

## Adaptability and stability of soybean for grain yield in shaded environments

Paulo Ricardo Américo Gloria<sup>1</sup>, Lucas Gomes da Silva Pereira<sup>1</sup>, José Cola Zanuncio<sup>2</sup>, Eder Matsuo<sup>3</sup>, Cristina Moreira Bonafé<sup>1</sup> and Anderson Barbosa Evaristo<sup>1\*</sup>

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**Abstract:** The aim of this study was to identify, through different methodologies, soybean cultivars with adaptability and stability for grain yield in environments with different levels of light restriction. The grain yield of sixteen cultivars was evaluated in environments with 25% and 48% restriction of photosynthetically active radiation (PAR) in the agricultural years 2019/2020 and 2021/2022. Based on the results, an adaptability and stability analysis was performed using the Eberhart and Russell, ANN (Artificial Neural Network) and GGE (Genotype plus Genotype-Environment interaction) methods. Grain yield varied with the levels of PAR restriction and agricultural years, being higher in environments A3 (2021/2022 25% PAR) and A1 (2019/2020 25% PAR), respectively. Cultivars NS7780, 8579RSF, NS8338 and RK6718 showed higher yield. The adaptability of cultivars AS3680, M7110, and 74177RSF was low, while that of NS8338 and NS7780 was high. Cultivars NS8338, 74177RSF, RK7518, and M6210 showed high phenotypic stability to environments.

**Keywords:** Glycine max, artificial neural network, photosynthetically active radiation

### INTRODUCTION


In integrated production systems, such as the agroforestry system, light interception modifies the quantity and quality of solar radiation according to the angle of solar rays' incidence, canopy dimension, leaf area, leaf geometry, and plant canopy architecture (Santos et al. 2018, Sgarbossa et al. 2020). The biomass production of crops depends on the photosynthetically active radiation absorbed by the leaves and the conversion and assimilation of photoassimilates. Light restrictions can change morphology, reduce growth and development, and consequently, reduce plant productivity (Sgarbossa et al. 2020).

Soybean [*Glycine max* (L.) Merrill], a source of high-quality protein and vegetable oil, is widely cultivated for human consumption, animal feed, and raw material for a wide variety of products (Mwiinga et al. 2020). In Brazil, soybean is one of the main grain crops produced in the agroforestry system (Leite et al. 2023). However, shading from tree components can reduce the plant's photosynthetic rate, decrease leaf mass per unit area, inhibit the transport of sucrose and its degradation into cellulose in the stem, leading to excessive stem elongation and branches without resistance and prone to lodging (Liu et al. 2016, Liu et al. 2017).



\*Corresponding author:

E-mail: anderson.evaristo@ufvjm.edu.br

 ORCID: 0000-0003-1236-0196

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<sup>1</sup> Universidade Federal dos Vales do Jequitinhonha e Mucuri, Rua da Glória, 187, Centro, 39100-000, Diamantina, MG, Brazil

<sup>2</sup> Universidade Federal de Viçosa, Avenida PH Rolfs, s/n, Campus Universitário, 36570-900, Viçosa, MG, Brazil

<sup>3</sup> Universidade Federal de Viçosa, Campus Rio Paranaíba, Rodovia BR 230, km 7, 38810-000, Rio Paranaíba, MG, Brazil

The high efficiency of soybean cultivation in the agroforestry system is linked to the choice of shading-tolerant cultivars (Wen et al. 2020). Genetic improvement seeks to develop cultivars with high yield, adapted, and stable for various regions and agroecological conditions (Milioli et al. 2018). However, there is complexity due to the significant influence of genotype x environment interaction on phenotypic expression. One way to take advantage of this interaction is to identify highly adaptable cultivars to different environments (Bornhofen et al. 2017).

Several study methods to evaluate the phenotypic stability of cultivars are described in the literature, differing in statistical methods (Milioli et al. 2018). Among them are univariate parametric models (Eberhart and Russell 1966), model based on artificial neural networks (ANN) (Nascimento et al. 2013), and multivariate parametric models through graphical biplot analysis (GGE) (Yan and Kang 2002).

The identification of soybean cultivars adapted to shaded environments should be further studied to increase grain yield in integrated production systems. The aim of this study was to identify, through different methodologies, soybean cultivars with adaptability and stability for grain yield in environments with different levels of shading.

## MATERIAL AND METHODS

### Locations and agricultural years

The experiments were conducted at Chácara Farm (lat 16° 26' 44.16" S, long 46° 54' 15.07" W), in the municipality of Unaí, Minas Gerais, Brazil, on clayey soil (49% clay, 23% sand, and 28% silt), in the agricultural year 2019/2020, and at Santa Paula Experimental Farm (lat 16° 26' 10.48" S, long 46° 54' 2.28" W) of the Institute of Agricultural Sciences, Federal University of Vales do Jequitinhonha and Mucuri, on clayey soil (45% clay, 9% silt, and 46% sand), in the agricultural year 2021/2022.

### Cultivars and growing environments

Sixteen soybean cultivars, with an indeterminate growth habit, recommended for soybean region 304 and widely used by farmers, were grown in rainfed environments without irrigation. The cultivars and their respective maturity groups (MG) were: NS 7667 IPRO (MG: 7.6), NS 7780 IPRO (MG: 7.8), NS 7901 RR (MG: 7.9), NS 8338 IPRO (MG: 8.3), RK 8115 IPRO (MG: 8.1), M7110 IPRO (MG: 6.8), 8579 RSF (Bonus) (MG: 7.9), RK 6719 IPRO (MG: 6.7), CD 2728 IPRO (MG: 7.2), RK 6316 IPRO (MG: 6.3), 8473 RSF (Desafio) (MG: 7.4), 74177 RSF (Foco) (MG: 7.4), RK 7518 IPRO (MG: 7.5), M 6210 IPRO (MG: 6.2) and AS 3680 IPRO (MG: 6.8).

Four experiments, divided into light restriction environments with measurement of photosynthetically active radiation (PAR), were set up per agricultural year. The growing environments with 25% and 48% of restriction of PAR (RPAR) were: A1 (agricultural season 2019/2020 in environment with 25% RPAR), A2 (2019/2020 under 48% RPAR), A3 (2021/2022 under 25% RPAR) and A4 (2021/2022 under 48% RPAR). The RPAR levels for the soybean cultivars were simulated in the field under shade nets with 18% and 38% light interception, providing 25% and 48% of RPAR. The photosynthetic photon flux density was measured with a photosynthetically active radiation meter (Apogee Quantum Meters – model M Q-200) once a month (during the soybean cycle), every hour, from 6 a.m. to 6 p.m., to determine the average PAR throughout the experiment.

Temperature was recorded hourly for each environment using a digital thermo-hygrometer with a datalogger. Precipitation was monitored with a manual rain gauge in the area and daily recording of precipitation (supplementary material).

### Field management

In the agricultural year 2019/2020, soybean seeds were treated before sowing with 160 mL kg<sup>-1</sup> of inoculant (1.0 x 10<sup>9</sup> viable cells of *Bradyrhizobium japonicum* per ml) and 2 mL kg<sup>-1</sup> of Standak Top (pyraclostrobin, thiophanate methyl, and fipronil). Sowing was done in November 2019, with a spacing of 0.5 m between rows, at depth of 3 to 5 cm, and the number of seeds according to the recommendation for each cultivar. In the agricultural year 2021/2022, the seeds were treated with Hober soy inoculant (*Bradyrhizobium japonicum*) (4.5 mL kg<sup>-1</sup> seeds) and with fertilizers Booster (3.5 mL kg<sup>-1</sup> seeds) and Infinity (5 mL kg<sup>-1</sup> seeds). Sowing was performed in October 2021 as in

the previous year.

Fertilization was based on soil analysis and recommendations for the crop. In the agricultural year 2019/2020, 290 kg ha<sup>-1</sup> of NPK (05-25-25) + 0.6% B + 0.06% Cu were applied at planting. Foliar fertilization was done with 250 mL ha<sup>-1</sup> of ExpertGrow (organic fertilizer formulated based on *Ascophyllum nodosum* seaweed extract and potassium hydroxide) and 1 kg ha<sup>-1</sup> of Quimifol K40 (10% N and 40% K<sub>2</sub>O). In the agricultural year 2021/2022, pre-planting broadcast fertilization was done with 68 kg ha<sup>-1</sup> of potassium chloride (58% K<sub>2</sub>O) and 10 kg ha<sup>-1</sup> of elemental sulfur (98% SO<sub>4</sub><sup>-2</sup>). At planting, 162 kg ha<sup>-1</sup> of triple superphosphate (42% P<sub>2</sub>O<sub>5</sub>) was applied. For foliar fertilization, 1.5 L ha<sup>-1</sup> of Complet Express® (fertilizer source of micronutrients and sulfur) was applied.

In both agricultural years, weed control was done with the application of the herbicide glyphosate. To control insects and fungal diseases, sprays with insecticides and fungicides were done according to the observed needs in each crop. Harvesting of the experiments began when the cultivars were at the R8 phenological stage (physiological maturity) according to the maturity of each one.

### Grain yield (GY)

The GY of the cultivars was evaluated based on the harvest of two central rows of 5 m per experimental unit. After the harvest, the plants were threshed in a stationary thresher to separate the grains, which were weighed and had their moisture content determined by a grain moisture meter (Gehaka model G650i), which was adjusted to 13%. GY was expressed in kg ha<sup>-1</sup>.

### Experimental design and statistical analysis

The experiments were set up in a randomized complete block design (RCBD) with 16 treatments (soybean cultivars) and three replications, each experimental unit consisting of four rows of plants, 6.5 meters in length, spaced 0.5 meters apart.

The data were subjected to individual analysis of variance to determine the residual variance of each environment for subsequent testing of variance homogeneity. For the joint analysis of environments, the relationship between the highest and lowest mean squared residuals (MSR) from the individual analysis of variance was evaluated, which should not exceed a 7:1 ratio (Banzatto and Kronka 2006). After confirming that this ratio was below “3.3:1” (Higher(MSR)/Lower(MSR)), the grain yield data were jointly analyzed. Subsequently, the data were subjected to adaptability and stability analysis using the methods of Eberhart and Russell (1966), ANN proposed by Nascimento et al. (2013), and GGE, *Genotype plus Genotype-Environment interaction*.

The method of Eberhart and Russell (1966) is based on simple linear regression fitting according to the mathematical model:  $Y_{ij} = \beta_{0i} + \beta_{1i} I_j + \delta_{ij} + \bar{\epsilon}_{ij}$ , where:  $Y_{ij}$  corresponds to the mean of genotype  $i$  in environment  $j$ ;  $\beta_{0i}$  is the overall mean of genotype  $i$ , considering all environments;  $\beta_{1i}$  is the linear regression coefficient, measuring the response of the  $i$ -th genotype to environmental variations;  $I_j$  is the coded environmental index;  $\delta_{ij}$  is the regression deviation for genotype  $i$  in environment  $j$ ;  $\bar{\epsilon}_{ij}$  is the average experimental error.

The parameter  $\beta_{0i}$  represents the overall mean GY of the genotype in all evaluated environments. Concerning the parameter  $\beta_{1i}$ , if  $\beta_{1i} < 1$  the cultivar has low adaptability, meaning it is a robust cultivar. If  $\beta_{1i} > 1$ , the cultivar is responsive to environmental variations, with high GY under conditions of higher luminosity. If  $\beta_{1i} = 1$ , the cultivars are recommended for all environments (high general adaptation).

In the ANN, data simulation for computational training purposes and classification of cultivars regarding adaptability and stability were performed using the *single hidden layer back-propagation* network, with one input layer, one hidden layer, and one output layer. The first layer with four inputs refers to the GY mean values evaluated in four environments. The number of neurons in the hidden layer ranged from 1 to 10. The output layer, composed of 6 neurons, represented the classification of the genotype into one of the six classes defined by Eberhart and Russell (1966). The necessary arguments for the network function, such as the number of neurons in the hidden layer, initial weight values, decay rate, and maximum number of iterations, were chosen considering the network that provided an error value of no more than 2% for the test dataset (Barroso et al. 2013, Nascimento et al. 2013).

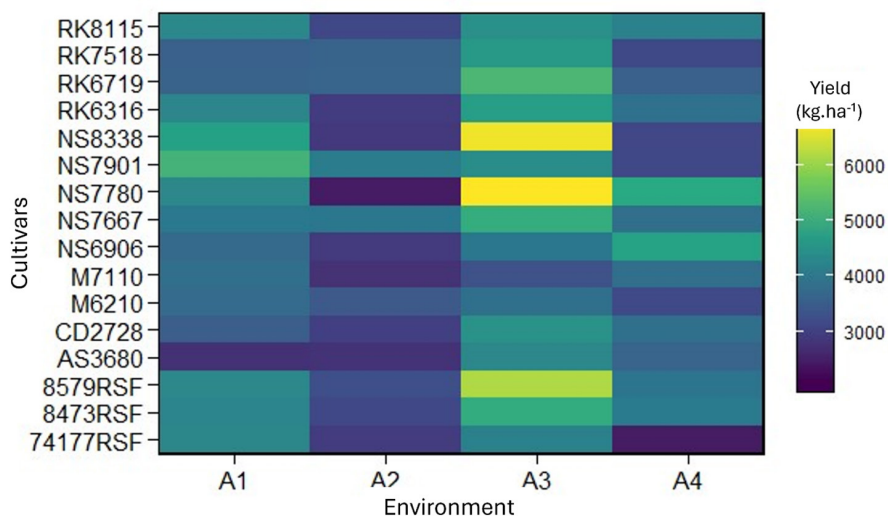
The GGE plot was created based on the data from the decomposition of means, graphically indicating which genotype had the best performance (Yan et al. 2000). The following GGE model was used:  $Y_{ij} - \mu_j = \lambda_1 V_{i1} \alpha_{j1} + \lambda_2 V_{i2} \alpha_{j2} + \varepsilon_{ij}$ , where:  $Y_{ij}$  corresponds to the average grain yield of genotype  $i$  in environment  $j$ ;  $\mu_j$  represents the overall mean yield of the genotypes in environment  $j$ ;  $\lambda_1 V_{i1} \alpha_{j1}$  is the first principal component (PC1) of the effect of genotypes + genotype x environment interaction;  $\lambda_2 V_{i2} \alpha_{j2}$  is the second principal component (PC2) of the effect of genotypes + genotype x environment interaction;  $\lambda_1$  and  $\lambda_2$  are the eigenvalues associated with PC1 and PC2, respectively;  $V_{i1}$  and  $V_{i2}$  are the scores of the first and second principal components, respectively, for the  $i$ -th genotype;  $\alpha_{j1}$  and  $\alpha_{j2}$  are the scores of the first and second principal components, respectively, for the  $j$ -th environment;  $\varepsilon_{ij}$  is the error associated with the model for the  $i$ -th genotype and  $j$ -th environment.

The analysis of adaptability and stability variance by the method of Eberhart and Russell (1966) was performed in the Genes program, and the GGE and ANN analyses were conducted in the R program using the metan package (multi-environment trials analysis) (Olivoto and Lúcio 2020), and nnet (Venables and Ripley 2002), respectively.

## RESULTS AND DISCUSSION

In environment A1 (2019/2020 and 25% RPAR), the grain yields of cultivars NS7901 (4.773 kg ha<sup>-1</sup>), RK8115 (4.185 kg ha<sup>-1</sup>), NS8338 (4.121 kg ha<sup>-1</sup>), 8473RSF (4.101 kg ha<sup>-1</sup>), and 74177RSF (4.065 kg ha<sup>-1</sup>) were the highest among the cultivars. In environment A2 (2019/2020 and 48% RPAR), the GY of cultivars NS7667 (3.805 kg ha<sup>-1</sup>), 8579RSF (3.515 kg ha<sup>-1</sup>), RK6719 (3.332 kg ha<sup>-1</sup>), 74177RSF (3.278 kg ha<sup>-1</sup>), and RK8115 (3.214 kg ha<sup>-1</sup>) was greater compared to the others. In environment A3 (2021/2022 and 25% RPAR), the GY of cultivars NS7780 (6.232 kg ha<sup>-1</sup>), NS8338 (5.675 kg ha<sup>-1</sup>), 8579RSF (5.462 kg ha<sup>-1</sup>), RK6719 (4.091 kg ha<sup>-1</sup>), and RK7518 (3.862 kg ha<sup>-1</sup>) was superior, while in environment A4 (2021/2022 and 48% RPAR), the cultivars with the highest GY were NS7780 (5.096 kg ha<sup>-1</sup>), 8579RSF (4.519 kg ha<sup>-1</sup>), NS6906 (4.156 kg ha<sup>-1</sup>), RK6719 (4.091 kg ha<sup>-1</sup>), and RK7518 (3.862 kg ha<sup>-1</sup>) (Figure 1).

In the agricultural years 2019/2020 and 2021/2022, the average soybean grain yield in Brazil was 3.379 kg ha<sup>-1</sup> and 3.029 kg ha<sup>-1</sup>, respectively. In the same agricultural years, the state of Minas Gerais produced an average of 3.747 kg ha<sup>-1</sup> (2019/2020) and 3.828 kg ha<sup>-1</sup> (2021/2022) (CONAB 2022, 2020). All cultivars with the best production performance in environments A1 (2019/2020 and 25% RPAR), A3 (2020/2021 and 25% RPAR), and A4 (2021/2022 and 48% RPAR) had an average yield above the national and Minas Gerais averages in both agricultural years. In environment A2 (2019/2020



**Figure 1.** Grain yield (kg ha<sup>-1</sup>) of soybean cultivars grown in different environments and levels of photosynthetically active radiation restriction (RPAR). Environments: A1: 2019/2020 harvest and 25% RPAR; A2: 2019/2020 harvest and 48% RPAR; A3: 2021/2022 harvest and 25% PAR; A4: 2021/2022 harvest and 48% PAR.

and 48% RPAR), only cultivars NS7667 and 8579RSF had an average yield above the national average; however, only NS7667 achieved an average yield higher than that of Minas Gerais in the agricultural year 2019/2020.

The grain yield results allowed inferring that in different environments, cultivars showed differentiated performance in response to environmental variations. In summary, the effects of location, crop year, and percentage of RPAR varied among themselves, combining predictable and unpredictable factors that led to different results among cultivars (Table 1S - supplementary material). Authors relate soybean GY to the interaction between genotypes and environments, classifying the causes of this interaction with physiological and adaptive factors (Vasconcelos et al. 2010, Freiria et al. 2018, Albuquerque et al. 2022). Genetic variations among soybean genotypes, involving morphological characteristics such as plant height and number of branches, allow for differences in the number of pods and grain yield per plant (Metwally et al. 2020).

The average yield of cultivars NS7780 (4.527 kg ha<sup>-1</sup>), 8579RSF (4.370 kg ha<sup>-1</sup>), NS8338 (3.993 kg ha<sup>-1</sup>), and RK6719 (3.964 kg ha<sup>-1</sup>) was higher in all four environments, while that of AS3680 (3.326 kg ha<sup>-1</sup>), M7110 (3.439 kg ha<sup>-1</sup>), and 74177RSF (3.511 kg ha<sup>-1</sup>) was lower (Table 1). The averages show heterogeneity among soybean cultivars. The genetic potential of cultivars in interaction with environmental conditions reflects on grain yield (Metwally et al. 2020). Cultivars that maintain high GY in different environments are highly valued by researchers in breeding programs due to the limitation of yield loss risks due to climatic circumstances (Habtegebriel 2022). Authors also highlight the grain yield in identifying cultivars that are adaptable and responsive to different edaphoclimatic conditions (Dallo et al. 2019, Carvalho et al. 2021).

The average grain yield of the cultivars per environment was 3.803 kg ha<sup>-1</sup> in A1, 3.092 kg ha<sup>-1</sup> in A2, 4.724 kg ha<sup>-1</sup> in A3, and 3.720 kg ha<sup>-1</sup> in A4. Compared with the average GY in Brazil in the agricultural years 2019/2020 (3.379 kg ha<sup>-1</sup>) and 2021/2022 (3.029 kg ha<sup>-1</sup>) (CONAB 2020, 2022), the averages in environments A1, A3, and A4 were higher, while the average in environment A2 was higher only in the agricultural year 2021/2022. Regarding the average GY in Minas Gerais (3.747 kg ha<sup>-1</sup> in 2019/2020 and 3.828 kg ha<sup>-1</sup> in 2021/2022) (CONAB 2020, 2022), the averages in environments A1 and A3 were higher in the agricultural year 2019/2020, but only the average in environment A3 was higher in both agricultural years.

In environments with less light restriction, grain yield was higher. Shading stress affects the morphology, biomass accumulation and distribution, and grain yield in soybean (Liu et al. 2010, Yao et al. 2017). In intercropping with corn,

**Table 1.** Estimation of adaptability and stability parameters of 16 soybean cultivars for grain yield based on the methodology of Eberhart and Russel (1966) and ANN - Artificial Neural Networks by Nascimento et al. (2013)

Cultivars	Eberhart and Russel (1966)				ANN	
	$\beta_0$ (average)	$\beta_1$	$\delta d$	R <sup>2</sup> (%)	Adap.	Stab.
AS3680	3,326	1.0439	15203.8	89.67	Overall	High
M6210	3,601	1.0431	-65178	99.35	Overall	High
RK7518	3,839	0.8502	6309	86.54	Overall	High
CD2728	3,759	1.0641	-11651	92.94	Overall	High
RK6719	3,964	1.0892	164689*	77.43	Overall	High
NS8338	3,993	1.7717**	88581.6	93.07	Favorable	High
8473RSF	3,918	1.0194	-12901	92.51	Overall	High
NS7667	3,963	0.6296	201063*	49.81	Overall	High
NS7901	3,858	0.7404	599319*	35.73	Overall	High
NS7780	4,527	2.0526**	38669*	86.23	Favorable	High
M7110	3,439	0.4515**	8289.66	63.86	Unfavorable	High
74177RSF	3,511	0.3054**	218753*	17.98	Unfavorable	High
8579RSF	4,370	1.1796	33528.9	90.12	Overall	High
RK8115	3,920	0.8352	-12480	89.16	Overall	High
RK6316	3,666	0.8698	-53121	96.81	Overall	High
NS6906	3,703	1.0544	153574*	77.14	Overall	High
Overall Mean	3,834					

$\beta_0$  – overall mean of the cultivar,  $\beta_1$  – linear regression coefficient for genotype, t - t-student test,  $\delta d$  – genotype regression deviation, R<sup>2</sup> – determination coefficient. \*, \*\* Significant at 5% (p<0.05) and 1% (p<0.01) probability of error by F test, respectively. Adap.: Adaptability. Stab.: Stability.

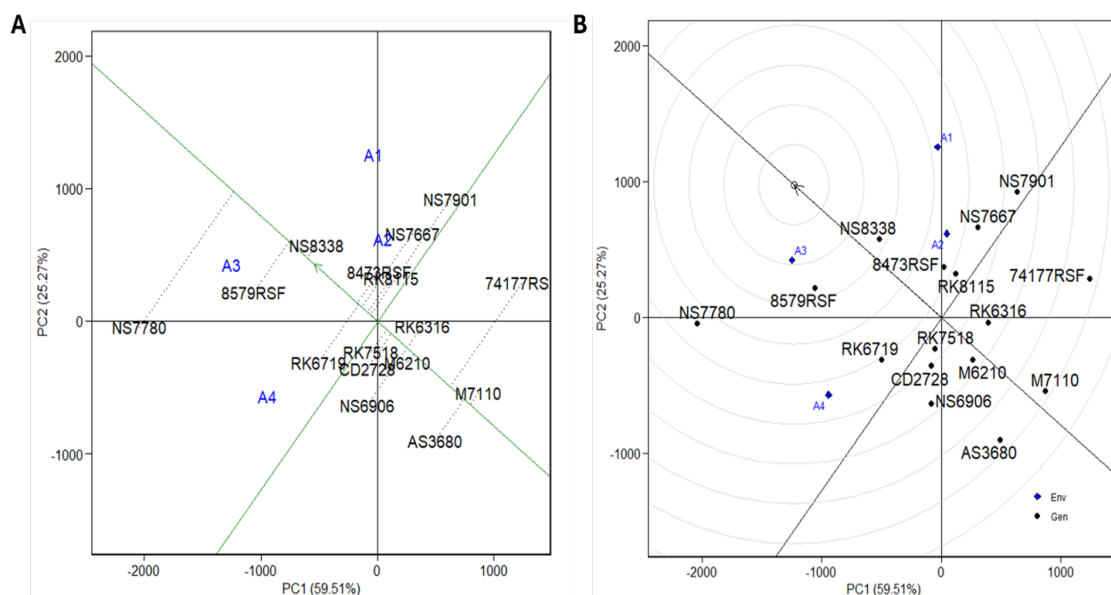
due to the decrease in intercepted light and consequently photosynthetic rate, GY was reduced (Yang et al. 2017). Photosynthesis in plants varies with light intensity and spectral components. Shading conditions or low light level increase the granular stacking of thylakoids and the content of photosynthetic pigments, but reduce net photosynthetic rate, decreasing GY (Yang et al. 2018).

Estimates of parameters by the method of Eberhart and Russel (1966) and classification by the ANN method regarding the adaptability and phenotypic stability of soybean cultivars are presented in Table 1. Cultivars M7110 and 74177RSF in both evaluated methods showed low adaptability, while cultivars NS8338 and NS7780 showed high adaptability for favorable environments, i.e., environments with less shading. The other cultivars for both methodologies showed general adaptation to the studied environments.

Identifying cultivars with specific adaptation is an efficient way to exploit the genotype x environment interaction, favoring crops under normally unfavorable environmental conditions (Melo et al. 2023). Soybean mechanisms of response to shading conditions vary with morphological characteristics, transpiration and photosynthetic rates, and stomatal conductance (Yang et al. 2014, Gong et al. 2014). Soybean genotypes tolerant to shading have fewer nodes on the main stem and lower lodging rates (Valladares and Niinemets 2008, Yu-shan et al. 2017, Sajad et al. 2019).

The regression deviation coefficient proposed by Eberhart and Russel (1966) indicated which cultivars have predictable behavior, referring to phenotypic stability. Cultivars with low predictability were: RK6719, NS7667, NS7901, NS7780, 74177RSF, and NS6906 (Table 1). The environment contributed to variation in grain yield among cultivars. GY is a quantitative trait with polygenic inheritance highly influenced by the environment, which may explain the variation in cultivar stability (Nascimento et al. 2023).

Analyzing stability using artificial neural networks (ANN) showed high stability for all cultivars under analysis. The low agreement (62% of cultivars) between the stability results of Eberhart and Russell (1966) and ANN is noted by Nascimento et al. (2013), who reported that the concept of stability in ANN is based on the work of Finlay and Wilkinson (1963), which considers stability as invariance rather than predictability. The nonlinear structure of ANNs can observe more complex characteristics of a dataset and does not require detailed information about the process to be modeled, attributed to self-learning (Nascimento et al. 2013).



**Figure 2.** Average performance vs stability (A) and classification of soybean cultivars (B) grown in different environments with active photosynthetically radiation restriction (RPAR) and agricultural year by GGE method. Environments: A1: 2019/2020 crop and 25% RPAR; A2: 2019/2020 crop and 48% RPAR, A3: 2021/2022 crop and 25% RPAR A4: 2021/2022 crop and 48% RPAR.

By the Eberhart and Russel (1966) and ANN methods of Nascimento et al. (2013), cultivar NS8338 stands out for showing high adaptability and stability, with high grain yield above the cultivars' average when grown in environments with restricted photosynthetically active radiation.

Using the GGE method to evaluate average performance vs stability, an average environmental coordinate (AEC) was plotted in the first biplot graph for cultivar evaluation (Figure 2A). Subsequently, a mean environment was represented by a small arrow, defined by the mean scores of PC1 and PC2 of the environments. Cultivars closer to the line passing through the biplot origin had higher phenotypic stability, namely NS8338, RK6316, RK7518, M6210, and M7110 (Figure 2A). Those farther from this line had lower phenotypic stability, such as cultivars NS7780, 74177RSF, and NS7901 (Figure 2A).

Figure 2B compares all cultivars with the "ideal" cultivar, represented by a small circle with an arrow pointing to it, defined as having the highest yield in all environments, meaning it has the highest average yield and is absolutely stable. Cultivar NS8338 surpassed all other cultivars, followed by 8579RSF, NS7780, and 8473RSF. Cultivars that deviated the most from the ideal cultivar were M7110, AS3680, and 74177RSF, with low GY in all environments (Figure 2B).

The methods employed have distinct criteria for assessing adaptability and stability, which explains some of the discrepancies in the results. Eberhart and Russell (1966) use simple linear regression, which indicates the behavior of each genotype based on environmental improvement. In turn, ANN captures more complex characteristics of the data set without the need for detailed information about the process to be modeled, due to its self-learning capabilities (Nascimento et al. 2013). In addition, the GGE method is based on the multiplicative main effect of the genotype plus the genotype-environment interaction (Yan et al. 2000). Thus, comparing the different statistical approaches used by these methods is a strategy that allows increasing confidence in the evaluation and classification of genotypes, improving the selection of superior cultivars for future crop recommendations under different environmental conditions (Yamamoto et al. 2021).

## ACKNOWLEDGMENTS

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## DATA AVAILABILITY

The datasets generated and/or analyzed during this research are available from the corresponding author upon request.

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## Adaptability and stability of soybean for grain yield in shaded environments

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