

Stability analysis of pure lines and a multiline of soybean in different locations

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Abstract: To compare agronomic performance and phenotypic stability among individual soybean lines and a multiline, eight cultivars and a mixture of them were evaluated in six environments in the 2014/2015 and 2015/2016 crop seasons. At maturity, grain yield (kg ha^{-1}), lodging score, plant height (cm), and first pod height (cm) were evaluated. In the 2015/2016 season, the grain oil and protein content were also evaluated. The GxE interaction was determined by the ecovalence method. The cultivar V-TOP RR had the highest yield, but it was also the highest contributor to the GxE interaction, while the multiline was one of the most stable. The average yield of the multiline did not differ from the average yield of the lines, i.e., the multiline was stable and high yielding. For the other traits, there were no differences between the averages of lines and the multiline by the confidence interval. The mixture of lines is an efficient strategy to increase phenotypic stability in soybean.

Keywords: *Glycine max* (L.) Merrill, genotype mixture, phenotypic stability, genotype-environment interaction.


INTRODUCTION

Soybean (*Glycine max* L. Merrill) is an important global crop. Brazil is the second largest soybean producer in the world, and planted area and production has continued to increase over the years (CONAB 2019). Soybeans are cultivated throughout most of Brazil, and has thus been exposed to a wide range of environments and climates, with differences in soil, temperature, rainfall, and photoperiod. The same soybean cultivar produces different yields depending on variations in environmental conditions. This condition is called the genotype x environment interaction (GxE) and it hinders identification of superior and stable cultivars for the same crop season (Branquinho et al. 2014).

According to Ramalho et al. (2012), there are three ways to determine the effect of the genotype x environment interaction. The first way is to identify specific genotypes for each environment. The second way is to perform ecological zoning and subdivide a region into more homogeneous subregions. The third and most used way is identification of cultivars with high phenotypic stability. This strategy consists of identifying genotypes that tend to remain more stable in the face of environmental variations (Becker and Léon 1988, Bruzi et al. 2007, Cruz et al. 2012). In order to evaluate performance in the desired environments and quantify the adaptability and stability of cultivars, it is common and essential to carry out multi-environment trials (Bhartiya et al. 2017).

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Phenotypic stability is an important concept for researchers interested in studying the genotype x environment interaction. “Stability” refers to the relative ability of genotypes to adapt to climatic fluctuations over the course of crop years within a given location (Ramalho et al. 2012). A population’s genetic structure may influence phenotypic stability; high heterogeneity and heterozygosity confer more stability to the population than high homogeneity and homozygosity (Beckér and León 1988, Acquaah 2016).

The main characteristic sought for in a cultivar in a breeding program is high yield, ideally together with good agronomic characteristics, such as easy management and resistance to major pests. However, these cultivars are often totally or almost completely genetically uniform (Trewavas 2001). For soybean, an autogamous plant, all individuals of a population are genetically identical. This feature facilitates management, but it can become a drawback; it has been reported in the literature that cultivars of self-pollinating plants have less capacity for individual buffering because they exhibit homozygosity in most loci, i.e., they are more susceptible to biotic and abiotic stresses (Acquaah 2016). Thus, the choice of strategies for better adaptation to both the predictable and unpredictable effects of environmental fluctuations requires careful selection of the population’s genetic structure (Bruzi et al. 2007). One strategy under research is the use of a multiline, i.e., a mixture of genotypes, which was developed to increase the heterogeneity of self-pollinating cultivars. In theory, a genotype mixture would have higher population homeostasis and therefore be more stable than the pure line, and this is important to minimize losses in the face of possible adversities in the field (Acquaah 2016).

In a study using *Arabidopsis thaliana* as a model to investigate intraspecific competition between plants at different levels of diversity, the genotypic diversity of *Arabidopsis* was shown to enhance population tolerance to nutritional stress and to a combination of nutritional and heat stress, showing an increase in yield and yield stability compared to the average of the monoculture. The yield from the mixture was as stable and almost as high as the best-performing monoculture throughout the experiment, sustaining the hypothesis that biodiversity increases phenotypic stability (Creissen et al. 2013).

Schutz and Brim (1968, 1971) performed studies on intergenotypic competition in soybean, evaluating the performance and stability of the mixtures. The mixtures tested exceeded or matched the yield of the best component of the mixture. The mixtures were also generally more stable than the pure lines. The authors concluded that the increase in yield was closely associated with the fact that there was no competition loss between plants, whereas the effects of complementary and super compensatory competitions seem to be essential to obtain a high degree of stability in a heterogeneous population. To date, there have been no reports on use of this strategy for soybean in Brazil.

The aim of the present study was to compare agronomic performance among individual soybean lines and the mixture of lines, and to evaluate and compare the phenotypic stability of the genotypes tested.

MATERIAL AND METHODS

The phenotypic data used in this study were obtained from experiments carried out in six environments (sites and crop season) in the state of Minas Gerais, Brazil, in the 2014/2015 and 2015/2016 crop years. The site characteristics, such as altitude, latitude, and longitude, are shown in Table 1.

Eight commercial cultivars from different origins and a mixture of them were evaluated (Table 2). The cultivars were chosen considering similarity among them as a criterion, because this similarity is a fundamental precept of multiline composition (Ramalho et al. 2012). All cultivars have the same pubescence color and belong to similar relative maturity groups. A priority was that each cultivar has a different reaction in relation to the major diseases that affect soybean cultivation.

Table 1. Site characteristics according to altitude (m), latitude, and longitude variables

Location	Altitude (m)	Latitude	Longitude
Lavras	918	21° 14' 43" S	44° 59' 59" W
Patos de Minas	832	18° 34' 44" S	46° 31' 04" W
Ijaci	920	21° 10' 12" S	44° 55' 31" W
Itutinga	969	21° 17' 52" S	44° 39' 28" W
Inconfidentes	869	22° 19' 01" S	46° 19' 40" W

Table 2. List of cultivars evaluated, the companies from which they came, the maturity group (M.G.) they belong to, and their disease resistance

Cultivars	Companies	M.G.	Disease Resistance
BMXFORÇA RR	Brasmax	6.2	stem canker, Phytophthora rot
CD 202 RR	Coodetec	6.4	stem canker, frog eye
CD 215 RR	Coodetec	5.9	Phytophthora rot
NA 5909 RG	Nidera	5.9	stem canker, frog spot, bacterial infection
NK 7059 RR	Syngenta	6.2	stem canker, bacterial pustule, frog eye, Phytophthora rot, cyst nematodes
V - MAX RR	Syngenta	6.2	stem canker, frog eye, cyst nematodes
V - TOP RR	Syngenta	5.9	frog eye, Phytophthora rot

A randomized complete block experimental design was used in a 6 x 9 factorial scheme, i.e., six environments and nine treatments (eight cultivars and the mixture of them), with three replications. Each plot consisted of two 4-m rows, at a spacing of 0.50 m between the rows and external border. Each treatment was composed of a different cultivar, except for the final treatment, which was composed of a mixture of all the cultivars in equal proportion.

The traits evaluated were grain yield in 60-kg bags per hectare (bg ha^{-1}), lodging score (Bernard et al. 1965), plant height (cm), first pod height (cm), and the oil and protein content in the grain (%). The percent of protein and oil content were determined in the whole grain by near infrared reflectance spectroscopy (NIRS), according to Heil (2010). Duplicate readings were made on grain from every treatment using the Thermo, Antaris II device with an integrating sphere and resolution of 4 cm.

Grain yield (bg ha^{-1}), lodging score, plant height (cm), first pod height (cm), and oil and protein content (%) were analyzed with R software (R Development Core Team 2012). The adjusted means were compared by the Scott-Knott (1974) test at 5% probability. Confidence intervals of the difference between two averages were also estimated using the SISVAR statistical program (Ferreira 2011). For the GxE interaction study, the Wricke (1965) ecovalence (ω_i) method was adopted. The contribution to the interaction was obtained for all treatments evaluated considering the grain yield trait. Ecovalence (ω_i) is given as follows: $\omega_i = \sum_{k=1}^n (Y_{ik} - Y_{i.} - Y_{.k} - Y_{..})^2$, where Y_{ik} is the phenotypic observation of the genotype "i" in the "k" environment; $Y_{i.}$ is the phenotypic observation of genotype "i" in all environments; $Y_{.k}$ is the phenotypic observation of the "k" environment for all genotypes; and $Y_{..}$ is the overall average. The relative contribution of each genotype to the genotype x environment interaction is estimated as follows: $\omega_i (\%) = \frac{\omega_i}{\sum \omega_i} \times 100$, where ω_i is the ecovalence value of genotype "i".

RESULTS AND DISCUSSION

In this study, the coefficient of variation (CV) and accuracy were used to measure experimental precision. The CV, according to Pimentel-Gomes (2009), is classified as low (<10%), medium (10-20%), high (20-30%), and very high (>30%). Accuracy is considered high above 70%, medium from 30% to 70%, and low below 30% (Resende and Duarte 2007). The CV ranged from 7.18% to 34.50%, and accuracy ranged from 81.11% to 97.55%, i.e. the experimental accuracy was generally good.

To evaluate the agronomic performance of different genotypes, having experiments conducted in several environments is one of the basic objectives of plant breeding programs (Bueno et al. 2006, Acquah 2016), and one way of evaluating these experiments is by performing a joint analysis among the environments and the crop years (Silva and Duarte 2006, Ward et al. 2019).

For all the traits evaluated, there was a significant difference for the sources of variation for genotypes, for environment, and for the genotype x environment interaction, suggesting that the response of cultivars did not coincide for all the characteristics in the different environments evaluated (Table 3). These differences among the lines tested can be explained because, although the genotypes are similar, there are genetic differences among each of the lines tested, due to their different genetic backgrounds, which, in association with environmental factors like temperature, water availability, and soil conditions, caused differences in phenotypic observations (Silva et al. 2017, Gesteira et al. 2018).

Table 3. Summary of joint analysis of variance for all traits evaluated, considering the six environments in two crop seasons (2014/2015 and 2015/2016) and summary of the joint analysis of variance for oil and protein contents in the grain for Lavras-MG, Ijaci-MG, Itutinga-MG, and Inconfidentes-MG

Source of variation	2014/2015 and 2015/2016 crop season					2015/2016 crop season		
	df	Mean Square				df	Mean Square	
		Lodging	Hei	Pod	Yield		Oil	Protein
Genotypes (G)	8	1.72*	852.00*	18.50*	244.10*	8	2.17*	8.89*
Environments (E)	5	10.43*	14,971.00*	722.50*	2,903.90*	3	13.29*	45.19*
Replicate	12	2.11*	138.00*	7.10	72.50	8	0.83	2.43*
G x E	40	1.01*	98.00*	10.60*	118.00*	24	0.63	1.21
Error	94	0.38	41.00	6.30	77.60	62	0.71	0.99
F _c (Genotypes)		4.63	20.68	2.92	3.15		3.07	8.98
CV (%)		34.50	7.18	15.10	21.16		3.89	2.62
Accuracy (%)		88.54	97.55	81.11	82.60		82.12	94.27
Overall mean		1.77	89.28	16.63	41.55		21.63	38.03

df: degrees of freedom; Hei: plant height (cm); Pod: first pod height (cm); Yield: bg ha^{-1} ; F_c: correction factor; CV: Coefficient of variation. * Significant to 95% confidence by the F test.

Significant effects of genotypes and environments were fundamental for the significance of the genotype x environment interaction (Bhartiya et al. 2017). The genotype x environment interaction represents the differential phenotypic response of genotypes grown under different environmental conditions that cannot be explained by genotypic or environmental components (Cruz et al. 2012). The results obtained by Silveira et al. (2018), studying the effects of adaptability and stability on grain yield in soybean, corroborate with the results obtained from this study, confirming that there are genotypes adapted to specific environments and possibly genotypes with generalist adaptation, which is the premise of the genotype x environment interaction. Previous studies in the southern region of Minas Gerais, Brazil, also reported the existence of genotype x environment interactions in the soybean crop (Gesteira et al. 2015, Soares et al. 2015, Silva et al. 2016, Silva et al. 2017).

The phenotypic averages of the nine genotypes for all traits evaluated are presented in Table 4. For all of them, a wide variation in the averages is observed. For lodging, the treatments had an average score lower than 2.12, showing that even in different environments, the plants did not lodge, i.e., the scores can be considered low according to the scale of Bernard et al. (1965), which ranges from 1 to 5, with 5 being the worst grade for lodging. For grain yield, the BRS 284 cultivar had the lowest performance. V-TOP RR stood out with the highest yield (48.03 bg ha^{-1}); however, this value was not statistically different from the multiline average, which was also high (42.28 bg ha^{-1}). For all traits evaluated, the average of the lines did not differ from that of the multiline by the confidence interval (Table 4).

Table 4. Phenotypic means of joint analysis for the traits evaluated in the six environments in two crop seasons (2014/2015 and 2015/2016)

Genotypes	Lodging	Hei.	Pod	Yield
BRS 284	2.12 b	83.11 c	14.30 b	35.67 b
BMX FORÇA RR	2.00 b	95.07 b	17.37 a	42.53 a
CD 202 RR	2.00 b	89.23 b	17.51 a	43.68 a
CD 215 RR	1.61 b	85.95 c	16.82 a	43.19 a
NA 5909 RG	1.06 a	75.97 d	16.63 a	36.55 b
NK 7059 RR	1.83 b	89.36 b	15.76 b	38.74 b
V - MAX RR	1.94 b	92.27 b	17.29 a	43.28 a
V - TOP RR	1.61 b	100.76 a	17.73 a	48.03 a
MULTILINE	1.72 b	91.80 b	16.25 a	42.28 a
Pure line means	1.77	88.97	16.68	41.46
Pure lines vs. multiline	0.05 (-0.37 to 0.47) ¹	-2.83 (-7.17 to 1.51) ¹	0.43 (-1.27 to 2.13) ¹	-0.82 (-6.79 to 5.15) ¹

Averages followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability. Hei.: plant height (cm); Pod: first pod height (cm); Yield: bg ha^{-1} .

¹ Confidence interval for difference between two means adopting 95% probability.

When using a mixture of genotypes, the average of the mixture is expected to equal or exceed the average of the pure lines composing it. In this study, the mean of the multiline did not differ statistically from the average of the pure lines, and this result is in agreement with the result obtained by Döring et al. (2015), who studied the performance and stability of different wheat populations, including pure lines and cultivar mixtures.

The oil and protein varied with both the variation genotype and environment (Table 3). The protein content and oil content in soybean are mainly determined by the genotype of each cultivar but can vary greatly depending on the environment to which the plant is exposed. The average oil content in the bean may range from 15% to 22%, while the protein content may range from 36% to 40% (Arslanoglu et al. 2011). The genotypes that had higher values for protein content, showed statistically lower oil content. These results support the idea that oil and protein contents have a negative association (Gesteira et al. 2018, Jiang et al. 2018, Matei et al. 2018). The oil and protein content is presented in Table 5. The values obtained in this study corroborate reports in the literature.

There is growing concern regarding the use of genetically uniform cultivars and monoculture as a production system. Although the consumer market most often promotes its use, monoculture restricts the ability of crops to tolerate varied biotic and abiotic stresses. For that reason, the use of cultivar mixtures, such as a multiline, may be an alternative to homogeneity, without loss of yield (Murphy et al. 2013). The use of a multiline is often reported in the literature as a strategy to contain pathogen infections in crops (Mundt 2002), but there are very few studies that quantify the stability of those mixtures.

The study of the genotype x environment interaction using accurate statistical tools undoubtedly contributes to greater efficiency of plant breeding programs (Pour-Aboughadareh et al. 2019). One way to study the genotype x environment interaction is to identify cultivars that have greater adaptability and phenotypic stability (Eeuwijk et al. 2016). In this study, the method used to identify cultivars with greater phenotypic stability was the ecovalence method of Wricke (1965). Among the nine genotypes evaluated, NA 5909 RG and V-TOP RR were the cultivars that most contributed to the genotype x environment interaction, with values of 24.57% and 21.30%, respectively. The genotypes that contributed least to the interaction and therefore had the highest phenotypic stability were BMX FORÇA RR (3.44%), NK 7059 (4.26%), and the multiline (4.52%) (Table 6). The multiline, in addition to being one of the most stable genotypes, showed one of the highest average yields (42.28 bg ha^{-1}). This finding is in accordance with the theory of Allard and Bradshaw (1964) that mixtures of genotypes are more stable because they have greater individual and population homeostasis. Döring et al. (2015) tested performance and stability among composite cross populations, variety mixtures and pure lines of winter wheat and observed that the blends tested were more stable than pure lines, just as observed in the present study. Moreover, this result is in line with the studies of Schutz and Brim (1971) in soybean, Helland and Holland (2001) in oat, and Bruzi et al. (2007) in common bean.

However, the BMX FORÇA RR and NK 7059 RR cultivars were also highly stable, contributing 3.44% and 4.26% to the interaction, respectively, and these values are not necessarily associated with the best yield averages. Although they

Table 5. Mean values of oil and protein content in the grain for Lavras-MG, Ijaci-MG, Itutinga-MG, and Inconfidentes-MG in the 2015/2016 crop year

Genotype	Oil (%)	Protein (%)
BRS 284	22.46 a	36.91 c
BMX FORÇA RR	21.31 b	39.18 a
CD 202 RR	22.18 a	36.77 c
CD 215 RR	21.68 b	37.68 b
NA 5909 RG	21.84 a	37.54 b
NK 7059 RR	21.17 b	38.89 a
V - MAX RR	21.15 b	38.06 b
V - TOP RR	21.40 b	39.11 a
MULTILINE	21.51 b	38.16 b
Pure line means	21.65	38.02
Pure lines vs. multiline	0.14 (-0.74 to 1.02) ¹	-0.14 (-1.48 to 1.20) ¹

The averages followed by the same letter in the columns belong to the same group by the Scott-Knott test at 95% reliability.

¹ Confidence interval for difference between two means adopting 95% probability.

Table 6. Mean values of Wricke Ecovalence (W_i) for grain yield

Genotype	Mean	Wi (%)
BRS 284	35.67	14.81
BMX FORÇA RR	42.53	3.44
CD 202 RR	43.68	8.53
CD 215 RR	43.19	10.01
NA 5909 RG	36.55	24.57
NK 7059 RR	38.74	4.26
V - MAX RR	43.28	8.56
V - TOP RR	48.03	21.30
MULTILINE	42.28	4.52
Total		100.00

are pure lines, according to Becker and Léon (1988), they may be as stable as heterogeneous populations, i.e., these data corroborate the literature. In soybean breeding programs in Brazil, the bulk strategy is adopted for management of segregating populations up to F_3/F_4 . During progeny evaluations, the bulk strategy is adopted within families. Under this condition, the cultivars obtained are actually a mixture of lines and not only a single pure line (Tokatlidis 2015). This corroborates the comments of Becker and Léon (1988) and reinforces that cultivars that are as stable as the heterogeneous populations can be identified.

Although plant breeding programs are very fast and efficient in launching higher yielding and adapted cultivars, climate change and pathogen evolution that affect crops and compromise yield are constant setbacks for agriculture. Therefore, the use of intelligent strategies that can help overcome such challenges is extremely important. Our results showed that the use of a multiline in soybean provided an agronomic performance as good as the performance of the best line tested and was still highly stable. From this, we conclude that the use of a multiline is an efficient strategy to increase phenotypic stability in soybean and may be useful in ensuring success of this crop in the field.

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