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International Journal of Current Research
Vol. 8, Issue, 08, pp.36875-36880, August, 2016

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

GENETIC DIVERGENCE AND CORRELATIONS BETWEEN CHARACTERS OF TRANSGENIC SOYBEAN CULTIVARS

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ARTICLE INFO

Article History:
Received 03rd May, 2016
Received in revised form
10th June, 2016
Accepted 15th July, 2016
Published online 31st August, 2016

Key words:

Glycine max, path analysis,
Multivariate analysis,
Dissimilarity, Canonical variables,
Pation of indirect effects.

ABSTRACT

This study aimed to evaluate the genetic divergence, the relative contribution of variables to the dissimilarity and the direct and indirect participation of the characters with the grain yield of commercial varieties of transgenic soybean first (RR) and second (intacta RR2 PRO) generation. Eight morphological characteristics of a quantitative nature were analyzed, 42 cultivars in the harvest 2014/2015 in Londrina, PR, Brazil, in a randomized complete block design with three replications. The Mahalanobis distance based the UPGMA cluster and canonical variable technique analysis. The relative contribution of the characteristics was calculated by Singh method and path analysis allowed us to estimate the direct and indirect participation of the variables with the grain yield. The 50% genetic distance was the formation of five groups by UPGMA method, with similar results to those obtained in the study of canonical variables. The largest relative contribution to the dissimilarity of the cultivars was provided by hundred grains mass, with the highest direct effect on grain yield, low participation of indirect effects.

Citation: Gustavo Henrique Freiria, Kawana Silva Bortolato, Fernando Modos Veiga Dias, Felipe Favoretto Furlan *et al.* 2016. "Genetic divergence and correlations between characters of transgenic soybean cultivars", *International Journal of Current Research*, 8, (08), 36875-36880.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important products in the Brazilian economy, especially in the supply of oil for domestic consumption, as a protein constituent in animal feed, as well as the one of the export products of the country (Hirakuri *et al*., 2014). With increasing demand for cultivars adapted, productive and tolerant to disease, pests and drought, among others, characteristics (Vieira *et al*., 2009). Wysmierski and Vello (2013) compared the genetic basis and the average coefficient of relatedness of soybean genotypes used in the United States, China, Japan and Brazil, and concluded that Brazilian soybean cultivars showed a narrow genetic base. However, studies indicate that in the primary gene pool is found a genetic variability enough to improve activities of the species, considering the use of landraces and improved cultivars (Yamanaka *et al*., 2007, Vieira *et al*., 2009).

Since the choice of progenitors meets criteria that allow increased variability and hence the expansion of the genetic base (Costa *et al*., 2004). In this sense, the study on the genetic diversity is needed, which can occur in two ways: quantitative and predictive. Among these two methods of assessing diversity, predictive methods are given greater prominence, by waiving the prior obtaining of hybrid combinations (Cruz *et al*., 2004). It is based on physiological, agronomical and molecular differences, quantified measure of dissimilarity, assessed by multivariate analysis techniques (Cross *et al*., 2004). The standardized average euclidean distances and the generalized Mahalanobis between genotypes pairs are used as measure of dissimilarity in the cluster methods. The latter, for providing correlations between the characteristics analyzed by matrix residual variances and covariances offers an advantage over the first (Cargnelutti Filho *et al*., 2008). One of the main objects of soybean breeding programs is to obtain cultivars with higher yield potential. However, the grain yield is a complex feature associated with the expression of different components. Thus, knowledge of the levels of these associations, through correlation studies allows the

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identification characters that can be used as indirect selection for yield (Hoogerheide *et al*., 2007). The simple study of correlations does not draw conclusions on the causes and effects of these relationships, the most suitable path analysis, which allows the separation of the correlation coefficient between the direct and indirect effects (Faria *et al*., 2015). In this context, this study aimed to evaluated the genetic divergence of 42 transgenic soybean cultivars of first (RR) and second (intacta RR2 PRO) generation through cluster methods and the relative contribution of variables to the dissimilarity, and the effects direct and indirect character with the grain yield.

MATERIAL AND METHODS

The experiment was conducted during the 2014/2015 crop in Londrina, Paraná, latitude 23 ° 20'23.45 "S, longitude 51 ° 12'32.28"W and altitude of 532 m. Climate classification according to Köppen Cfa, with average annual rainfall from 1400 to 1600 mm (Almeida, 2015). Sowing took place on December 4th, 2014, in experimental design with randomized complete block design with three replications, with 42 treatments. The experimental unit consisted of four rows of five meters (m) and spaced apart by 0.45 m. The treatments consisted of 42 soybean cultivars from different breeding programs, 22 with genes of first genaration for resistance to glyphosate (RR), described as: BALU 3711 RR, BMX 6968 RSF, BMX Potência RR, BMX Turbo RR, BRS 359 RR, CD 202 RR, CD 2611 RR, FTS 1154 RR, FTS 2125 RR, FTS 4164 RR, FPS Antares RR, FPS Iguaçu RR, FPS Jupiter RR, FPS Solimões RR NA 5909 RG, NS 6700 RR, NS 6823 RR , SYN 1059 RR SYN 1163 RR, SYN 1258 RR, SYN 1259 RR, TMG 1264 RR. And 20 with genes of second generation for resistance to glyphosate and the main soybean caterpillars (Intacta RR2 PRO), described as: AS 3610 IPRO, BMX 6160 RSF IPRO, BMX 7166 RSF IPRO, BS 2595 IPRO, CD 2620 IPRO, CD 2644 IPRO CD 2720 IPRO, FTS 4160 IPRO FPS Solar IPRO, M 5917 IPRO, M 5947 IPRO, M 6210 IPRO, M 6410 IPRO, NS 6006 IPRO, SYN 13561 IPRO, SYN 13595 IPRO, SYN 13671 IPRO, TEC 5833 IPRO, TMG IPRO 7060, TMG 7062 IPRO.

Table 1. Summary of the analysis of variance for eight quantitative characters of 42 soybean cultivars

FV	GL	Average Square							
		DTF	PHF	DTM	PHM	IFP	LI	YED	MHG
Block	2	1.37	203.1	24.67	29.05	37.72	0.39	267621.19	0.52
Cult.	41	17.98**	119.45**	33.21**	404.17**	18.57*	0.59**	311181.17**	9.71**
Error	82	2.19	19.37	3.95	29.09	10.47	0.21	99765.36	0.28
CV (%)		3.36	10.79	1.86	5.93	21.96	26.12	12.64	4.38

* $P < 0.05$ and ** $p < 0.01$ according to the F test DTF: days to flowering; PHF: plant height at flowering; DTM: days to physiological maturity; PHM: plant height at maturity; IFP: insertion of the first pod; LI: lodging index; YED: grain yield; MHG: hundred mass grain.

The seeds were treated with the product Standak Top® (fipronil, thiophanate methyl and pyraclostrobin) in 200 mililiters (ml) dose of the commercial product per 100 kg of seed and inoculated at the time of seeding with *Bradyhizobium japonicum* strains at a concentration of 10^9 cells viable ml^{-1}. Cultural practices such as weed control, pests and diseases were carried out through the application of agrochemicals in accordance with the recommendations proposed by Embrapa (2011). Harvest was done manually, after R8 development stage, when 95% of the pods had the typical staining mature pod eliminating the two outer rows and 0.5 m from each end of the central lines as boundary totaling an area of 3.6 m^2. The variables measured were: plant height at flowering (PHF); plant height at maturity (PHM); insertion of the first pod (IFP); grain yield (YED), obtained after the track of the plants of the useful area of the plot and extrapolated to kg ha^{-1}, with humidity correction of grain to 13%; Mass of one hundred grains (MHG), weighing two subsamples of hundred grains for each repeating field, with correction for 13% moisture. Were also determined the days to flowering (DTF); days to maturity (DTM); lodging index (LI), grade scale ranging from one (all or most upright plants) to five (more than 80% of the plants in the useful area of the plot bedridden), in the maturation period.Data were subjected to analysis of variance and F test ($p < 0.05$). When found significant effect of treatment, the means were grouped by the Scott-Knott test ($p < 0.05$). The genetic distance between all pairs of genotypes was estimated by Mahalanobis distance, which served as the basis for the hierarchical clustering method Unweighted Pair-Group Method Using in Arithmetic Average (UPGMA). The grouping was tested by means of canonical variables and the relative importance of the characteristics was calculated by Singh method (1981), made path analysis between the variables in relation to grain yield, using the computer program Genes (Cruz, 2008).

RESULTS AND DISCUSSION

All features, by analysis of variance were significant by F test ($p < 0.05$) (Table 1). These results show the variability among cultivars. The average results of evaluated characters of 42 cultivars are shown in Table 2. The plant height at maturity was the feature that allowed the greater discrimination of cultivars with six significantly different groups. The highest value was the cultivar CD 2720 with 123.90 cm and the lowest cultivar FPS Solimões with 69.40 cm. This same behavior was observed for plant height at flowering, where the cultivar CD 2720 is located in the greater height group and cultivate FPS Solimões in the group of genotypes showed the lowest plants.

The number of days to flowering oscillated 39-49 and the cycle (days to maturity) ranged from 102 to 112. According Meotti *et al*. (2012) there is great variability among soybean cultivars for sensitivity to photoperiod and temperature, and the first is considered the determining factor of adaptation of cultivars and rate of development, affecting plant height and cycle.All cultivars showed the insertion of the first pod up to 10 cm, with the exception of cultivar FPS Solimões.

Table 2. Average eight quantitative characters of 42 soybean cultivars

Cultivars	DTF	DTM	PHF	PHM	IFP	LI	YED	MHG
	-------days-----		------------cm----------				Kg ha^{-1}	g
AS 3610	46b	104c	54.4a	95.8d	17.3a	2.0b	2543.2b	10.60d
BALU 3711	46b	111a	40.0c	88.8e	15.7a	1.2c	2555.6b	11.06d
BMX 6160	43c	108b	35.7c	91.4d	19.2a	1.6c	3012.3a	13.23b
BMX 6968	44b	111a	44.1b	91.3d	17.3a	2.0b	2444.4b	13.71b
BMX 7166	46b	111a	42.1b	98.8c	15.7a	2.3b	2802.5a	11.90c
BMX Potência	48a	112a	44.2b	97.9c	16.6a	1.8c	2172.8c	10.09d
BMX Turbo	44b	107c	32.7c	74.4f	10.3b	1.7c	2253.1c	11.73c
BRS 359	44b	105c	46.0b	99.3c	13.6b	1.9c	2271.6c	11.82c
BS 2595	40d	108b	34.6c	96.8d	14.2a	1.5c	2518.5b	10.78d
CD 202	43c	108b	46.9b	112.4b	16.8a	2.7a	2055.6c	12.23c
CD 2611	45b	105c	37.7c	82.2e	16.9a	1.8c	2407.4c	14.01b
CD 2620	43c	103d	38.7c	104.9c	14.9a	1.9c	2987.7a	13.19b
CD 2644	44b	104d	45.4b	87.3e	19.2a	2.9a	2506.2b	14.03b
CD 2720	47a	111a	55.7a	123.9a	15.6a	2.0b	2222.2c	12.52c
FTS 1154	39d	102d	34.0c	61.6g	10.0b	1.1c	2524.7b	10.75d
FTS 2125	43c	104c	35.2c	77.0f	10.7b	1.2c	2617.3b	11.34c
FTS 4160	43c	103d	38.7c	91.2d	14.4a	1.4c	2796.3a	10.97d
FTS 4164	46b	111a	53.8a	83.2e	16.6a	1.7c	2114.8c	10.32d
FPS Antares	46b	109b	40.8c	102.1c	16.0a	1.3c	2197.5c	10.24d
FPS Iguaçu	39d	102d	36.8c	90.1d	11.2b	1.7c	1851.9c	14.12b
FPS Júpiter	42c	105c	34.1c	78.0f	13.2b	1.1c	2444.4b	10.77d
FPS Solar	45b	106c	42.2b	97.9c	15.8a	1.7c	2666.7b	12.56c
FPS Solimões	39d	111a	27.9c	69.4g	9.7b	1.7c	2185.2c	9.70d
M 5917	44b	104c	34.8c	79.6e	14.1a	1.2c	2500.0b	11.35c
M 5947	44b	110a	37.9c	94.0d	17.4a	1.4c	2500.0b	11.70c
M 6210	49a	111a	51.9a	97.3c	12.3b	1.8c	1944.4c	9.51d
M 6410	48a	110a	43.4b	92.2d	15.9a	1.5c	2512.3b	10.06d
NA 5909	44b	106c	36.0c	80.9e	15.6a	1.1c	2567.9b	11.95c
NS 6006	44b	102d	38.3c	77.1f	14.9a	1.7c	2808.6a	12.59c
NS 6700	43c	111a	40.4c	101.6c	16.9a	1.6c	2308.6c	11.91c
NS 6823	45b	109b	42.1b	91.6d	15.3a	2.2b	2500.0b	10.30d
SYN 1059	43c	108b	34.6c	85.1e	11.1b	1.6c	2598.8b	10.86d
SYN 1163	44b	107b	50.7a	102.8c	14.9a	2.8a	2265.4c	10.89d
SYN 1258	45b	108b	39.0c	86.2e	11.6b	1.8c	1919.8c	9.83d
SYN 1259	43c	107b	34.2c	81.1e	12.3b	1.4c	2709.9b	10.43d
SYN 13561	39d	103d	32.9c	85.9e	12.9b	2.2b	2722.2b	13.86b
SYN 13595	42c	103d	44.3b	101.9c	16.9a	2.2b	2740.7b	13.75b
SYN 13671	48a	103d	44.0b	102.6c	17.0a	1.7c	2493.8b	12.46c
TEC 5833	45b	102d	43.7b	84.6e	17.2a	1.7c	3098.8a	14.97b
TMG 1264	45b	108b	42.8b	96.1d	12.1b	2.1b	2277.8c	10.84d
TMG 7060	43c	111a	39.1c	88.3e	13.4b	1.8c	3222.2a	17.37a
TMG 7062	44b	111a	41.1c	95.7d	16.1a	2.7a	3074.1a	16.53a

Means followed by the same letter in the column do not differ at 5% significance by Scott-Knott test. DTF: days to flowering; PHF: plant height at flowering in centimeters (cm); DTM: days to physiological maturity; PHM: plant height at maturity in centimeters (cm); IFP: insertion of the first pod in centimeters (cm); LI: lodging index; YED: grain yield in Kg ha^{-1}; MHG: hundred mass grain in grams (g).

Table 3. Estimation of direct and indirect effects of the characters on grain yield

Effect	DTF	PHF	DTM	PHM	IFP	LI	MHG
Direct on YED	0.157	-0.349	-0.087	-0.088	0.281	-0.085	0.528
Indirect via DTF		0.103	0.062	0.068	0.067	0.016	-0.034
Indirect via PHF	-0.228		-0.077	-0.227	-0.172	-0.171	0.010
Indirect via DTM	-0.035	-0.019		-0.026	-0.011	-0.010	0.017
Indirect via PHM	-0.038	-0.057	-0.026		-0.043	-0.041	-0.010
Indirect via IFP	0.121	0.139	0.035	0.138		0.091	0.087
Indirect via LI	-0.009	-0.042	-0.010	-0.040	-0.028		-0.030
Indirect via MHG	-0.113	-0.016	-0.010	0.058	0.163	0.189	
Total (Pearson´s correlation)	-0.145	-0.241	-0.207	-0.116	0.258	-0.011	0.569

Almeida *et al* (2011), points out that plants with low height of first pod (<10 cm) can cause losses in mechanical harvesting. For this characteristic was the formation of two distinct groups with averages of 16.23 and 10.82 cm, respectively. The cultivars with higher lodging index were the CD 202, CD 2644, SYN 1163 and TMG 7062. The average yield was 2498.00 kg ha^{-1}. Sowing in December is considered late for the state of Paraná, which results in early flowering, reduced cycle and plant height, such morphological changes can implicate the grain yield (Meotti *et al*., 2012). Dallacort *et al*. (2006) studied the best sowing dates for soybeans in the state of Paraná and found that sowing in December had the lowest productivity. The genotypes were separated into three groups, the BMX 6160 cultivars, BMX 7166, CD 2620, FTS 4160, NS 6006, TEC 5833, 7060 GMT and 7062 GMT, with an average of 2975.31 kg ha^{-1}, joined the group showed the highest grain yields about 13.91 and 27.29% more productive than the cultivars leased in the second and third group, respectively. The mass of one hundred grains ranged from 9.51 to 17.37 g and showed a positive correlation coefficient of 0.569 with grain yield (Table 3), results similar to those obtained by Almeida *et al*. (2010) and Rigon *et al*. (2012) in soybean, low share of indirect effects with the main component. As the mass of one hundred grains, days to flowering characteristics and first pod had positive direct effects on production. However, the characteristic days to flowering with pronounced participation of negative indirect effects, especially plant height at flowering, with -0.228, showed a negative correlation coefficient with grain yield.

The presence of negative indirect effects show the difficulty to select only based on the behavior of the main variable (Hoogerheide *et al*., 2007). The other characters showed negative direct effects on productivity but low contributions, results similar to those obtained by Rigon *et al*. (2012). The coefficient of determination was found to be 0.46, showing that 46% of grain yield is explained by the effect of the variables analyzed. Similar amounts of residual effect and coefficient of determination were found by Nogueira *et al*. (2012) in the study of correlations between characters: number of days to flowering and maturity, grain yield, average number of seeds per pod and weight of 100 grains, 90 soy genotypes. It should be noted that the production is a quantitative character with large modifiers alleles, which give little effect, being strongly influenced by environmental conditions, with low heritability (Rigon *et al*., 2012). The relative contributions of each characteristic in the genetic dissimilarity, second method of Singh (1981), are presented in Table 4. The greatest contribution parameter for the genetic divergence was the mass of one hundred grains with 54.18%, followed by height plant maturation with 15.06%, days to maturity with 10.63% and days to flowering with 9.78%, which together contributed 89.65% of all dissimilarity among the 42 cultivars.

Table 4. Relative contribution of agronomic traits for genetic dissimilarity of 42 soybean cultivars as criterion Singh (1981)

Agronomical trait	Amount (%)
1- Days to flowering	9.78
2- Plant height at flowering (cm)	5.18
3- Days to physiological maturity	10.63
4- Plant height at maturity (cm)	15.06
5- Insertion of the first pod (cm)	1.42
6- Lodging index	3.42
7- Grain yield (Kg ha^{-1})	0.33
8- Hundred mass grain (g)	54.18

DTF: days to flowering; PHF: plant height at flowering; DTM: days to physiological maturity; PHM: plant height at maturity; IFP: insertion of the first pod; LI: lodging index; YED: grain yield; MHG: hundred mass grain.

Almeida *et al*. (2011) evaluated the genetic divergence in twelve genotypes of soybean under conditions of irrigated lowland, in the southern state of Tocantins, and found that among the characters studied, the number of days to maturity (39.49%), weight hundred seeds (26.56%) and the number of days to flowering (13.59%) were the most efficient in explaining dissimilarity between cultivars. The characteristics evaluated grain production was less contributed to genetic divergence only 0.33%, this result can be justified because it is commercial genotypes, improved to high grain yield and all adapted to the study area. This low relative contribution to genetic dissimilarity in soybeans, regarding grain yield was also verified by Santos *et al*. (2011, 2013). However, this characteristic is essential in genetic diversity studies, since the choice of possible parents, as well as genetic dissimilarity, should consider the high grain yield for obtaining superior progenies (Elias *et al*. 2007). The recognition group of materials with high similarity by observing the set of distances is inefficient due to the high number of available measures, which makes necessary to use groupings of methods in the evaluation and characterization assembly genotypes (Azevedo *et al*., 2004). According Cargnelutti Filho *et al*. (2008) drastic changes in level dendrogram indicate union of heterogeneous cultivars. Therefore, we used 50% genetic distance as a criterion for defining the groups by UPGMA method (Figure 1). The application of the cluster, based on the dissimilarity matrix of Mahalanobis, separated individuals into five groups. Group I concentrated 76.16% of genotypes, group II and III were made only by cultivars FPS Solimões and CD 2720, respectively. Six cultivars formed the fourth group and TMG 7062 cultivars and TMG 7060 gave rise to Group V. The formation of these groups indicates genetic variability among the studied cultivars (Ferreira Júnior *et al*., 2013). According to Elias al. (2007), grouped materials in the same group are genetically similar.

The V group focused more productive cultivars and most massive hundredfold; averaging 3148.15 kg ha^{-1} and 16.95 g, respectively. The CD 2611 cultivars, CD 2644, Iguaçu FPS, 13595 SYN, SYN 13561 and TEC 5833, grouped in Group IV, were the earliest with an average cycle of 103 days. Group III, composed only by CD 2720 genotype, had the most disparate characteristics of the high number of days to flowering, plant height at flowering and maturation. Inverse observed to grow FPS Solimões (Group II) had the lowest plant height at flowering and maturation, first pod and mass hundredfold; to 27.89 cm, 69.45 cm, 9.67 cm and 9.70 g, respectively.

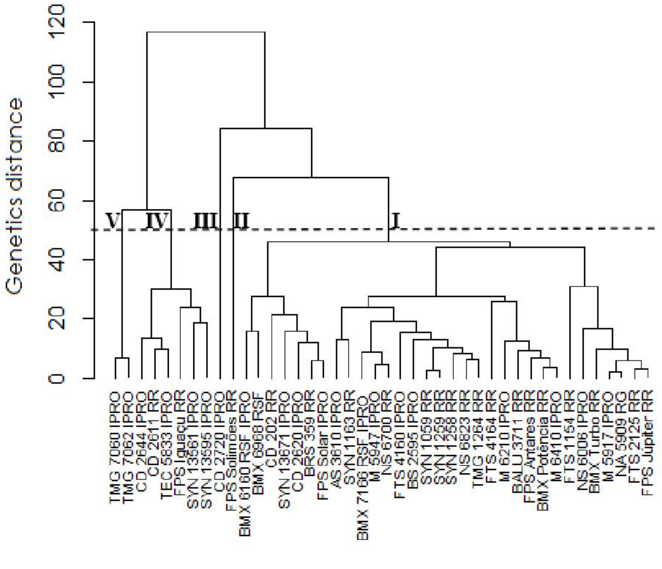

Figure 1. Dendrogram obtained by UPGMA, from dissimilarity measures between 42 soybean cultivars, based on the Mahalanobis distance

In the analysis based on the canonical variables (CV), 78.12% of the total variance was explained by the CV1 (57.19%) and CV2 (20.93%), setting a two-dimensional graphic representation (Figure 2).

There was a correlation between cluster analysis UPGMA and the canonical variables, taking into account the grouping of cultivars, subject to cultivate FTS 1154, that unlike observed by UPGMA cluster, which is the Group I, introduced separately by the method of canonical variables. This result is similar to those obtained by Santos *et al*. (2011), in which approximately 80% of the total variance explained was found by the first two canonical variables in the study of genetic divergence among 48 soybean cultivars. And the pattern of similarity of genotypes is also given by the graphic dispersion of canonical variables in relation to the grouping by UPGMA method. Rocha *et al*. (2009) found small differences between the results obtained by the UPGMA method and the canonical variables in the study of genetic divergence among cherry tomato accessions group.

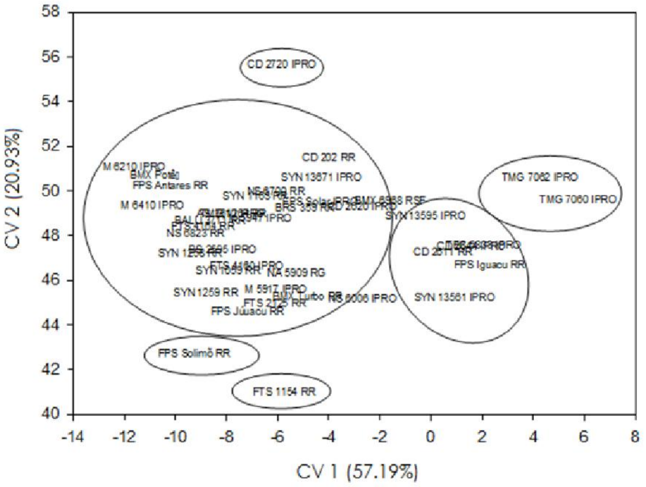

Figure 2. Graphical dispersion of 42 soybean cultivars in relation the canonical variables (VC1 and VC2) for eight quantitative characters

Conclusions

There is genetic divergence among soybean cultivars, which allows the identification of dissimilar genotypes. The UPGMA and the canonical variables are consistent with each other. The one hundred grains mass presents the greatest contribution to the dissimilarity between cultivars. The highest correlation with grain yield is with the mass of one hundred grains with low participation of indirect effects.

Acknowledgements

I thank Prof. Dr. Leandro Simões Azeredo Gonçalves for all the support statistic.

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