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Multiline is a strategy for homeostasis and Asian Soybean Rust Management in Agriculture

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ABSTRACT. Two studies were undertaken with the aim of assessing the phenotypic stability and progression of ASR severity in soybean cultivars within a tropical altitude climate. In the first study, six commercial soybean cultivars containing the INOX® and a multiline cultivar were evaluated in 24 environments during the 2017/18 and 2018/19 growing seasons, assessing yield and full maturity. Stability was assessed using the ecovalence and Annicchiarico confidence index methods. To evaluate ASR progress in the second study, plants were maintained in a greenhouse and inoculated with *Phakopsora pachyrhizi* uredospores. Disease severity, area under the disease progress curve, above-ground biomass, number of pods, number seeds, number of seeds per pod, 100-seed weight, total grain weight, and harvest index were assessed. The multiline cultivar exhibited yield performance statistically similar to the higher-yielding INOX® cultivars, including the cultivar TMG 7067 IPRO, with the highest stability and lowest risk. In terms of ASR progression severity, the multiline cultivar exhibited

statistically superior performance compared to M6410 IPRO, demonstrating enhanced responses for the variables SEV, AUDPC, NBS/P, 100SW, TGW and HI. It can be concluded that the use of resistant cultivars and multiline is effective in reducing the severity of ASR. The employment of multiline is a valuable strategy for the management of Asian soybean rust.

Key words: Genotype × Environment interaction; Adaptability; *Phakopsora pachyrhizi*; Severity; Yield; *Glycine max* L.

INTRODUCTION

The soybean crop is highly affected by the environment. Soybean growth, development, and consequently, grain yield are affected by interactions among cultivars, environmental factors such as disease management, and other predictable or unpredictable environmental factors. Due to the effect of environmental factors on phenotypic expression, a genotype × environment (G × E) interaction typically occurs; i.e., different lines and/ or cultivars respond differently to a given environment (Van Eeuwijk et al., 2016). Furthermore, the genetic structure of the population is expected to affect the magnitude of the G × E interaction. Thus, identification of more stable individuals in the face of environmental variations is of great importance, and it has been the aim of many studies (Carneiro et al., 2019).

A possible strategy for increasing crop stability is to increase biological diversity, due to the effects of compensation and complementation in heterogeneous materials. Heterogeneous line are typically more stable over time than homogeneous lines (Acquaah et al., 2016, Carneiro et al., 2019). The adoption of a multiline cultivar is an alternative to ensure greater heterogeneity in completely homogeneous crops. A multiline cultivar is a mixture of genotypes that have similar morphological and phenological traits and, when possible, different disease resistance alleles of different genes (Carneiro et al., 2019).

Several studies have been carried out to compare the performance of pure lines and mixtures of genotypes. For example, Nogueira et al., (2005) considered coffee, Bruzi et al., (2007) for common bean, and Carneiro et al., (2019) for soybean crops. Multiline and mixtures of cultivars have successfully reduced the incidence of pathogens compared to the incidence obtained for pure lines in some crops, such as apples, rice, sorghum, and common bean (Valério et al., 2018). However, there are no reports of the use of this strategy for disease resistance in soybean crops in tropical regions.

Asian soybean rust (ASR), which is caused by the fungus *Phakopsora pachyrhizi*, is one of the main disease problems in soybean crops (Kato et al., 2022). Asian soybean rust causes damage by reducing the number of pods (NP), the number of bean seeds (NBS), and the weight of bean seeds and pods due to premature defoliation of the plant. Disease control requires a combination of crop practices to minimize damage and loss. Fungicide application is the strategy most commonly used to control ASR, although some populations of the pathogen have shown degrees of tolerance to certain active ingredients (Müller et al., 2021).

Thus, two studies were carried out with the intention of increasing knowledge regarding soybean crops. The first one was to assess the stability of soybean genotypes in

different environments and identify cultivars that contribute less to the G × E interaction under a highland tropical climate. In the second, we aimed to quantify the effectiveness of soybean lines against infection by ASR.

MATERIAL AND METHODS

The experiments were conducted during the 2017/2018 and 2018/2019 crop season. In the first year, experiments were conducted in two locations, Lavras and Ijaci, in the Minas Gerais state (MG). In the second year, experiments were carried out in four locations in MG (Lavras, Ijaci, Itutinga, and Inconfidentes). In each year, the experiments were sown within the same time period. Four experiments were conducted each year and in each location; thus, in combining the total number of sites and experiments, we have eight environments (2 locations × 4 experiments) in the first year and 16 environments (4 locations × 4 experiments) in the second year, for a total of 24 environments.

For the 2017/18 crop year, the plots consisted of two 4-m rows, with a 0.5-m spacing between rows. For 2018/19, the plots consisted of four 5-m rows with a 0.5-m spacing between rows; and the two central rows were used as the plot area for data collection. A randomized complete block experimental design was used with three replicates in a factorial arrangement (24 environments × 7 genetic treatments). Six genetic treatments consisted of different commercial soybean cultivars with INOX® technology, and one genetic treatment consisted of a multiline cultivar, i.e., a mixture of genotypes in equal proportion, with high population homeostasis.

The full maturity trait, in days (90% of the plants in the plot showing pod maturity, stage R8), and grain yield trait (determined from the harvest of the plots) were evaluated (Fehr and Caviness, 1977). After standardizing grain moisture to 13%, the yield in kg ha^{-1} was calculated according to the area of each plot.

Data from the same treatment in different environments were compared for the same response variable (Figure S3). The outlier values were analyzed by the quantiles of the data distribution (McHugh, 2003). The environments were analyzed individually for normality of the residues based on the Shapiro-Wilk test (Shapiro and Wilk, 1965) (Figure S2) and analyzed to detect homogeneity of variance by the maximum *F*-ratio (Hartley, 1950), according to the model:

$$y_{ij} = \mu + \beta_j + \theta_i + e_{ij} \quad \text{(Eq. 1)}$$

where y_{ij} is the value for the trait analyzed in genotype i in block k for site j; μ is the constant associated with all observations, assumed to be fixed; β_j is the effect of block *j*, assumed to be fixed; θ_i is the effect of genotype *i*, assumed to be fixed; and e_{ij} is the effect of the error associated with the observation of genotype *i* in block *j*, assumed to be random ($e_{ij} \sim N(0, \sigma^2_{e_{ij}})$).

Figure S2- Residual Normality Test by Shapiro-Wilk (Supplementary Material)

Figure S3- Flowchart for decision making in data analysis (Supplementary Material)

The joint analysis for the environments was conducted using the mixed model approach, according to the model:

$$y_{ijk} = \mu + \theta_i + A_j + \theta A_{ij} + \beta/A_{kj} + e_{ijk} \quad \text{(Eq. 2)}$$

where y_{ijk} represents the value for the trait analyzed in genotype i in block k for environment j; μ is the constant associated with all observations, assumed to be fixed; θ_i is the effect of cultivar i, assumed to be fixed; A_j is the effect of environment j, assumed to be random (A_j ~ N (0, σ^2_{Aj})); θA_{ij} is the effect of interaction between cultivar i and environment j, assumed to be random (θA_{ij}~ N (0, $\sigma^2_{\theta A_{ij}}$)); β/A_{kj} is the effect of block k within environment j, assumed to be fixed; and e_{ijk} is the error effect associated with the observation of cultivar i in environment j and block k, assumed to be random, with a structure of residual variances and covariances represented by a diagonal matrix $e_{ijk} \sim N(0, \bigoplus_{j=1}^{j} I\sigma^2_{e_{ijk}})$.

The experimental precision was analyzed by the coefficient of variation (CV) (Pimentel, 2009) and by accuracy estimation (Resende and Duarte, 2007). The coefficient of variation was calculated by the equation:

$$CV = \left(\frac{\sqrt{MSE}}{\bar{X}}\right) * 100 \quad \text{(Eq. 3)}$$

where MS_E is the mean square of the residue and $\bar{X}$ is the overall average. Accuracy was calculated by the equation:

$$Accuracy = \sqrt{1 - \frac{1}{F_c}} \quad \text{(Eq. 4)}$$

where F_c is the value of the *F*-test for the effect of cultivars.

The stability of the cultivars was evaluated by the method of Wricke (1964) by using the phenotypic means for grain yield. The ecovalence of each genotype W_i was estimated by partitioning the variance of the cultivar × environment interaction according to the following expression:

$$W_i = \sum_{j=1} \left(\bar{y}_{ij} - \bar{y}_{i.} - \bar{y}_{.j} + \bar{y}_{..}\right)^2 \quad \text{(Eq. 5)}$$

where $\bar{y}_{ij}$ is the adjusted mean of cultivar i in environment j; $\bar{y}_{i.}$ is the adjusted mean of cultivar i in the evaluated environments; $\bar{y}.j$ is the adjusted mean of environment j; and $\bar{y}_{..}$ is the overall mean. The relative contribution (W_i(%)) of each cultivar to the interaction per environment is given by:

$$W_i(\%) = \frac{W_i}{\sum_i W_i} \times 100 \quad \text{(Eq. 6)}$$

where W_i is the ecovalence value of genotype i. For the confidence index (I_i) of Annicchiarico (1992), the following model was used:

$$I_i = \bar{Y}_{i.} - Z_{(1-\alpha)}(\sigma_{i.}) \quad \text{(Eq. 7)}$$

where $\bar{Y}_{i.}$is the mean of cultivar i; $Z_{(1-\alpha)}$ is the value in the standardized normal distribution at which the cumulative distribution function reaches the value with a significance level α (preset by the author at 0.05); and $\sigma_{i.}$ is the standard deviation.

The difference and confidence interval were also estimated with a significance level of $P < 0.05$ for two means, adopting the average effect of the multiline cultivar compared to the average of the INOX® cultivars.

The second experiment was carried out in Lavras (MG) in a greenhouse located in the Plant Pathology sector of the Federal University of Lavras (UFLA). A randomized complete block statistical design was used with four replicates. Each replicate consisting of four 5-L pots, with three plants per pot. Six soybean cultivars with INOX® technology were used, as well as one multiline cultivar and one susceptible cultivar (M6410 IPRO).

The plants were kept in a greenhouse and inoculated with uredospores of *P. pachyrhizi*. Leaves with uredinal rust lesions were collected at UFLA and immediately immersed in water to remove the uredospores. Then, a spore suspension was prepared in distilled water containing Tween 20 (0.1 mL L^{-1}). For inoculation, 10 mL of the suspension (9×10^4 uredospores mL^{-1}) per pot were used. The uredospore suspension was sprayed once at the pour point on the abaxial surface of the leaves when the plant was at the R3 developmental stage. After inoculation, the plants were kept in a dark, humid chamber inside a greenhouse for 12 hours, with humidity close to 100% to favor germination and penetration of the pathogen.

Disease severity (SEV), which was defined as the percentage of leaf area covered with disease symptoms, was quantified using a diagrammatic scale (Godoy et al., 2006). The evaluations began fifteen days after inoculation of the plants with the *P. pachyrhizi* inoculum. The data was analyzed using the model:

$$y_{ijk} = \mu + \theta_i + \tau_k + \theta\tau_{ik} + \beta_j + (\beta\theta_{ij}) + (\beta\tau_{jk}) + e_{ijk} \quad \text{(Eq. 8)}$$

where y_{ijk} is the effect of cultivar *i* in block *j* during evaluation *k*; μ is a constant associated with all observations, assumed to be fixed; θ_i is the effect of cultivar *i*, assumed to be fixed; τ_k is the effect of evaluation *k*, assumed to be fixed; $\theta\tau_{ik}$ is the effect of interaction between cultivar *i* and evaluation *k*, assumed to be fixed; β_j is the effect of block *j*; $(\beta\theta_{ij})$ is the error effect associated with the cultivar effect during evaluations, assumed to be fixed; $(\beta\tau_{jk})$ is the error associated with the evaluation effect, assumed to be fixed; and e_{ijk} is the general error associated with cultivar *i* in block *j* during evaluation *k*.

Severity was assessed eight times, with an interval of two or three days between evaluations. Two trifoliate leaves per plant were evaluated (one from the upper third and one from the lower/middle third), for a total of 72 leaflets evaluated per plot. Mean SEV was an estimate of the mean disease severity for each line. The area under the disease progress curve (AUDPC) was obtained by:

$$AUDPC = \sum_{i=1}^{n}\left[\frac{Y_{i+1}+Y_i}{2} x\,(T_{j+1} - T_j)\right] \quad \text{(Eq. 9)}$$

where Y_i is SEV at the time of evaluation *i*; Y_{i+1} is SEV at the time of evaluation *i*+1; T_j is the time of evaluation *j*, in number of days; and T_{j+1} is the time of evaluation *j*+1. This data was analyzed with the model of equation one.

At harvest the following traits were evaluated: above-ground biomass (AB), number of pods (NP), number of bean seeds/pod (NBS/P), 100 seed weight (100SW), total grain weight (TGW), and harvest index (HI). Above-ground biomass (AB) was determined by harvesting and weighing the three plants of each pot (12 plants). Subsequently, NP and the number of bean seeds (NBS) were counted, and NBS/P was also determined. The grain

weight obtained in each replicate was corrected for a moisture content of 13% to obtain TGW. The 100 seed weight (100SW) determination was made by counting 100 grains and weighing them; and HI was determined as the ratio between TGW and AB.

The data obtained from both studies were analyzed using the R environment (R Core Team, 2020) version 4.1.3. Data were manipulated and graphs were generated using the Tidyverse tools. Fixed model analyses were conducted using the *stats* package, while mixed model analysis was carried out using the *sommer* package. Adjusted means were obtained using the *emmeans* package. The Scott-Knott test (1974) with a significance level of $P < 0.05$ for mean comparisons was applied using the *ExpDes* package. The Dunnett test was used to assess the effect of the multiline in relation to the INOX® cultivars using the *multcomp* package.

RESULTS

In the individual variance analysis, a significant difference was observed among environments for days to maturity (DTM – 4, 7, 10, 12, 14, 15, 16, 17, 18, 19, 20, and 21). The grain yield exhibited no significant variation across environments 4, 9, 10, 11, 12, 13, 14, 22, and 24 highlighting the effect of the location and year interaction (Table 1). The environment effect was not significant in joint analysis. However, the cultivar (C) × environment (E) interaction was significant for both traits evaluated (Table 2). For joint analysis, the variances showed heteroscedasticity, based on the distribution of maximum F values ($P < 0.05$).

Table 1. Summary of analysis of individual variance for the variables of the days to maturity (DTM) and grain yield in six commercial soybean cultivars and one multiline, evaluated in 24 environments applying the mixed model approach.

Envir.	DFe	Days to maturity (DTM)					Grain yield				
		MS_E	CVe	F-Gen	*r̂gg*	p-Res	MSE	CVe	F-Gen	r̂gg	p-Res
1	12	9.38	2.49	2.99	0.82	0.73	231550.83	11.72	3.13*	0.82	0.42
2	12	5.5	1.89	2.92	0.81	0.55	337801.81	12.13	5.74*	0.91	0.29
3	12	8.96	2.36	1.68	0.64	0.45	120808.3	6.76	5.96*	0.91	0.68
4	12	3.94	1.6	8.99*	0.94	0.93	286079.22	10.21	1.74	0.65	0.47
5	12	5.27	1.73	1.58	0.61	0.11	88275.78	11.44	14.59*	0.97	0.61
6	12	9.39	2.51	2.05	0.72	0.31	63279.17	8.40	23.74*	0.98	0.21
7	12	7.24	2.04	4.59*	0.88	0.53	73702.04	7.97	30.29*	0.98	0.19
8	12	3.51	1.42	1.04	0.2	0.14	282099.95	16.97	6.74*	0.92	0.37
9	12	2.4	1.32	2.83	0.8	0.04	426382.14	17.42	1.26	0.45	0.81
10	12	0.89	0.79	8.18*	0.94	0.18	260841.74	12.22	1.90	0.69	0.45
11	12	3.34	1.51	1.82	0.67	0.92	242403.01	10.79	1.95	0.7	0.97
12	12	4.11	1.64	6.13*	0.91	0.89	663521.01	15.42	2.97	0.81	0.39
13	12	7.14	2.33	1.51	0.58	0.07	720289.06	19.93	1.28	0.47	0.25
14	12	2.92	1.45	7.13*	0.93	0.15	296247.24	13.64	2.87	0.81	1.00
15	12	1.02	0.86	36.51*	0.99	0.24	224851.31	11.62	4.93*	0.89	0.75
16	12	1.57	1.06	13.42*	0.96	0.02	169932.48	9.78	6.26*	0.92	0.74
17	12	1.78	1.14	8.98*	0.94	0.23	83586.72	8.85	4.32*	0.88	0.99
18	12	4.13	1.73	5.39*	0.9	0.31	117778.95	10.51	6.65*	0.92	0.33
19	12	4.98	1.9	3.60*	0.85	0.34	117748.47	10.12	3.54*	0.85	0.18
20	12	2.83	1.46	3.33*	0.84	0.2	102352.8	9.87	3.88*	0.86	0.2
21	12	1.05	0.86	4.00*	0.87	0.14	131911.52	8.87	6.23*	0.92	0.87
22	12	2.52	1.33	0.46	0.68	0.59	233658.57	11.74	2.39	0.76	0.65
23	12	2.45	1.31	0.17	0.42	0.44	212286.14	10.56	6.29*	0.92	0.79
24	12	3.29	1.51	0.36	0.6	0.97	203646.19	9.85	2.03	0.71	0.64
						9.51**					11.38**

*Significant at $P < 0.05$. ** Significant based on maximum F distribution ($P < 0.05$) of the Hartley test. Envir: environment. DFe: Error degrees of freedom; MS_E: Mean square of the error; CVe: Coefficient of variation; F-Gen: Stimulated value; *r̂gg:* Accuracy, P- Res: P-value. Environment: 2017/2018 – 1-4 Lavras, 5-8 Ijaci; 2018/2019 – 9-12 Lavras, 13-16 Ijaci, 17-20 Itutinga, 21-24 Inconfidentes.

Table 2. Summary of joint variance analysis for grain yield traits in kg ha^{-1} and absolute maturity in days in six commercial soybean cultivars and one multiline, evaluated in 24 environments.

SV	Effect	Grain yield	Absolute maturity
Environment (E)	A	52474.81^{ns}	0.80^{ns}
Block/E	F	49842443.86*	2626.39*
Cultivar (C)	F	738263.78*	15.78*
C × E	A	152039.86*	1.16*
σ^2_{ej} ~ j = 1	A	216305.52	11.70
σ^2_{ej} ~ j = 2	A	341246.23	5.54
σ^2_{ej} ~ j = 3	A	120268.90	7.79
σ^2_{ej} ~ j = 4	A	263677.10	4.68
σ^2_{ej} ~ j = 5	A	97689.28	4.39
σ^2_{ej} ~ j = 6	A	66780.00	9.61
σ^2_{ej} ~ j = 7	A	82231.94	8.58
σ^2_{ej} ~ j = 8	A	309107.53	4.03
σ^2_{ej} ~ j = 9	A	369261.68	2.34
σ^2_{ej} ~ j = 10	A	247875.69	0.82
σ^2_{ej} ~ j = 11	A	243244.96	2.88
σ^2_{ej} ~ j = 12	A	631864.59	3.97
σ^2_{ej} ~ j = 13	A	652677.58	6.26
σ^2_{ej} ~ j = 14	A	279078.20	3.45
σ^2_{ej} ~ j = 15	A	211461.42	1.65
σ^2_{ej} ~ j = 16	A	168210.19	1.58
σ^2_{ej} ~ j = 17	A	78627.51	1.84
σ^2_{ej} ~ j = 18	A	115970.57	3.97
σ^2_{ej} ~ j = 19	A	122985.05	4.58
σ^2_{ej} ~ j = 20	A	97376.51	2.72
σ^2_{ej} ~ j = 21	A	134415.26	1.00
σ^2_{ej} ~ j = 22	A	220866.67	2.38
σ^2_{ej} ~ j = 23	A	227022.21	2.39
σ^2_{ej} ~ j = 24	A	194228.49	3.01
Normality of the residuals (*p*-value)		0.4015	0.0778

SV: Source of variation; A: Effect assumed as random - estimated variance component. F: Effect assumed as fixed - quadratic component estimated by the model and σ^2_{ej}: Residual variance in environment *j*. * Significant by the *F*-test for fixed effects (F) and Likelihood Ratio Test (LRT) for random effects (A) at a significance level of P < 0.05.

Regarding the cultivar factor, the multiline was in the highest grain yield group (4157 kg ha^{-1}), with 481.66 kg ha^{-1} more than the lower-yielding INOX® cultivars (TMG 7363 RR, TMG 7060 IPRO, TMG 7262 RR). Based on the data, phenotypic correlation between grain yield traits and full maturity was not observed since there was no consistent pattern among the higher-yielding cultivars and an increase in the phenological cycle (Table 3).

Comparison of grain yield showed a huge variation in amplitude – 863.44 kg ha^{-1}. The amplitude of days to full maturity was lower than the amplitude measured for grain yield. The multiline is one of the most stable genotypes, contributing 13.66% to the G × E interaction, using W_i (Table 3). In addition to exhibiting relatively high yield and stability, the multiline also exhibits lower risk compared to four other INOX® cultivars, as indicated by Annichiarico's confidence index.

The TMG 7067 IPRO cultivar led the highest-yielding cultivar group, combining high stability and low risk. In this context, we can expect it to have an average yield 7.60% higher than the overall average yield, while for the multiline, the expected increase is 2.76%. In the second experiment, conducted in a greenhouse, the CV exhibited good accuracy for all evaluated traits, with values exceeding 20% only for disease severity analysis.

Table 3. Joint phenotypic means for the grain yield in kg ha^{-1} and absolute maturity in days traits for the different cultivars, mean values of Wricke's ecovalence (W_i), and Annicchiarico's confidence index (I_i).

Cultivar	Yield			Absolute maturity
	Grain yield	W_i%	I_i%	
TMG 7067 IPRO	4432.70 a	14.46	107.60	123.48 a
TMG 7063 IPRO	4286.66 a	11.00	105.03	121.08 b
MULTILINE	4157.86 a	13.66	102.76	121.69 b
TMG 7062 IPRO	4104.40 a	17.40	101.81	120.79 b
TMG 7363 RR	3895.24 b	13.77	96.38	121.43 b
TMG 7060 IPRO	3564.47 b	14.15	85.11	122.23 b
TMG 7262 RR	3569.26 b	15.55	85.28	118.36 c
Means of cultivars	3975.49	-	-	121.23
Multilines *vs.* Cultivars	191.93	-	-	0.75
Lower limit[1]	-12.12	-	-	-0.75
Upper limit[1]	395.97	-	-	2.26
Fc (Cultivars)	23.58	-	-	85.60
Accuracy (%)	97.86	-	-	99.41
CV (%)	11.96	-	-	1.69
Overall mean	4001.52	-	-	121.30

Means followed by the same letter belong to the same group according to the Scott-Knott test at $p < 0.05$. [1] Confidence interval for the difference between two means, adopting $P < 0.05$

Precision, measured by accuracy, was high for all traits except for NP (61.26%). Using Dunnett's test ($P < 0.05$), the disease severity of the cultivar without INOX® biotechnology was higher than that of the multiline. For the other cultivars, no significant differences were detected (Table 4). A similar pattern was observed for the traits AUDPC, 100SW, TGW, and HI. The multiline did not differ from any of the other cultivars for NP and AB. The M6410 IPRO cultivar exhibited lower performance for the NBS/P ratio.

The susceptible cultivar showed higher SEV than the other genotypes from the beginning of the evaluations (Figure 1). Inoculation with *P. pachyrhizi* in R3 caused a significant reduction in NBS and consequently in NBS/P. In the current study, the mean value of 100SW for the INOX® cultivars was 13.92 g, while for the multiline cultivar, it was 14.69 g. This outcome highlights the effectiveness of INOX® and multiline technologies compared to the susceptible cultivar, which had an average 100SW of 8.23 g. Considering only 100SW, there was a 56% reduction in grain weight for the susceptible cultivar compared to the multiline.

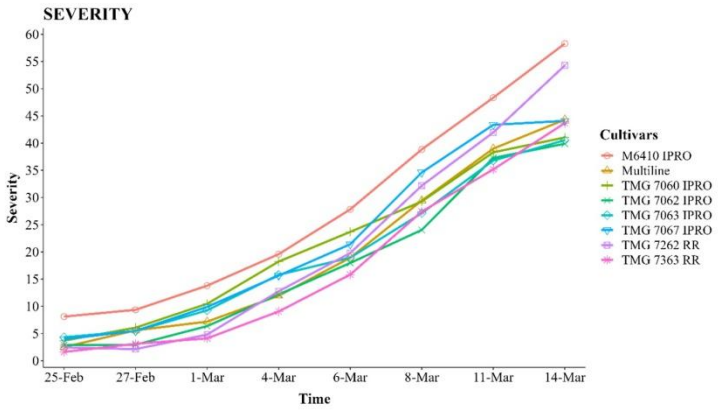

Figure 1. Asian soybean rust (ASR) severity curve (%) as a function of evaluation date for the evaluated genotypes.

Table 4. Joint means for the following traits: rust severity (SEV), area under the disease progress curve (AUDPC), number of bean seeds (NBS), number of pods (NP), number of bean seeds per pod (NBS/P), 100 seed weight (100SW) in g, total grain weight (TGW) in g, above-ground biomass (AB) in g, and harvest index (HI) in percentage for the different cultivars.

Cultivars	SEV	AUDPC	NBS	NP	NBS/P	100SW	TGW	AB	HI
TMG 7363 RR	17.49 a^{ns}	311.32 a^{ns}	363.50 a^{ns}	183.50 a^{ns}	1.96 a^{ns}	14.29 a^{ns}	52.09 a^{ns}	105.50 a^{ns}	0.49 a^{ns}
TMG 7062 IPRO	17.94 a^{ns}	332.28 a^{ns}	328.50 a^{ns}	195.50 a^{ns}	1.67 b^{ns}	14.79 a^{ns}	48.51 a^{ns}	106.50 a^{ns}	0.45 a^{ns}
TMG 7063 IPRO	19.78 a^{ns}	352.85 a^{ns}	344.50 a^{ns}	188.00 a^{ns}	1.82 b^{ns}	12.92 a^{ns}	44.48 a^{ns}	104.75 a^{ns}	0.43 a^{ns}
MULTILINE	19.86 a	356.40 a	329.00 a	183.75 a	1.78 b	14.69 a	48.30 a	103.75 a	0.46 a
TMG 7262 RR	21.29 a^{ns}	382.87 a^{ns}	362.25 a^{ns}	168.50 a^{ns}	2.02 a*	14.42 a^{ns}	52.44 a^{ns}	100.00 a^{ns}	0.52 a^{ns}
TMG 7060 IPRO	21.34 a^{ns}	383.61 a^{ns}	390.50 a^{ns}	192.75 a^{ns}	2.03 a*	13.22 a^{ns}	51.86 a^{ns}	110.50 a^{ns}	0.47 a^{ns}
TMG 7067 IPRO	22.30 a^{ns}	410.41 a^{ns}	353.25 a^{ns}	202.50 a^{ns}	1.73 b^{ns}	13.88 a^{ns}	49.37 a^{ns}	119.50 a^{ns}	0.41 a^{ns}
M6410 IPRO	28.02 b*	510.55 b*	237.50 b^{ns}	168.25 a^{ns}	1.44 c*	8.23 b*	19.49 b*	67.99 b^{ns}	0.29 b*
Cultivars Means	20.0.2	362.23	357.08	188.49	1.88	13.92	49.80	107.79	0.47
Multiline *vs.* Cultivars	-1.31	-5.82	-28.08	-4.71	-0.09	0.78	-1.50	-4.04	-8,00E-04
Lower limit[1]	-7.40	-68.86	-58.98	-10.70	-0.27	-0.83	-8.82	-13.07	-0.04
Upper limit[1]	4.78	57.22	2.82	1.28	0.09	2.37	5.83	4.99	0.04
Fc (Cultivars)	19.17	4.03	3.17	1.60	15.47	13.99	6.54	2.54	5.88
Accuracy (%)	97.36	86.70	83.31	61.26	96.71	96.36	92.04	77.87	91.09
CV (%)	20.22	16.06	14.89	10.40	5.55	8.67	18.71	18.43	12.72
Overall mean	21.00	380.04	338.62	185.34	1.81	13.31	45.82	102.31	0.45

1 Confidence interval for the difference between two means, adopting $P < 0.05$. * Significant, ns non-significant at $P < 0.05$ by Dunnett's test

DISCUSSION

Experimental precision, assessed by the coefficient of variation, was good. This can be explained by the large number of environments available. The larger the number of replications, the better the estimates and precision of inferences and recommendations (Ramalho et al., 2012). However, other statistics, such as heritability, the coefficient of determination, the *F*-test value for genotype, the Fasoulas differentiation index (Fasoulas, 1983), and selective accuracy have been proposed (Cargnelutti and Storck, 2009) to evaluate experimental precision. As these statistics are associated with greater genetic variability and lower residual variances, they are more appropriate than the coefficient of variation and the minimum significant difference (MSD) (Cargnelutti and Storck, 2009).

The genotype by environment (G×E) interaction is responsible for differences in genotypic performance in different growing environments and is one of the main challenges in a plant breeding program for cultivar selection and recommendation. Therefore, evaluation in multi-environment trials is necessary to assess the presence and magnitude of the G×E interaction. One way to evaluate these experiments is to conduct a joint analysis between environments and crop years, as adopted in this study.

Under experimental conditions where interactions between two independent variables (e.g., location and year, different storage parameters, etc.) are statistically proven, joint analysis enables the combination of both variables to represent the environment, which can serve as a basis for stability analysis (Flajšman et al., 2018), as was done in this study. However, the environmental context is often poorly characterized in multi-environment datasets used for linking crop growth conditions to eco-physiological processes, thereby compromising the representativeness of the environment.

Although the G×E interaction was significant in joint analysis, it is possible to identify locations where the cultivars did not display different responses, regardless of the genotype. This consistency confirms the presence of genotypes adapted to specific environments and, possibly, genotypes with overall adaptability, which is the essence of the G×E interaction. These results corroborate those obtained by Silva et al., (2022), who also reported the existence of G×E interactions in soybean crops in previous studies in the southern region of MG, Brazil.

Studying the G × E interaction using precise statistical tools undoubtedly contributes to greater efficiency in plant breeding programs. One way to study the G×E interaction is to identify cultivars with greater adaptability and phenotypic stability. In this study, two distinct but complementary analyses were carried out, as reported by Wricke (1965) and Annicchiarico (1992). Using the Wricke (W_i) method, cultivars with greater agronomic stability can be identified, that is, those that contribute less to the interaction and have more predictable responses in spite of temporary variations caused by the environment. The multiline cultivar had one of the smallest deviations for grain yield within the different environments, indicating greater stability. The genetic structure of a population can affect phenotypic stability; high heterogeneity and heterozygosity confer more stability to the population than high homogeneity and homozygosity (Aquaah, 2016).

In theory, a mixture of genotypes, as in the case of multilines, would have greater population homeostasis and would therefore be more stable than pure lines, which is important for minimizing losses in the face of possible adversities in the field (Aquaah, 2016). These results are consistent with those found by Carneiro et al., (2019), where the multiline evaluated was one of the most stable cultivars. In the present study, although the multiline was not the most stable, it performed as well as the highest yielding cultivar. Increasing productivity and reducing days to maturity are among the main objectives of soybean breeding programs (Carneiro et al., 2019), and multilineage has performed well in this regard. Its cycle was just three days longer than that of the earlier cultivar (TMG 7262 RR) and it presented a higher yield than the same.

The Annicchiarico method considers the ideal cultivar to be the one with the lowest risk of adoption, because it has the highest confidence index . Thus, the multiline was one of the cultivars that contributed least to the interaction. Miranda (1999) found that the stability × cycle ecovalence is inversely proportional to the cycle, and affirmed that the earlier the maturity of the material, the lower its stability, correlating the results found in this study.

Asian soybean rust (ASR) is a global threat to soybean production (Meira et al., 2020). One of the main management strategies includes the use of genetic resistance, which has been exploited by developing cultivars carrying resistant genes. However, among the recommended soybean cultivars, none are resistant enough to eliminate the use of fungicides to control ASR. This pathogen is characterized by a broad host range, a consequence of genes that contribute to a diverse and complex virulence pattern (Hartman et al., 2005).

Disease severity assessment shows that the use of multiline is a promising strategy for obtaining long-lasting resistance in cultivated plants, especially in the soybean immunity pathosystem (without visible symptoms) specific to *P. pachyrhizi*, in which the pathogen shows wide variability. Therefore, it is important to evaluate the response of multiple lines in the search for greater stability and resistance to ASR in soybean growing.

In a study by Sacon et al., (2020), the authors concluded that the INOX® cultivars evaluated (TMG 7062, TMG 7161, and TMG 7261) delayed disease progression; however, only TMG 7161 showed tolerance to the presence of the inoculum in the 2016/17 and 2017/18 seasons. The virulence of ASR varies in different field environments, and shows annual and regional variations as well, which makes it important to know the full diversity of *P. pachyrhizi*, its virulence, and how soybean cultivars react to pathogens in different grain-producing regions.

The use of genetic resistance to ASR in the host plant offers a sustainable alternative to the use of fungicides, as it can bring about long-term pathogen management (Godoy et al.,2016). Eight Rpp loci for resistance to *P. pachyrhizi* have been identified and mapped in the soybean genome so far (Rpp1 to Rpp7): Rpp1 from PI 200492 (Hyten et al., 2007), Rpp1-b from PI 594538A (Chakraborty et al., 2007), Rpp2 from PI 230970 (Silva et al., 2008), Rpp3 in PI 462312 (Hyten et al., 2009), Rpp4 in PI 459025 (Silva et al., 2008), Rpp5 in PI 200456 (Garcia et al., 2008), Rpp6 in PI 567102B (Li et al., 2012), and Rpp7 in PI 605823 (Childs et al., 2017). However, none of these resistance genes is effective against all currently known soybean rust pathotypes (Childs et al., 2017), so combining use of resistant cultivars and prudent application of fungicides in an integrated disease management program is the best strategy for ASR control.

R-genes confer different degrees of resistance (brown-red lesions) or immunity (no visible symptoms) to specific *P. pachyrhizi* pathogens. This qualitative immunity/resistance can be quickly overcome by the pathogen. An R gene pyramid is an efficient strategy to increase the durability and resistance spectrum of soybean cultivars (Mundt, 2018), and the adoption of multilines also appears to be an efficient strategy for that purpose. In order to analyze inheritance of the gene of resistance to *P. pachyrhizi* in TMG 803 (a line with INOX® biotechnology), and to identify microsatellite markers linked to that resistance gene, Matsuo et al., (2014) concluded that the resistance of the cultivar TMG 803 is governed by a gene with complete dominance, mapped as the resistance locus Rpp4, of linkage group G.

Em another study, Aoyagi et al., (2020), when characterizing the occurrence of three local soybean races to ASR, concluded that the resistances of these are related to the Rpp1 and Rpp3 loci, implying the utilization of these for the development of cultivars resistant to ASR.

Although plant breeding programs are very efficient at releasing adapted and higher-yielding cultivars, climate changes and the evolution of pathogens that compromise crop yields are constant challenges for agriculture. Therefore, the use of intelligent strategies that can help overcome these challenges is of utmost importance (Carneiro et al., 2019). Our results showed that the use of multiline in soybean provided agronomic performance as good as the best pure line tested and provided quite stable yield. It can be concluded that the use of resistant cultivars and multiline is effective in reducing the severity of ASR. The employment of multiline is a valuable strategy for the management of Asian soybean rust.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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