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Genotype x environment interaction and stability of soybean cultivars for vegetative-stage characters

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ABSTRACT. In order to obtain the certificate of cultivar protection, it is necessary to prove its distinctiveness, homogeneity, and stability. Currently, there are 37 descriptors for differentiating soybeans cultivars. However, they are still not enough and, as a result, it is necessary to create, identify, and evaluate new descriptors. This study was aimed at evaluating the genotypic and environment interaction (GxE) and determining the stability of eight soybean cultivars for five vegetative-stage descriptors. The research was done in a greenhouse of the Soybean Breeding and Genetic Studies Program of Universidade Federal de Uberlândia. The treatments were composed of eight soybean cultivars, sown in two different growing seasons (January 25, 2014 and November 27, 2014). The experiments were carried out in randomized complete blocks with three replications and each experimental plot consisted of one pot with four soybean plants. The characters evaluated were: length of hypocotyl (LH), length of epicotyl (LE), length of unifoliolate leaf petiole (LUP), length of first trifoliate leaf petiole (LTLP), and rachis length of terminal leaflet of the first trifoliate leaf (RL). The data achieved from the trials were undergone genetic-statistical analyses by the GENES software. For all analyzed characters, the existence of genetic variability was observed emphasizing the vegetative-stage descriptors' utility to differentiate soybean cultivars. The occurrence of GxE interaction was detected for all characters assessed, mainly of complex nature, except by RL, which was of simple nature. The most stable cultivars for the vegetative-stage descriptors analysed were UFUS 7415 and UFUS Impacta.

Key words: *Glycine max*; Descriptor; Distinctiveness

INTRODUCTION

The soybean [*Glycine max* (L.) Merrill] crop is originated from China and had its cultivation initiated in Brazil in the year of 1882 in the State of Bahia, although it was in the State of Rio Grande do Sul where the crop found favorable climatic conditions to its best development (Proque et al., 2014). Nowadays, Brazil is the second largest worldwide producer of soybeans and in the growing season of 2016/2017 reached a cultivated area of 33,889.9 million hectares, which led to a record production of 113,923.1 million of tons of grains (CONAB, 2017).

The soybean international value is mainly due to its versatility as raw material for edible oils, animal food, high percentage of protein, and its wide utilization in human diet (Alvez et al., 2013). Also, the crop is responsible for redeeming a whole socially and economically importance as it enables the creation of new jobs and leads to a raise of Gross National Product (GNP) in the agribusiness sector (Neto and Martins, 2016).

Investments on soybean genetic breeding have enlarged the development of cultivars with wide adaptation to Brazilian edaphoclimatic conditions and increased the resistance to biotic and abiotic factors (Mateus and Silva, 2013). The great incentive for genetic breeding happened with the creation of the Cultivar Protection Law, in 1997. In the first eight years after its implementation, it was released more cultivars than in the last 30 years that preceded its creation (Vidal, 2015). A cultivar is liable of protection since it meets the requirements of innovation, distinctiveness (D), homogeneity (H), and stability (S) (Viana et al., 2011).

In order to evaluate D, H, and S, it is necessary the implementation of DHS tests, which are carried out according to each species particularity. Several characteristics are taken into consideration in a variety of development stages of a crop, and such, are named minimum descriptors (Vieira et al., 2009). For soybean crop, there are 37 mandatory and additional descriptors to differentiate cultivars (MAPA, 2009); nevertheless, they are still limited to distinguish candidate cultivars from cultivars already protected (Nogueira et al., 2008).

Some studies have shown the identification of possible descriptors for differentiating soybean cultivars. Nogueira et al. (2008), studying 11 soybean cultivars in four different growing seasons, concluded that the length of hypocotyl, epicotyl, unifoliolate leaf petiole, first trifoliate leaf petiole, and rachis length of terminal leaflet of the first trifoliate leaf can be useful as additional descriptors in soybeans.

Matsuo et al. (2012) evaluating 85 genotypes in 4 experiments also found genetic variability for length of hypocotyl, epicotyl, first trifoliate leaf petiole, and rachis of the first trifoliate leaf. In studies of quantitative trait locus (QTL) mapping, Liang et al. (2014) have demonstrated that the length of hypocotyl is a quantitative characteristic controlled by 4 additive genes.

Considering that the additional descriptors of vegetative phase mentioned previously are of quantitative nature, the aim of this research is to evaluate the genotypic and environment interaction (GxE), as well as to determine the stability of eight soybeans cultivars for five different descriptors of vegetative phase.

MATERIAL AND METHODS

The experiment was done in a greenhouse of Soybean Breeding and Genetic Studies Program of Federal University of Uberlândia (18º52'S; 48º20'W and 805 m in altitude) located in a farm named Capim Branco belonging to the Federal University of Uberlândia. It was evaluated eight soybeans cultivars (UFUS Milionária, UFUS 7910, UFUS Riqueza, UFUS 7415, UFUS Guarani, UFUS Impacta, UFUS Xavante, and UFUS Vila Rica) in two different growing seasons, January 25, 2014 and November 27, 2014.

The experiments were carried out in randomized complete blocks with three replications. Each experimental plot consisted of four soybean plants cultivated in one pot of 5-L capacity, previously filled with substrate made up of 2/3 soil and 1/3 organic matter. On the substrate, it was done a sowing fertilization with N (20 kg/ha), P_2O_5 (200 kg/ha), and K_2O (100 kg/ha). The fertilizers were incorporated into the substrate followed by a homogenization process. The pots were irrigated before sowing in order to facilitate the opening of holes. Nine holes were opened three centimeters long in each pot and one seed was placed in each one of them. The seeds were previously treated with Vitavax-Thiram 200 SC in the dosage of 300 mL for 100 kg seeds. Also, it was performed a seed inoculation with the bacteria *Bradyrhizobium japonicum* in the dosage of 100 mL for 50 kg of seeds. In the phenological stage V3, it was conducted a foliar application of cobalt and molybdenum in the dosage of 3 and 12 g/ha, respectively, attempting to optimize the air nitrogen fixation. All pots were daily irrigated. After seedling emergence, they were thinned to four plants per pot.

The following characters were evaluated: a) length of hypocotyl (LH): length in mm, from the soil surface to cotyledonal node, at vegetative stage V2; b) length of epicotyl (LE): length in mm, starting from the cotyledonal node to the first trifoliate node, at vegetative stage V3; c) length of unifoliolate leaf petiole (LUP): length in mm from petiole insertion on the main stem to the insertion on the unifoliate leaf, at vegetative stage V3; d) length of first trifoliate leaf petiole (LTLP): length in mm, from the leaf insertion on the main stem to the insertion of lateral leaflets, at V4; e) rachis length of terminal leaflet of the first trifoliate leaf (RL): length in mm, of the rachis of the first trifoliate leaf, at V4. The Fehr and Caviness (1997) scale was adopted in order to correctly identify the vegetative stages.

The data achieved were submitted to genetic-statistical analyses by the GENES software (Cruz, 2013). Analyses of variance were carried out for all evaluated characteristics basing on the mean of four plants of each experimental plot in consideration to assessing the existence of genetic variability between the eight cultivars.

According to approaches of the variance components, the coefficient of genotypic determination (H^2) was estimated by an estimator:

$$H^2 = \frac{\hat{\phi}_g}{\frac{QMT}{r}} \quad \text{(Equation 1)}$$

where $\hat{\phi}$g: quadratic genetic component; QMT: genotypic mean square; and r: number of repetition.

The joint analyses of variance were achieved following the statistical model described below: $Y_{ij} = \mu + G_i + E_j + GE_{ij} + \varepsilon_{ij}$, where μ is the overall mean; G_i: is the effect of the ith genotype (cultivar); E_j: is the effect of the jth environment (growing season); GE_{ij}: is the interaction of the ith genotype with the jth environment; and ε_{ij}: is the random error.

The effects of genotypic variation source were considered fixed by the fact that they were pre-determined genotypes and do not represent any population sample (Cruz et al., 2012). For the joint analysis, it was verified the existence of residual variance homogeneity obtained through individual analysis by the quotient of highest and lowest mean squared error, adopting the value 7, the maximum accepted value according to Pimentel-Gomes (2000). When the interaction was significant, its decomposition was proceeded by estimating the percentage of simple parts following the Cruz and Castoldi (1991) methodology, and also, estimating the phenotypic and genotypic correlations for the same characteristics between the pairs of environments as described next.

The coefficient of phenotypic correlation was estimated by the following equation:

$$r_f = \frac{COV(Y_{ij}, Y_{ij`})}{\sqrt{\hat{V}(Y_j)\hat{V}(Y_{j`})}} \quad \text{(Equation 2)}$$

where $COV(Y_{ij}, Y_{ij})$: phenotypic covariance of an X characteristic assessed in the environment j and j'; $\hat{V}(Y_j)$: phenotypic variance of an X characteristic in the environment j; and $\hat{V}(Y_{j`})$: phenotypic variance of an X characteristic in the environment j'.

The coefficient of genotypic correlation was estimated using the subsequent expression:

$$rg = \frac{\hat{\varphi}_{g(jj')}}{\hat{\varphi}_{g(jj')} + \hat{\varphi}_{ga(jj')}} \quad \text{(Equation 3)}$$

where: $\hat{\phi}_{g(jj')}$: genetic variability of an X characteristic between the environment j and j'; e $\hat{\phi}_{ga(jj')}$: interaction variability. The statistical significance of the phenotypic correlation coefficient was tested by t test in 1 and 5% levels of probability.

To evaluate the cultivars stability regarding the vegetative stage characters, it was used the method proposed by Wricke (1986) that has as parameter the stability and ecovalence. To estimate ecovalence, the sum of squares of the GE interaction was decomposed in the parts corresponding to each location, according to the following expression:

$$\omega_i = r\sum_j (y_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..})^2 \quad \text{(Equation 4)}$$

where: r: replications; Yij: mean of genotype i in environment j ; $\bar{y}$ i: mean of genotype i; $\bar{y}$ j: average environment j; $\bar{y}$: overall mean.

RESULTS AND DISCUSSION

The genetic variability is the necessary condition for a feature to be useful in differentiating cultivars. It was observed the existence of genetic variability for the characteristics LH, LE, LTLP, and RL at the level of $P \leq 0.01$ by the F test (Table 1) and in the level of $P \leq 0.05$ (Table 1) for the length of the leaf petiole unifoliate indicating the usefulness of these characters to distinguish soybean cultivars. These results corroborate those obtained by Nogueira et al. (2008) evaluating eleven cultivars in four sowing dates and detected genetic variation for the same characters.

Table 1. Summary analysis of variance of quantitative descriptors of vegetative phase in 8 soybean cultivars grown in two sowing dates in Uberlandia, Minas Gerais.

SV	d.f.	Mean square				
		LH	LE	LUP	LTLP	RL
Genotypes (G)	7	53.82**	798.12**	13.49*	427.02**	39.97**
Environment (E)	1	71.79**	2935.78**	17.48^{ns}	2191.19**	115.48**
GxE	7	33.06**	463.68**	26.84**	719.70**	17.57*
Error	32	9.25	32.74	5.11	77.26	6.30

LH = length of the hypocotyls; LE = length of the epicotyls; LUP = length of unifoliate leaf petiole; LTLP = length of first trifoliate leaf petiole; RL = rachis length of terminal leaflet of the first trifoliate leaf. * and **Significant at the level of 5 and 1%, respectively, by the F test; ^{ns}not significant; d.f. = degrees of freedom; SV = sources of variation.

Low magnitude variation coefficient (VC) values indicate experimental precision (Silva et al., 2011). In soybean experiment to evaluate the cultivation and use value, VC up to 20% at most is accepted (MAPA, 2009).

The variation coefficient for the analyzed characters (Table 2) ranged from 8.27% for LH and 27.37% for LR. Nogueira et al. (2008) evaluated 16 soybean cultivars and found VC of less than 16.3% for the same characters in this study, except for LR, which obtained high VC, the highest being 39.1%, which agrees with the data obtained in this study. The high variation coefficient values for LR can be associated with non-homogeneous characteristics along with the cultivar development process. In another study, Liang et al. (2014) made QTL mapping and noted that LH presented continuation data distribution ranging from 7 to near 16 cm, also too close to those found in this experiment.

Table 2. Coefficient of variation and coefficient of genotypic determination (H^2) of the vegetative phase descriptors in eight soybean cultivars at two sowing dates in the city of Uberlândia, Minas Gerais.

Characters	VC (%)	H^2 (%)
LH	8.27	82.81
LE	10.92	95.90
LUP	13.11	62.09
LTLP	8.89	81.91
RL	27.37	84.25

LH = length of the hypocotyl; LE = length of the epicotyl; LUP = length of unifoliolate leaf petiole; LTLP = length of first trifoliate leaf petiole; RL = rachis length of terminal leaflet of the first trifoliate leaf. VC = coefficient of variation; H^2 = coefficient of determination genotypic.

It was observed for all the traits H^2 provided estimates of over 70% (Table 2), indicating high genetic influences on phenotypic expression, except for LUP that was 62.09%. Silva et al. (2016), in a study with 10 soybean cultivars, obtained for the same characters analyzed estimates of H^2 ranging from 78.4 to 92.3%, denoting similarity of results.

Analyzing the medium of characters, it was observed that LH ranged from 32.81 to 43.61 mm respectively for the UFUS 7910 and UFUS Impacta on January sowing (Table 3). In November sowing, the same character ranged from 27.86 to 39.97 mm, respectively, for UFUS Guarani and UFUS Impacta. The overall average for the January sowing was 38 mm. Nogueira et al. (2008), in four experiments with eleven soybean cultivars, had an average of 33 mm, which is similar to that obtained in this study.

Table 3. Average hypocotyl and epicotyl length to eight cultivars grown in a greenhouse in two sowing dates in Uberlandia, Minas Gerais.

Cultivars	LH (mm)		LE (mm)	
	JAN	NOV	JAN	NOV
UFUS Milionária	39.27^{abA}	38.91^{aA}	29.46^{dB}	44.92^{abcA}
UFUS Carajás	32.81^{bA}	37.47^{abA}	79.56^{aA}	47.33^{abcB}
UFUS Riqueza	36.3^{abA}	35.17^{abA}	38.17^{cdA}	37.75^{bcA}
UFUS 7415	41.19^{abA}	37.94^{aA}	77.8^{aA}	60.33^{aB}
UFUS Guarani	38.35^{abA}	27.86^{bB}	62.93^{abA}	53.83^{abA}
UFUS Impacta	43.61^{aA}	39.97^{aA}	55.57^{bcA}	40.58^{bcB}
UFUS Xavante	38.1^{abA}	31.5^{abA}	62.94^{abA}	33.42^{cB}
UFUS Vila Rica	34.33^{abA}	35.57^{abA}	75.19^{aA}	38.33^{bcB}
Average	38.00	35.50	60.15	44.53

LH = length of the hypocotyl; LE = length of the epicotyl. Jan = sowing the month of January 2014; Nov = sowing the month of November 2014. Means with the same capital letter horizontally and with the same letter vertically do not differ statistically from each other at 1% probability by the Tukey test.

In both sowing dates, the cultivar UFUS Impacta showed high average for the LH, while the others cultivars had different estimates in the two periods analyzed.

In January sowing, the average LE ranged from 29.46 to 79.56 mm to cultivars UFUS Milionária and UFUS 7910, respectively (Table 3). In November sowing, the same character oscillated from 33.42 to 60.33 mm to cultivars UFUS Xavante and UFUS 7415, respectively. The overall average for January sowing was 60.15 mm. Matsuo et al. (2012), in an experiment with 22 soybeans genotypes and five measurements, obtained an average of 58.95 mm corroborating the data obtained in this study.

There was predominantly inconsistency between the upper middle, lower and middle of the cultivars to the LE character in different sowing periods.

In January sowing, the average of the character LUP ranged from 11.79 to 21.20 mm to cultivars UFUS Guarani e UFUS Xavante, respectively (Table 4). In November sowing, the same character oscillated from 15.42 to 20.17 mm to cultivars UFUS Xavante and UFUS Guarani, respectively. The overall average for January sowing was 16.59 mm. Silva (2013), in experiments with 16 genotypes and 16 replications, obtained an overall media of 15.2 mm, which is very close to that found in this study.

In LUP, it was ascertained that in January sowing the cultivate UFUS Guarani obtained the lowest average, while in November sowing the same cultivar had the highest average group. The same occurs with the cultivar UFUS Xavante, which obtained the highest average in January and the lowest average in November.

Table 4. Average length of petiole unifoliate leaf, rachis length of terminal leaflet of the 1st trifoliate leaf and rachis of the terminal leaflet of the 1st trifoliate leaf in 8 cultivars grown in a greenhouse in two sowing dates in Uberlandia, Minas Gerais.

Cultivars	LUP (mm)		LTLP (mm)		RL(mm)	
	JAN	NOV	JAN	NOV	JAN	NOV
UFUS Milionária	13.91^{bcA}	16.25^{aA}	76.55^{bB}	128.83^{aA}	6.73^{aB}	16.67^{abA}
UFUS 7910	14.95^{abcA}	17.67^{aA}	99.15^{abB}	121.08^{abA}	10.68^{aB}	17.11^{aA}
UFUS Riqueza	16.76^{abcA}	19.75^{aA}	89.89^{abA}	88.17^{dA}	8.33^{aA}	8.56^{cA}
UFUS 7415	15.38^{abcA}	17.78^{aA}	111.99^{aA}	108.61^{abcdA}	6.12^{aA}	7.78^{cA}
UFUS Guarani	11.79^{cB}	20.17^{aA}	78.68^{bB}	117.25^{abcA}	8.11^{aA}	11.25^{bcA}
UFUS Impacta	18.79^{abcA}	16.83^{aA}	84.24^{abA}	92.42^{cdA}	5.69^{aA}	7.67^{cA}
UFUS Xavante	21.20^{aA}	15.42^{aB}	99.84^{abA}	94.28^{bcdA}	8.52^{aA}	10.03^{cA}
UFUS Vila Rica	20.33^{abA}	18.92^{aA}	96.17^{abA}	93.97^{bcdA}	6.75^{aA}	6.69^{cA}
Average	16.59	17.80	91.89	105.33	7.61	10.70

LUP = length of unifoliolate leaf petiole; LTLP = length of first trifoliate leaf petiole; RL = rachis length of terminal leaflet of the first trifoliate leaf; JAN = sowing in 25/01/2014; NOV = sowing in 27/11/2014. Means with the same capital letter horizontally and with the same letter vertically do not differ statistically from each other at 1% probability by Tukey test.

In January sowing, the average of character LTLP ranged from 76.55 to 111.99 mm to cultivars UFUS Milionária and UFUS 7415, respectively (Table 4). In November sowing, the same character ranged from 88.17 to 128.83 mm to cultivars UFUS Riqueza e UFUS Milionária, respectively. The overall average for January sowing was 91.89 mm. Silva et al. (2016), in a study with ten cultivars in two sowing dates, obtained an overall average of 82.16 mm.

The results for LTLP showed inconsistency as the upper and lower average. The cultivar UFUS Milionária, which showed the lowest average in sowing of January obtained in November sowing the highest estimate of the group.

The character LR in January sowing ranged from 5.69 to 10.68 mm to cultivars UFUS Impacta and UFUS 7910, respectively (Table 4). In November sowing, the same character ranged 6.69 to 17.11 mm to cultivars UFUS Vila Rica and UFUS 7910, respectively. The overall average for sowing in January was 7.61 mm. Silva (2013) in experiments with 16 genotypes and 16 replications obtained overall media 7.8 mm, corroborating the results. For the character LR, the cultivar UFUS 7910 obtained the highest average in both periods of sowing.

In the Table 1, we verified the occurrence of interaction GxE for all characters, indicating differential behavior of genotypes with environmental oscillation, that is, between the sowing dates. These results agree with those obtained by Nogueira et al. (2008), which also detected occurrence of interaction GxE in research with 15 soybean cultivars in four sowing dates, being two summer and two winter seasons.

According to Cruz et al. (2012), the existence of GxE interaction is associated with two factors. The first, called simple, is provided by the difference between genotypes; the second, called complex, is given by the absence of correlation between the genotypes. The interaction is considered simple nature when the decomposition GxE exceeds 50%.

By the decomposition of interaction GxE in simple and complex part by Cruz and Castoldi (1991) methodology, it was found that all the characters had complex nature interaction with the exception of character LR, which was considered simple nature (Table 5).

Nogueira et al. (2008), in a study with 16 cultivars in different sowing times, observed low values for the estimation of simple parts in LH, LE, and LTLP, high values for LUP and high and low values for LR.

Estimates of phenotypic and genotypic correlations between the sowing dates for all characters were low, except for the LR, whose estimate was 0.57 and 0.63 (Table 5), corroborating with the complex nature of the interaction detected by GxE decomposition.

Table 5. Phenotypic correlation coefficient (rf) and genotypic (rg) between the two sowing dates and decomposition of interaction in simple and complex part of Cruz and Castoldi (1991) for quantitative traits of the vegetative phase in soybean.

Characters	S (%)	C (%)	Classification of interaction	$rf_{Jan-Nov}$	$rg_{Jan-Nov}$
LH	14.10	85.90	Complex	0.24^{ns}	0.33
LE	41.90	58.10	Complex	0.34^{ns}	0.36
LUP	-1.98	101.98	Complex	-0.41*	-1.60
LTLP	-9.67	109.67	Complex	-0.26^{ns}	-0.30
RL	67.79	32.21	Simple	0.57*	0.63

LH = length of the hypocotyls; LE = length of the epicotyls; LUP = length of unifoliolate leaf petiole; LTLP = length of first trifoliate leaf petiole; RL = rachis length of terminal leaflet of the first trifoliate leaf; Jan = sowing in January 25, 2014; Nov = sowing in November 27, 2014. * and **Significant at 5 and 1%, respectively, by the *t*-test; ^{ns}not significant.

Considering the characters of vegetative phase allows differentiate cultivars, but has complex GxE interaction to use them as an alternative to distinguish cultivars, becoming important to identify standards cultivars for characterization of cultivar candidates for protection. In this respect, the stability study becomes important because it allows the identification of genotypes that are more stable.

Table 6 shows the results of stability analysis by Wricke (1986) method, which considers the genotype more stable one that presents the lowest values of Wi, meaning less contribution to the GxA interaction. In general, the most stable cultivar for the descriptors of the vegetative phase was UFUS 7415 and UFUS Impacta.

Table 6. Parameter stability (Wi) of Wricke (1986) method, for 8 soybean cultivars grown in a greenhouse in two sowing dates in Uberlândia, Minas Gerais.

Cultivars	Wi (%)				
	LH	LE	LUP	LTLP	RL
UFUS Milionária	2.81	44.70	1.02	44.72	56.98
UFUS 7910	32.75	12.71	1.82	2.10	13.53
UFUS Riqueza	1.12	10.70	2.53	6.91	10.10
UFUS 7415	0.42	0.15	1.13	8.49	2.54
UFUS Guarani	41.90	1.98	41.01	18.69	0.01
UFUS Impacta	0.92	0.19	7.99	0.84	1.55
UFUS Xavante	11.20	8.91	39.02	10.83	3.09
UFUS Vila Rica	8.80	20.80	5.48	7.34	12.17

LH = length of the hypocotyls; LE = length of the epicotyls; LUP = length of unifoliolate leaf petiole; LTLP = length of first trifoliate leaf petiole petiole length of the first trifoliate leaf; RL = rachis length of terminal leaflet of the first trifoliate leaf.

For LH character, the most stable cultivars were UFUS 7415 and UFUS Impacta, with Wi parameter values of 0.42 and 0.92%, respectively. For the LE character, the cultivars, which less contributed to the interaction, were UFUS 7415 and UFUS Impacta, with 0.15 and 0.19%, respectively. In LUP, the most stable cultivars were UFUS Millionaire and UFUS 7415 with values of 1.02 and 1.13%, respectively. To LTLP, the most stable cultivars were UFUS 7910 and UFUS Impacta with 2.10 and 0.84%, respectively. And for the character LR, the cultivars that less contributed in the interaction were UFUS 7415 and UFUS Guarani with 2.54 and 0.01%, respectively.

CONCLUSIONS

LH, LE, LUP, LTLP, and RL allow to differentiate soybean cultivars. The interaction genotypes by environment are complex nature for LH, LE, LUP, and LTLP. The character LR is simple in nature. In general, the most stable cultivars for the descriptors of the vegetative phase were UFUS 7415 and UFUS Impact.

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