). With 15 measurements, it was possible to obtain 90% reliability for the hypocotyl length, petiole length of trifoliate leaf and the angle formed by the insertion of the petioles of the unifoliate leaf for all methods used; and, for the rachis length of the first trifoliate leaf, 37 measurements would be necessary for reliability of 90% by methods of ANOVA, CP(correl), CP(cov) and AE(correl).

Key words: *Glycine max* (L.), soybean breeding, number of evaluation, stability.

INTRODUCTION

One of the major components of the global financial market are the agricultural Commodities, within the soybean (*Glycine max* (L.) Merrill) stands out, in relation to the volume sold. Brazil is the world's second largest producer of soybeans with 102,110 million tons produced in 2015/2016, in a total planted area of 33,228 million hectares, and presents the greater growth in planted area in the Brazilian agribusiness (Embrapa, 2016).

The genetic improvement programs of the oleaginous have been intensively acted in the development of new cultivars in Brazil, mainly after 1,997, when it was sanctioned the Law of Protection of Plant Varieties (LPC)

n°. 9,456, April 25, 1,997, regulated by Decree n°. 2,366, November 5, 1,997 (Neto et al., 2005). For a cultivar to be protected, it is necessary to prove that it is distinct, uniform and stable. The distinctiveness of a cultivar refers to a clear difference from any other variety whose existence on the date of the period of protection is recognized (Grilli, 2005).

The differentiation of cultivars is performed by a minimum margin of descriptors that are specific to each species (Neto et al., 2005). Currently, about 38 descriptors are used between the mandatory and the additional to differentiate genotypes of soybean, nevertheless, they are still insufficient to distinguish cultivars (Nogueira et al., 2008). Therefore, there is the need of expanding that list.

The identification of morphological descriptors evaluated in the early stages of plant development should be preferred, once it enables to obtain fast results and it is not necessary to wait for adult plants, thereby accelerating the work of the breeder.

Nevertheless, in literature, there is few detailed information about the amount of plants which should be measured to determine the number of evaluations needed in order to estimate the difference between the evaluated materials, in order for the selected genotype maintain its characteristic in future generations. According to Cruz et al. (2004) and Paula Ferreira et al. (2010), this expectation may be proved by the repeatability coefficient of the studied characteristic, and being possible to estimate when the measurement of the character is performed repeatedly in a particular individual.

The concept of repeatability can be stated as the correlation between measurements of a given character in the same individual, whose assessments were repeated in time or in space. It expresses the proportion of the total variance that is explained by the variations provided by genotype and by permanent changes attributed to the common environment (Cruz et al., 2004). Many authors such as da Silva et al. (2014), Lessa et al. (2014), Lira et al. (2009), Ribeiro et al. (2015) have been studying repeatability and morphological descriptors for breeding and preservation of cultivars for different crops, showing that the study has impact and importance worldwide.

In the tests involving regularly evaluated genotypes, it is possible to estimate the repeatability coefficients of the variables studied, that is, the probability that this result will be repeated in future evaluations. Also, it is possible to estimate the number of phenotypic observations required, for a certain character, which must be performed on each individual so that discrimination (or selection) between the genotypes is carried out with a certain degree of reliability and time and labor economy (Cruz and Regazzi, 1997).

There are several methods used to estimate the repeatability, as the variance analysis, principal components and structural analysis (Abeywardena, 1972; Cruz and Regazzi, 2001; Mansour et al., 1981).

The objective of this study was to estimate the repeatability coefficient of some morphological descriptors in the early stages of development of soybean and the minimum number of evaluations necessary to predict the real value of genotypes.

MATERIALS AND METHODS

The experiments were conducted and evaluated in a greenhouse at the city of Viçosa, Minas Gerais – Brazil (20°45'14" S; 42°52'54" W; altitude of 408 m).

Five experiments were conducted in a completely randomized design, in which each experimental unit consisted of a plant, and the experiments one, two and three were conducted with five replicates for each treatment and the experiments four and five were conducted with sixteen repetitions. All experiments were grown in pots containing 3 dm^3 of soil with 1/3 of organic matter and seeding depth standardized at 3 cm. After germination, the plants were conducted according to culture recommendations.

In Experiment 1, from September to October 2011, 124 genotypes (UV10-01, UV10-02, until UV10-122, Bossier and BRS Valiosa RR) were evaluated. In Experiment 2, from November to December 2011, 93 soybean genotypes (UV1-001, UV1-002, until UV1-090, BRS Valiosa RR, Bossier and MG/BR-46) were evaluated. In Experiment 3, from February to March 2012, 90 genotypes (UV100B01, UV100B02 up to UV88, Bossier and MG/BR-46) we evaluated. In Experiment 4, from December 2011 to April 2012, 16 genotypes (BRS Maxixe, BRS Candle, BRS 278 RR, BRS 271 RR, BRS Tracajá, UFV TN 105 AP, MGT 401 RR, BRS MG 68, MGT 801, FMT Tucunaré, MGT 123 RR, MGT 127 RR, TMG 1176 RR, TMG 7188 RR, Msoy 7211 RR, Msoy 7908 RR) we evaluated. And, in Experiment 5, between June and November 2012, 16 genotypes (BRS Maxixe, BRS Candle, BRS 278 RR, BRS 271 RR, BRS Tracajá, UFVTN 105 AP, MGT 401 RR, BRS MG 68, MGT 801, FMT Tucunaré, MGT 123 RR, MGT 127 RR, TMG 1176 RR, TMG 7188 RR, Msoy 7211 RR, Msoy 7908 RR) were also evaluated.

In all experiments, two trefoil completely developed, the variables hypocotyl length (CH), plant height (ALV3), epicotyl length (CE), the first internode (CPIN), the petiole of the first trifoliate leaf (CPFT), the petiole of the unifoliate leaf (CPFU) and the rachis of the first trifoliate leaf (CRFT), were measured at V3. The evaluations were performed using a digital pachymeter. In Experiments 2, 3, 4 and 5 the angle formed by the insertion of the petioles of the unifoliate leaf (AIFU), was also measured, with a protractor.

Initially, the variance analysis was performed, in order to identify the existence of genetic variability between genotypes, based on the characters analyzed in each experiment. Only for the characters with significant differences between the genotypes ($p < 0.05$), the repeatability study was conducted. The repeatability coefficients (r) were estimated by the variance analysis (ANOVA); principal

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Table 1. Variance analysis of morphological descriptors for soybeans: CH, ALV3, EC, CPIN, CPFT, CPFU, CRFT and AIFU.

V. S.	G.L	CH	ALV3	CE	CPIN	CPFU	CPFT	CRFC	AIFU
		----------Experiment 1 (124 genotypes and 5 repetitions)----------							
Genotypes	123	2.01*	147.77*	8.49*	9.74*	0.95*	10.34*	0.71*	-
Residue	496	0.34	8.35	0.76	0.69	0.13	1.12	0.09	-
Overall average		2.89	29.54	6.49	6.08	2.16	10.12	0.81	-
CV%		20.08	9.78	13.47	13.66	16.65	10.45	37.21	-
		----------Experiment 2 (93 genotypes and 5 repetitions)----------							
Genotypes	92	1.43*	47.63*	8.35*	8.63*	1.57*	17.70*	0.51*	541.60*
Residue	372	0.36	4.46	0.84	0.54	0.16	1.15	0.15	98.14
Overall average		3.59	18.54	8.06	5.04	2.28	10.44	0.85	62.35
CV%		16.80	11.38	11.39	14.60	17.68	10.27	45.94	15.89
		----------Experiment 3 (90 genotypes and 5 repetitions)----------							
Genotypes	89	2.60*	101.51*	2.86*	4.97*	0.64*	10.02*	0.14*	454.80*
Residue	360	0.39	5.10	0.39	0.34	0.10	0.68	0.06	97.49
Overall average		3.59	18.54	8.06	5.04	2.28	10.44	0.85	62.35
CV%		14.95	8.51	9.61	12.29	19.56	11.12	43.78	15.32
		----------Experiment 4 (16 genotypes and 16 repetitions)----------							
Genotypes	15	6.65*	505.98*	23.39*	24.38*	2.07*	8.87*	0.45*	2025.88*
Residue	240	0.22	13.96	0.48	0.54	0.08	0.84	0.06	110.73
Overall average		3.39	24.69	6.19	4.46	1.40	7.91	0.78	63.59
CV%		13.61	15.12	11.13	16.48	19.65	11.57	31.87	16.51
		----------Experiment 5 (16 genotypes and 16 repetitions)----------							
Genotypes	15	6.26*	248.75*	23.79*	9.83*	3.30*	11.89*	0.55*	1476.42*
Residue	240	0.18	3.91	0.40	0.20	0.04	0.36	0.03	83.75
Overall average		3.45	17.52	4.91	2.94	1.52	6.21	0.72	54.89
CV%		12.20	11.28	12.92	15.10	13.55	9.67	24.32	16.67

* Significant at 5% probability by F-test.

components based on matrices of correlation [CP(correl)] and of variances and covariance phenotypic characteristics [CP(cov)]; and structural analysis, based on the correlation coefficient [AE(correl)] and of variances and covariance [AE(cov)]. The minimum number of measurements necessary to predict the real value of individuals, on the basis of the determination coefficients (R^2) pre-established (0.80, 0.85, 0.90, 0.95 and 0.99), was obtained according to the methodology described by Cruz et al. (2004). Statistical analyzes were performed in the Program Genes: Biometria (Cruz, 2013).

RESULTS AND DISCUSSION

All the characters studied had significant genotype effect ($p < 0.05$) in the experiments in which they were evaluated, indicating that the soybean genotypes differ among themselves, which reinforces the importance of repeatability studies to determine the minimum number of plants to be measured to predict their real value (Table 1). Nogueira et al. (2008) and Matsuo et al. (2012) also identified variability in CH, EC, and CPFT CRFT among genotypes, in four different periods of seeding. Furthermore, Nogueira et al. (2008) reported large genetic influence for most of the characteristics in different periods, showing little environmental effect.

The repeatability coefficients for the CH, ranged from 0.369 to 0.705. The lowest value was obtained by ANOVA in experiment 2 and the largest one by CP(correl) in Experiment 5. For the ALV3, the lowest value (0.659) was obtained in experiment 2 by the methods of the AE(correl) and AE(cov) and the highest (0.823) in Experiment 5 by the method CP(cov). While for the EC the same coefficient was lower in experiment one (0.724) by the method of ANOVA and higher in experiment 5 (0.823) by the method of CP(cov). The magnitude of the determination coefficients for the CH and CE was greater than or equal to 75.20% by all methods in the five experiments evaluated, and lower than the lowest value obtained by Matsuo et al. (2012), equal to 82.5%. For the ALV3, the values for the

Table 2. Estimate of repeatability coefficients (*r*) and determination coefficients (Det), using the different methods for the morphological descriptors of soybean: CH, ALV3, CE, CPIN and CPFU.

Methods*	-------CH-------		------ALV3-----		------CE--------		------PIN-------		------CPFU-----	
	*R*ˆ	Det	*R*ˆ	Det	*R*ˆ	Det	*R*ˆ	Det	*R*ˆ	Det
	----------Experiment 1 (124 genotypes and 5 repetitions)----------									
ANOVA	0.499	83.273	0.770	94.353	0.669	91.009	0.724	92.919	0.526	85.417
CP(cov)	0.533	85.096	0.774	94.487	0.671	91.085	0.728	93.053	0.610	85.904
CP(correl)	0.529	84.886	0.772	94.410	0.670	91.034	0.726	92.989	0.616	89.324
AE(correl)	0.529	84.862	0.771	94.406	0.670	91.021	0.726	92.980	0.679	89.048
AE(cov)	0.527	84.761	0.771	94.381	0.670	91.030	0.726	92.964	0.532	85.442
	----------Experiment 2 (93 genotypes and 5 repetitions) ----------									
ANOVA	0.369	74.516	0.660	90.644	0.641	89.907	0.750	93.735	0.636	89.714
CP(cov)	0.377	75.152	0.661	90.693	0.648	90.202	0.753	93.841	0.643	89.989
CP(correl)	0.377	75.179	0.660	90.662	0.647	90.147	0.753	93.846	0.639	89.857
AE(correl)	0.376	75.082	0.659	90.630	0.645	90.075	0.753	93.844	0.639	89.834
AE(cov)	0.372	74.794	0.659	90.624	0.644	90.061	0.752	93.801	0.636	89.741
	----------Experiment 3 (90 genotypes and 5 repetitions) ----------									
ANOVA	0.534	85.130	0.791	94.977	0.562	86.529	0.729	93.076	0.506	83.678
CP(cov)	0.539	85.397	0.795	95.091	0.583	87.477	0.742	93.510	0.532	85.038
CP(correl)	0.537	85.309	0.794	95.080	0.564	86.603	0.737	93.330	0.511	83.958
AE(correl)	0.537	85.283	0.794	95.072	0.561	86.459	0.737	93.323	0.508	83.754
AE(cov)	0.535	85.198	0.793	95.040	0.563	86.550	0.733	93.206	0.507	83.730
	----------Experiment 4 (16 genotypes and 16 repetitions) ----------									
ANOVA	0.652	96.767	0.688	97.240	0.750	97.961	0.732	97.767	0.623	96.360
CP(cov)	0.692	97.288	0.767	98.141	0.773	98.194	0.770	98.169	0.655	96.819
CP(correl)	0.692	97.295	0.746	97.916	0.771	98.172	0.760	98.062	0.631	96.471
AE(correl)	0.688	97.240	0.742	97.876	0.769	98.154	0.757	98.032	0.654	96.820
AE(cov)	0.680	97.142	0.728	97.713	0.761	98.078	0.742	97.871	0.658	96.857
	----------Experiment 5 (16 genotypes and 16 repetitions) ----------									
ANOVA	0.682	97.163	0.797	98.429	0.784	98.307	0.753	97.992	0.827	98.705
CP(cov)	0.702	97.415	0.823	98.669	0.804	98.495	0.791	98.378	0.849	98.898
CP(correl)	0.705	97.449	0.808	98.535	0.800	98.464	0.773	98.199	0.842	98.842
AE(correl)	0.701	97.402	0.804	98.502	0.799	98.451	0.769	98.157	0.842	98.837
AE(cov)	0.686	97.216	0.803	98.485	0.790	98.369	0.761	98.073	0.829	98.727

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

determination coefficients were greater than 90% (Table 2). The CPIN presented, in experiment 1, the lowest repeatability coefficient (0.724) by ANOVA and the highest (0.791) in Experiment 5 by CP(cov), associated with the determination coefficients greater than 93% (Table 2). The repeatability coefficient estimated for CPFU presented magnitude of 0.849 by CP(cov) to 0.506 by ANOVA, in the fifth and third experiment, respectively. The determination coefficients ranged from 98.898 to 83.678% (Table 2). Whereas, for the CPFT (Table 3), the repeatability coefficients ranged from 0.375 (ANOVA), in Experiment 4, to 0.749 [CP(cov)], in Experiment 2; and the determination coefficients presented magnitude of 89.190% in Experiment 1 and 97.411% in Experiment 5.

For the CRFT, the repeatability coefficients ranged from 0.196 to 0.581, with the lowest one obtained by the method of the AE (cov), in Experiment 3, and the largest by methods CP(correl), AE(correl) and AE(cov), in Experiment 1. The same character had determination coefficients that ranged between 54.931 and 94.977%, in

Table 3. Estimate of repeatability coefficients (*r*) and determination coefficients (Det), using the different methods for the morphological descriptors of soybean: CPFT, CRFT, AIFU, COVG and DVG.

Methods*	-------CPFT--------		-------CRFT------		-------AIFU-------	
	***R*ˆ**	**Det**	***R*ˆ**	**Det**	***R*ˆ**	**Det**
	----------Experiment 1 (124 genotypes and 5 repetitions)----------					
ANOVA	0.623	89.190	0.578	87.268	-	-
CP(cov)	0.631	89.531	0.583	87.467	-	-
CP(correl)	0.627	89.369	0.581	87.400	-	-
AE(correl)	0.627	89.345	0.581	87.384	-	-
AE(cov)	0.627	89.362	0.581	87.385	-	-
	----------Experiment 2 (93 genotypes and 5 repetitions)----------					
ANOVA	0.742	93.504	0.319	70.084	0.475	81.880
CP(cov)	0.749	93.709	0.388	75.983	0.540	85.445
CP(correl)	0.745	93.581	0.408	77.507	0.565	86.655
AE(correl)	0.744	93.571	0.390	76.169	0.551	85.976
AE(cov)	0.744	93.555	0.380	75.364	0.523	84.587
	----------Experiment 3 (90 genotypes and 5 repetitions) ----------					
ANOVA	0.734	93.249	0.198	55.231	0.423	78.565
CP(cov)	0.736	93.298	0.199	55.395	0.491	82.818
CP(correl)	0.734	93.235	0.201	55.756	0.524	84.616
AE(correl)	0.733	93.224	0.197	55.156	0.506	83.671
AE(cov)	0.733	93.200	0.196	54.931	0.474	81.819
	----------Experiment 4 (16 genotypes and 16 repetitions) ----------					
ANOVA	0.375	90.564	0.283	86.347	0.405	77.309
CP(cov)	0.464	93.276	0.371	90.404	0.471	81.673
CP(correl)	0.439	92.607	0.331	88.799	0.502	83.460
AE(correl)	0.414	91.879	0.291	86.797	0.483	82.352
AE(cov)	0.401	91.454	0.295	86.998	0.451	80.439
	----------Experiment 5 (16 genotypes and 16 repetitions) ----------					
ANOVA	0.666	96.962	0.516	94.457	0.520	94.534
CP(cov)	0.702	97.411	0.540	94.946	0.600	95.993
CP(correl)	0.695	97.335	0.542	94.977	0.554	95.209
AE(correl)	0.691	97.275	0.530	94.752	0.533	94.815
AE(cov)	0.685	97.201	0.519	94.522	0.535	94.846

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

experiments 3 and 5, respectively. The AIFU presented magnitude of 0.405 by the method of ANOVA and 0.600 in the CP(cov) in Experiment 4 and 5, respectively, associated with the determination coefficient greater than 77.00% (Table 3).

The repeatability coefficients and the prediction of real value for the CH, CE, CPIN, CPFT and CRFT in soybean genotypes were studied by Matsuo et al. (2012). The authors reported that estimates of repeatability coefficients for the CH were low, ranging from 0.345 to 0.793, for the CE ranged from 0.478 to 0.914, for the CPIN, from 0.428 to 0.865, for CPFT, from 0.163 to 0.645 and for CRFC the coefficients ranged from 0.216 to 0.553, with prediction of real value average of 84.72%. The results obtained in Experiments 1 and 2 of this study were similar to that of Matsuo et al. (2012) for the variable CH, however, the magnitude of the repeatability coefficient found in the other experiments and for the other characteristics were superior. This difference in the magnitude of the values of repeatability varies with the

Table 4. Number of evaluations needed associated with different determination coefficients (R^2), estimated for the CH and ALV3 in five experiments based on different methodologies*.

R^2	CH					ALV3				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
					ANOVA					
0.80	4.0	6.8	3.5	2.1	1.9	1.2	2.1	1.1	1.8	1.0
0.85	5.7	9.7	4.9	3.0	2.6	1.7	2.9	1.5	2.6	1.4
0.90	9.0	15.4	7.9	4.8	4.2	2.7	4.6	2.4	4.1	2.3
0.95	19.1	32.5	16.6	10.2	8.9	5.7	9.8	5.0	8.6	4.9
0.99	99.4	169.3	86.5	52.9	46.2	29.6	51.1	26.2	45.0	25.3
					CP(cov)					
0.80	3.5	6.6	3.4	1.8	1.7	1.2	2.1	1.0	1.2	0.9
0.85	5.0	9.4	4.8	2.5	2.4	1.7	2.9	1.5	1.7	1.2
0.90	7.9	14.9	7.7	4.0	3.8	2.6	4.6	2.3	2.7	1.9
0.95	16.6	31.4	16.2	8.5	8.1	5.5	9.7	4.9	5.8	4.1
0.99	86.7	163.7	84.6	44.2	42.0	28.9	50.8	25.6	30.0	21.4
					CP(correl)					
0.80	3.6	6.6	3.4	1.8	1.7	1.2	2.1	1.0	1.4	1.0
0.85	5.0	9.4	4.9	2.5	2.4	1.7	2.9	1.5	1.9	1.3
0.90	8.0	14.9	7.7	4.0	3.8	2.7	4.6	2.3	3.1	2.1
0.95	16.9	31.4	16.4	8.5	8.0	5.6	9.8	4.9	6.5	4.5
0.99	88.1	163.4	85.2	44.0	41.5	29.3	51.0	25.6	33.7	23.6
					AE(cov)					
0.80	3.6	6.6	3.5	1.8	1.7	1.2	2.1	1.0	1.4	1.0
0.85	5.1	9.4	4.9	2.6	2.4	1.7	2.9	1.5	2.0	1.4
0.90	8.0	14.9	7.8	4.1	3.8	2.7	4.7	2.3	3.1	2.2
0.95	16.9	31.5	16.4	8.6	8.1	5.6	9.8	4.9	6.6	4.6
0.99	88.3	164.3	85.4	45.0	42.3	29.3	51.2	25.7	34.4	24.1

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

nature of the character, with the genetic properties of the population, with the conditions in which individuals are developed and if the genotype of the individual, where repeated measurements are made, is stabilized (Cruz et al., 2004).

The low estimates of the repeatability coefficients, in general, less than 0.4, result in difficulties for the breeder to identify the best genotypic values from the phenotypic average analysis obtained (Ferreira et al., 1999). According to Neto et al. (2002), these estimates indicate dissimilarity in the repetition of character between one assessment and another.

Neto et al. (2002) observed irregularities of behavior between successive evaluations for diameter, lap height and weight of liquid in heart of palm, due to low magnitude of repeatability coefficients, less than 0.4. Matsuo et al. (2009) stated that the selection of strains with resistance to powdery mildew, based on the results of the group of genotypes adapted in Goiás, Brazil, would not be a good alternative, since it showed repeatability coefficients of less than 0.4.

It was observed, in general, in the present study, that the greater the repetitions number, greater the results for the repeatability coefficient (r) and lower the numbers of evaluations needed. Nevertheless, this occurred in function of the genetic properties of analyzed genotypes. According to Danner et al. (2010), the repeatability and determination coefficients obtained among the individuals selected from a strawberry guava tree were high in relation to those found in surinam cherry tree, because the genotypes of strawberry guava tree presented greater phenotypic stability, due to the increased selection pressure and management used to plants.

Considering a confidence level of 90%, and taking into account the greatest value within each method and between experiments, to predict the real value of CH in soybean genotypes, would be necessary 16 evaluations by the method of ANOVA, and 15 by methods of CP(correl), CP(cov) and AE(cov). For the ALV3, would be necessary 5 evaluations based on all four methods used (Table 4). For CE and CPIN (Table 5) the number of measurements would be 7 and 4 respectively, by ANOVA,

Table 5. Number of evaluations needed associated with different R^2, estimated for the CE and CPIN in five experiments based on different methodologies*.

R^2	CE					CPIN				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
	ANOVA									
0.80	2.0	2.2	3.1	1.3	1.1	1.5	1.3	1.5	1.5	1.3
0.85	2.8	3.2	4.4	1.9	1.6	2.2	1.9	2.1	2.1	1.9
0.90	4.4	5.1	7.0	3.0	2.5	3.4	3.0	3.3	3.3	3.0
0.95	9.4	10.7	14.8	6.3	5.2	7.2	6.4	7.1	6.9	6.2
0.99	48.9	55.6	77.1	33.0	27.3	37.7	33.1	36.8	36.2	32.5
	CP(cov)									
0.80	2.0	2.2	2.9	1.2	1.0	1.5	1.3	1.4	1.2	1.1
0.85	2.8	3.1	4.1	1.7	1.4	2.1	1.9	2.0	1.7	1.5
0.90	4.4	4.9	6.4	2.6	2.2	3.4	3.0	3.1	2.7	2.4
0.95	9.3	10.3	13.6	5.6	4.6	7.1	6.2	6.6	5.7	5.0
0.99	48.4	53.8	70.9	29.1	24.2	37.0	32.5	34.4	29.6	26.1
	CP(correl)									
0.80	2.0	2.2	3.1	1.2	1.0	1.5	1.3	1.4	1.3	1.2
0.85	2.8	3.1	4.4	1.7	1.4	2.1	1.9	2.0	1.8	1.7
0.90	4.4	4.9	7.0	2.7	2.2	3.4	3.0	3.2	2.8	2.6
0.95	9.4	10.4	14.7	5.7	4.7	7.2	6.2	6.8	6.0	5.6
0.99	48.8	54.1	76.6	29.5	24.7	37.3	32.5	35.4	31.3	29.0
	AE(cov)									
0.80	2.0	2.2	3.1	1.2	1.0	1.5	1.3	1.4	1.3	1.2
0.85	2.8	3.1	4.4	1.7	1.4	2.1	1.9	2.0	1.8	1.7
0.90	4.4	5.0	7.0	2.7	2.3	3.4	3.0	3.2	2.9	2.7
0.95	9.4	10.5	14.9	5.7	4.8	7.2	6.2	6.8	6.1	5.7
0.99	48.8	54.5	77.5	29.8	24.9	37.4	32.5	35.4	31.8	29.7

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

CP(correl), CP(cov) and AE(cov). While for the character CPFU would be required at least 9 evaluations by ANOVA, 8 by CP(cov) and 9 by methods of the CP(correl) and AE(cov), and for the CPFT the real value would be obtained with evaluation of 15 individuals by the method of ANOVA, 11 by the CP(cov), 12 by the CP(correl) and 13 by AE(cov) (Table 6).

Taking into account the same confidence level (90%), the prediction of real value to the CRFC would be necessary to evaluate 36 individuals by the methods CP (cov) and CP (correl) and 37 individuals by ANOVA and AE(cov). While for the characteristic AIFU would be needed 13 evaluations by ANOVA, 10 by the CP(cov) and AE(cov and 9 by CP(correl) (Table 7).

The results for CH, CE and CRFT corroborate with the obtained values by Matsuo et al. (2012), however they present lower predicted values to CPIN and CPFT, which probably is due to the genetic properties of different genotypes analyzed in the two studies.

The increase in accuracy for 95% implies the need for a greater number of evaluations and this would increase the costs and time for obtaining the results, but if labor-intensive enough, these factors do not impede the realization of the test in any of the characteristics studied.

In general, based on estimates of repeatability and reliability of 95%, considering the methods and experiments average, would be necessary 17 evaluations for hypocotyl length, 6 for plant height in V3, 9 for epicotyl length, 7 for the first internode length, 12 for petiole length of unifoliate leaf and petiole length of trifoliate leaf, 36 for rachis length, 18 for opening angle of the petioles of unifoliate leaf.

Analyzing the relationship between repeatability and number of measurements, it can be stated that: when the

Table 6. Number of evaluations needed associated with different R^2, estimated for the CPFU and CPFU in five experiments based on different methodologies*.

R^2	CPFU					CPFT				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
	ANOVA									
0.80	3.1	2.3	3.9	2.4	0.8	2.4	1.4	1.4	6.7	2.0
0.85	4.5	3.2	5.5	3.4	1.2	3.4	2.0	2.1	9.4	2.8
0.90	7.1	5.2	8.8	5.4	1.9	5.5	3.1	3.3	15.0	4.5
0.95	14.9	10.9	18.5	11.5	4.0	11.5	6.6	6.9	31.7	9.5
0.99	77.8	56.8	96.6	59.8	20.8	60.0	34.4	35.8	165.0	49.6
	CP(cov)									
0.80	3.0	2.2	3.5	2.1	0.7	2.3	1.3	1.4	4.6	1.7
0.85	4.3	3.2	5.0	3.0	1.0	3.3	1.9	2.0	6.5	2.4
0.90	6.8	5.0	7.9	4.7	1.6	5.3	3.0	3.2	10.4	3.8
0.95	14.4	10.6	16.7	10.0	3.4	11.1	6.4	6.8	21.9	8.1
0.99	74.9	55.1	87.1	52.0	17.7	57.9	33.2	35.6	114.2	42.1
	CP(correl)									
0.80	3.1	2.3	3.8	2.1	0.8	2.4	1.4	1.5	5.1	1.8
0.85	4.4	3.2	5.4	2.9	1.1	3.4	1.9	2.1	7.2	2.5
0.90	7.0	5.1	8.6	4.7	1.7	5.4	3.1	3.3	11.5	3.9
0.95	14.8	10.7	18.2	9.9	3.6	11.3	6.5	6.9	24.3	8.3
0.99	77.0	55.9	94.6	51.4	18.6	58.9	34.0	35.9	126.4	43.4
	AE(cov)									
0.80	3.1	2.3	3.9	2.1	0.8	2.4	1.4	1.5	5.7	1.8
0.85	4.4	3.2	5.5	3.0	1.1	3.4	1.9	2.1	8.0	2.5
0.90	7.0	5.1	8.7	4.8	1.7	5.4	3.1	3.3	12.7	4.0
0.95	14.8	10.8	18.4	10.0	3.6	11.3	6.5	6.9	26.9	8.5
0.99	77.3	56.0	96.0	52.3	18.6	59.0	34.0	36.0	140.0	44.4

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

repeatability is high, the increase in the number of measurements will result in little increase in accuracy, in relation to which it would have if an individual was evaluated by means of a single observation. When repeatability is low, the increase of repeated measurements can result in a significant increase in precision; and, with intermediate levels of repeatability, is rarely beneficial to do more than three measures in each individual for each character (Cruz et al., 2004; Cargnelutti Filho and Gonçalves, 2011). Dos Santos et al. (2016) also found similar results to the present work.

The results indicate the need for further studies with the aim of better understanding about the effect of genotypes, the influence of environmental conditions and genotype x environment interaction for the additional descriptors, aiming to estimate repeatability and determination coefficients and the number of evaluations needed in order to estimate the difference between the evaluated materials.

Conclusions

The length of the first internode requires a smaller amount of measurements in comparison to other characteristics measured in the early stages of soybean development for the same level of reliability.

With six measurements, reliability levels of 95 and 90% were obtained for plant height in V3 and length of the first internode, respectively, by the methods of the ANOVA, CP(correl), CP(cov) and AE(correl).

With seven measurements, 90% of accuracy was obtained for the epicotyl length, for all methods used; 85% for the petiole length of the unifoliate leaf, by method

Table 7. Number of evaluations needed associated with different R^2, estimated for the CRFT and AIFU in five experiments based on different methodologies.*

R^2	CRFC					AIFU				
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
	ANOVA									
0.80	2.9	8.5	16.2	10.1	3.8	-	4.4	5.5	3.7	3.8
0.85	4.1	12.1	23.0	14.3	5.3	-	6.3	7.7	5.2	5.5
0.90	6.6	19.2	36.5	22.8	8.5	-	10.0	12.3	8.3	8.7
0.95	13.9	40.6	77.0	48.1	17.8	-	21.0	25.9	17.6	18.3
0.99	72.2	211.3	401.2	250.5	92.9	-	109.5	135.0	91.6	95.3
	CP(cov)									
0.80	2.9	6.3	16.1	6.8	3.4	4.5	3.4	4.1	2.7	3.1
0.85	4.1	9.0	22.8	9.6	4.8	6.4	4.8	5.9	3.8	4.4
0.90	6.4	14.2	36.2	15.3	7.7	10.1	7.7	9.3	6.0	6.9
0.95	13.6	30.0	76.5	32.3	16.2	21.3	16.2	19.7	12.7	14.7
0.99	70.9	156.5	398.6	168.1	84.3	111.1	84.3	102.7	66.1	76.4
	CP(correl)									
0.80	2.9	5.8	15.9	8.1	3.4	4.0	3.1	3.6	3.2	3.2
0.85	4.1	8.2	22.5	11.4	4.8	5.6	4.4	5.2	4.6	4.6
0.90	6.5	13.1	35.7	18.2	7.6	8.9	6.9	8.2	7.2	7.2
0.95	13.7	27.6	75.4	38.3	16.1	18.8	14.6	17.3	15.3	15.3
0.99	71.4	143.6	392.8	199.8	83.8	98.1	76.2	90.0	79.7	79.6
	AE(cov)									
0.80	2.9	6.3	16.3	9.7	3.5	4.3	3.3	3.9	3.5	3.5
0.85	4.1	8.9	23.0	13.8	5.0	6.1	4.6	5.5	5.0	5.0
0.90	6.5	14.1	36.6	21.9	8.0	9.6	7.3	8.8	7.9	7.9
0.95	13.7	29.7	77.2	46.2	16.8	20.4	15.5	18.5	16.6	16.7
0.99	71.5	154.9	402.5	241.0	87.7	106.1	80.7	96.6	86.6	87.2

*Estimation methodologies of the repeatability coefficient: ANOVA: Variance analysis with one factor; CP(cov): principal components obtained from the matrix of covariance; CP(correl): principal components obtained from the correlation matrix; AE(correl): structural analysis based on theoretical eigenvalue of the correlation matrix or correlation average; and AE(cov): structural analysis based on theoretical eigenvalue of the covariance matrix.

of CP(cov) and 90% by methods of ANOVA, CP(correl), AE(correl).

With 15 measurements, it was possible to obtain 90% reliability for the hypocotyl length, petiole length of the trifoliate leaf and the angle formed by the insertion of the petioles of the unifoliate leaf for all methods used; and, for the rachis length of the first trifoliate leaf, would be necessary 37 measurements for reliability of 90% by methods of ANOVA, CP(correl), CP(cov) and AE(correl).

CONFLICT OF INTERESTS

The authors have declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors would like to thank CNPq (process 142182/2016-8) for the financial support, resources provided and grant.

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