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MULTILINE AIMING AT PHENOTYPIC STABILITY AND RICE BLAST RESISTANCE

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Abstract
This study aimed to verify the efficiency of multilines in reducing blast progress and their potential benefits to phenotypic stability in rice. The experiments were conducted in the 2016/17 and 2017/18 agricultural years. A randomized block design was performed with three replications, evaluating 12 lines and a multiline, which consisted of five lines from the Cultivation and Use Value (CUV) test. The multiline presented an estimated grain yield above the average of experiments of around seven bags ha^{-1} and superior performance in early flowering, justifying the high phenotypic stability for these characters. In this case, the line selection for composing the multiline was favorable and efficient, highlighted by a higher agronomic performance than most lines of the CUV test. The multiline is an adequate strategy to provide higher phenotypic stability and reduce blast progress in the field.

Keywords: *Oryza sativa* L. Plant breeding. *Pyricularia grisea.* Varietal mixture.

1. Introduction

In the middle of the last century, upland rice (*Pyricularia grisea*) gained prominence in Brazil after opening new agricultural areas in the Cerrado region for pasture implantation. Over the following decades, rice culture stood out in Brazilian agriculture. However, there has been a significant reduction in the cultivated area over the last few years due to lower crop adherence to the incorporation of new agricultural areas. It is worth noting that the occurrence of several biotic and abiotic stresses is a relevant factor that provides high fluctuations in upland rice cultivation in the country. Despite the adversities, Brazil is among the top ten in the world ranking, producing around 12 million tons of rice, and standing out as the largest producer outside the Asian continent (Faostat 2016; Conab 2018).

In recent years, crop-breeding programs have launched cultivars with increasingly high yield potential (Botelho et al. 2018). These programs aim to obtain plants with higher resistance to diseases, higher grain quality, a flowering season suitable to the most diverse regions, and cultivars tolerant to lodging and water deficit, aiming to meet the consumer market and the requirements and needs of producers.

Multilines appear as a viable and efficient alternative for developing cultivars resistant to diseases because they stand out for productive stability compared to a single strain with several resistance genes. According to Mundt et al. (2002), this is due to the appropriate correspondence between the resistance alleles in line mixtures and the avirulence genes of the pathogen. Successfully using multilines requires identifying lines with different resistance reactions to the relevant pathogens of a given culture and selecting those with good compensation capacity in the mixture, that is, lines with satisfactory complementation.

A valuable component for studying multilines and breeding strategies is the interaction between genotypes and environments, requiring estimation and attribution to the responsible genotypes and environments. Hence, breeders have recommended using adaptability and stability analyses to minimize the effects of this interaction (Cruz et al. 2004).

There is little information in the current literature, mainly in Brazil, on using multilines for rice blast resistance and production stability because these investigations require evaluations at different locations and consecutive years. Given the above, this study aimed to verify the efficiency of multilines in reducing blast progress and their potential benefits to phenotypic stability in rice.

2. Material and Methods

Cultivation and Use Value (CUV) tests were conducted in five municipalities in Minas Gerais and São Paulo (Brazil) over the agricultural years of 2016/2017 and 2017/2018 (Table 1).

Table 1. Identification of agricultural years, experiment locations, geographical characteristics, and corresponding environments.

Agricultural year	Locations	Altitude (m)	Latitude	Longitude	Environment identification
2016/17	DAG[1]/Lavras/MG	919	21°14’43” S	45°00’00” W	1
	Epamig Patos/MG	832	18°34’44” S	46°31’04” W	2
	Epamig Lambari/MG	896	21°58’02” S	45°20’48” W	3
2017/18	Muquém[2] Lavras/MG	918	21°14’43” S	44°59’59” W	4
	Epamig Lavras/MG	919	21°14’43” S	45°00’00” W	5
	Epamig Patos/MG	832	18°34’44” S	46°31’04” W	6
	Epamig Lambari/MG	896	21°58’02” S	45°20’48” W	7
	Unesp Registro/SP	25	24°29’16” S	47°50’38” W	8

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This study used 13 lines of the Cultivation and Use Value (CUV) tests of the Upland Rice Genetic Improvement Program of the Federal University of Lavras (UFLA), in partnership with Embrapa (Brazilian Agricultural Research Corporation) and Epamig (Agricultural Research Company of Minas Gerais), evaluated over the two agricultural years (Table 2).

Table 2. Identification of lines used in the experiment.

Identification	Lines	Resistance reaction
1	BRS Esmeralda	Moderately resistant
2	CMG 2119	Moderately resistant
3	BRSMG Caçula	Susceptible
4	CMG 2187	Moderately resistant
5	CMG 2188	Resistant
6	CMG 2085	Moderately resistant
7	CMG ERF 221-4	Moderately resistant
8	CMG ERF 221-7	Moderately resistant
9	CMG 1896	Moderately resistant
10	CMG ERF 221-9	Moderately resistant
11	CMG ERF 221-19	Moderately resistant
12	CMG ERF 221-29	Moderately susceptible
13	Multiline	-

Five upland rice lines (CMG ERF 221-4, CMG ERF 221-9, CMG ERF 221-29, BRSMG Caçula, and CMG 2085) composed the multiline. Line selection started by sorting the average harvest data before initiating this experiment (2015/2016). The lines in the multiline must be isogenic, similar to the agronomic characters. Therefore, genotype selection to establish the cultivar mixtures was based on the similarity between the agronomic attributes of the lines, such as plant grain height, width, length, days for flowering, and yield.

The BRSMG Caçula cultivar is highly susceptible to diseases, thus representing a source of inoculum in the mixture, differently from other lines. The same number of seeds of each strain formed the multiline to be implanted in the field based on the standard planting density for cultivating rice. The experiments were implemented in a randomized block design (RBD) with three replications, plots consisting of five 4-meter rows spaced at 35 cm, the three central lines representing the working area, and a sowing density of 80 seeds per linear meter.

Direct seeding was performed in all locations with irrigation at 80% of field capacity relative to the crop, using the sprinkler system. Furrow opening and fertilization were done mechanically, fertilization used 400 kg ha^{-1} of the formulated 8-28-16, and the sowing density was 80 seeds per linear meter. For weed control, the Pendimethalin herbicide was applied immediately after planting, before the emergence of rice plants. Thirty days after the emergence of rice seedlings, Cyhalofop-butyl and Metsulfuron methyl herbicides were applied.

The days for flowering were evaluated during the experiments when the plot presented approximately 50% of flowering plants. Grain productivity (kg ha^{-1}) was assessed with grain weight in the plot after harvest and dried to 13% moisture. Disease severity was also investigated, and blast occurrence on the leaf and panicle neck was evaluated according to the notes by the International Rice Research Institute (Irri 1996). The assessments were performed in favorable periods for the infection and development of each disease in the field. In this case, leaf blast was evaluated during intense vegetative development, and neck blast was studied in pasty grain and grain maturation phases, with higher crop susceptibility to the disease.

Statistical analyses were performed with R statistical software (2015), and the means were grouped for all characteristics using the Scott and Knott test (1974) at 5% probability.

Genotype adaptability and stability were analyzed according to the results of the genotype-environment interaction (GxE). The evaluation was performed with the GGE biplot model (Genotype and Genotype - Environment Interaction) by Yan et al. (2000), which considers the main genotype effect and the interaction between genotypes and environments. The matrices and graphs were obtained with R software (2015) using the GGE biplot package. The biplot graphs from the scores improved the understanding of the interrelationship between genotypes and environments, according to Yan and Tinker (2006), and they were constructed by decomposing the averages, showing the best genotype performance with the lowest severity of the associated disease. The biplots were built from the first two main components of the treatment effect and the genotype-environment interaction.

The analysis of agronomic character averages evaluated in the treatments also used the graphic method by Nunes et al. (2005). Hence, the means from the joint analysis of lines and the multiline were used and standardized relative to the average among all treatments, obtaining the Z_{ij} value. Considering that the standardized variable can assume positive and negative values, constant four was added to Z_{ijq} values to make them permanently positive. The generated graphics showed that axis dimensions (phenotypic characters) corresponded to standardized Z_{ijq} values.

3. Results

High accuracy values and good coefficient of variation estimates were obtained. The joint analysis of variance involving all eight environments showed a significant difference for variation genotype sources, environment, and GxE (Table 3) by the F test, analyzing grain yield and the number of days for flowering.

Table 3 shows the adjusted means of the 12 lines and the multiline for the characters initially described after the joint analysis. For the grain yield character, the averages ranged from 3068.7 to 4350.6 kg ha^{-1} between BRS Caçula and CMG ERF 221-29 lines, respectively, with an overall average of 3927.5 kg ha^{-1}. The number of days for flowering ranged from 76.5 to 93.7 days for BRS Caçula and CMG 2188 lines, respectively.

It is worth noting that the multiline performed significantly compared to grain yield and the number of days for flowering. For productivity, the multiline presented an estimated mean of 4275.5 kg ha^{-1}, higher than all its constituent lines (Table 3). As for the number of days for flowering, the average was 83 days, considered an early material compared to other genotypes.

Table 3. Means of grain yield (GY), in kg ha^{-1}, and the number of days for flowering (NDF), in days, in all evaluated environments.

Genotypes	GY	NDF
BRS Esmeralda	3601.1 b	89.9 d
CMG 2119	4289.1 a	89.0 e
BRSMG Caçula	3068.7 c	76.5 i
CMG 2187	3689.2 b	92.1 b
CMG 2188	3989.7 a	93.7 a
CMG 2085	4102.3 a	85.2 g
CMG ERF 221-4	4021.4 a	88.8 e
CMG ERF 221-7	4286.6 a	92.1 b
CMG 1896	3784.7 b	86.6 f
CMG ERF 221-9	4052.4 a	90.1 c
CMG ERF 221-19	4132.2 a	91.4 c
CMG ERF 221-29	4350.6 a	89.8 d
Multiline	4275.5 a	83.0 h
Mean	3972.6	88.3
CV (%)	19.95	1.7
Accuracy	89.1	99.7

Means followed by the same letter do not differ at the 5% significance level by the Scott Knott test (1974).

Figure 1 indicates the graphical method by Nunes (2005), which uses polar graphs where the phenotypes inherent to each variable for a given genotype generate 'full ball' and 'withered ball' graphs. This method consists of standardizing the means of the variables, with the highest values referring to the best performance of a given characteristic. It is a recent methodology compared to others in the literature, easy to interpret, and efficient in identifying genotype performance against the evaluated characters. Therefore, the 'full ball' shape describes a line that behaves above average for all or almost all variables. However, the 'withered ball' format refers to the deficient strain, with below-average performance for some variables.

Figure 2 shows the agronomic character ideotypes in the set of environments represented in the center of concentric circles. Thus, the genotypes closest to the circle center are the closest to the ideal. Figure 3 shows the biplot built with SVP = 2, scale = 0, centered on G + GxE and PC1 vs. PC2 (Primary). The environments were divided into sectors according to the red lines from the origin of the biplot. This division occurs according to the variation of the genotype group in a given group of environments.

The performance of a given genotype or environment is observed concerning the x-axis. The more to the right of the biplot center, the lower the performance relative to the average due to the association with higher severity scores. The more to the left, the higher the performance relative to the average (Figure 3). Thus, the distribution concerning the x-axis reflects the behavior of lines for grain yield, the number of days for flowering, and disease severity (leaf and neck blast) in these genotypes, considering the respective environments.

Figure 4 presents the biplots for grain yield, the number of days for flowering, and the severity of leaf and neck blast. They were centralized in G + (GxE) (centralization = 2) and with SVP = 1. The line with the arrow passing through the graph origin is the axis of the medium environment, and the arrow indicates the direction of higher yields. The line perpendicular to the axis of the medium environment refers to genotype stability, in which the smaller the distance between the genotype of this line, the higher its stability, and the larger the distance from the abscissa axis, the higher the genotype contribution to the interaction.

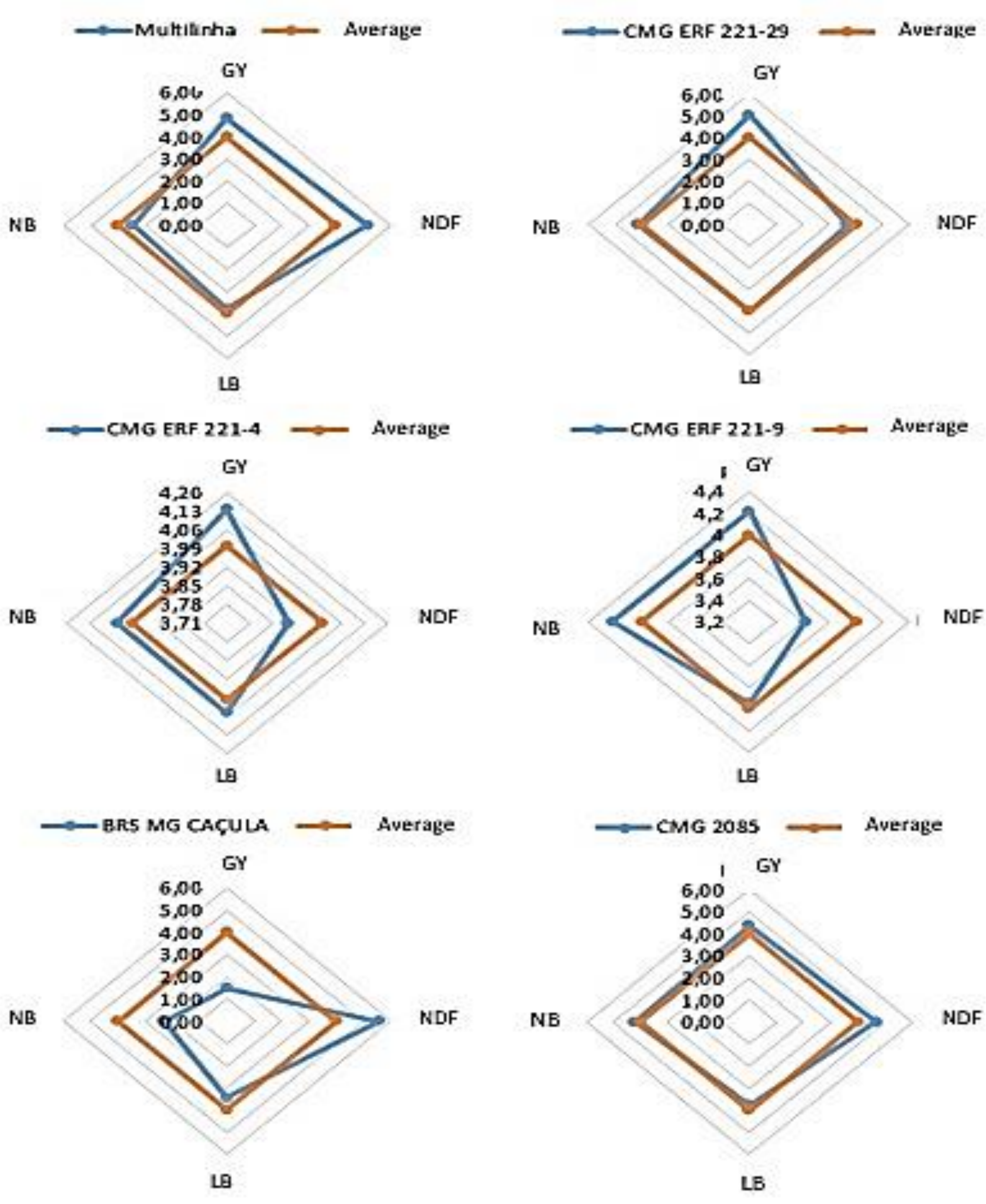

Figure 1. Graphical representation of the performance of the multiline and its constituent genotypes regarding grain yield, the number of days for flowering, and severity of leaf and neck blast. The red line represents the average of each character associated with the standardized z-value, in this case, the constant four. The vertices of each polygon indicate the performance of each character.

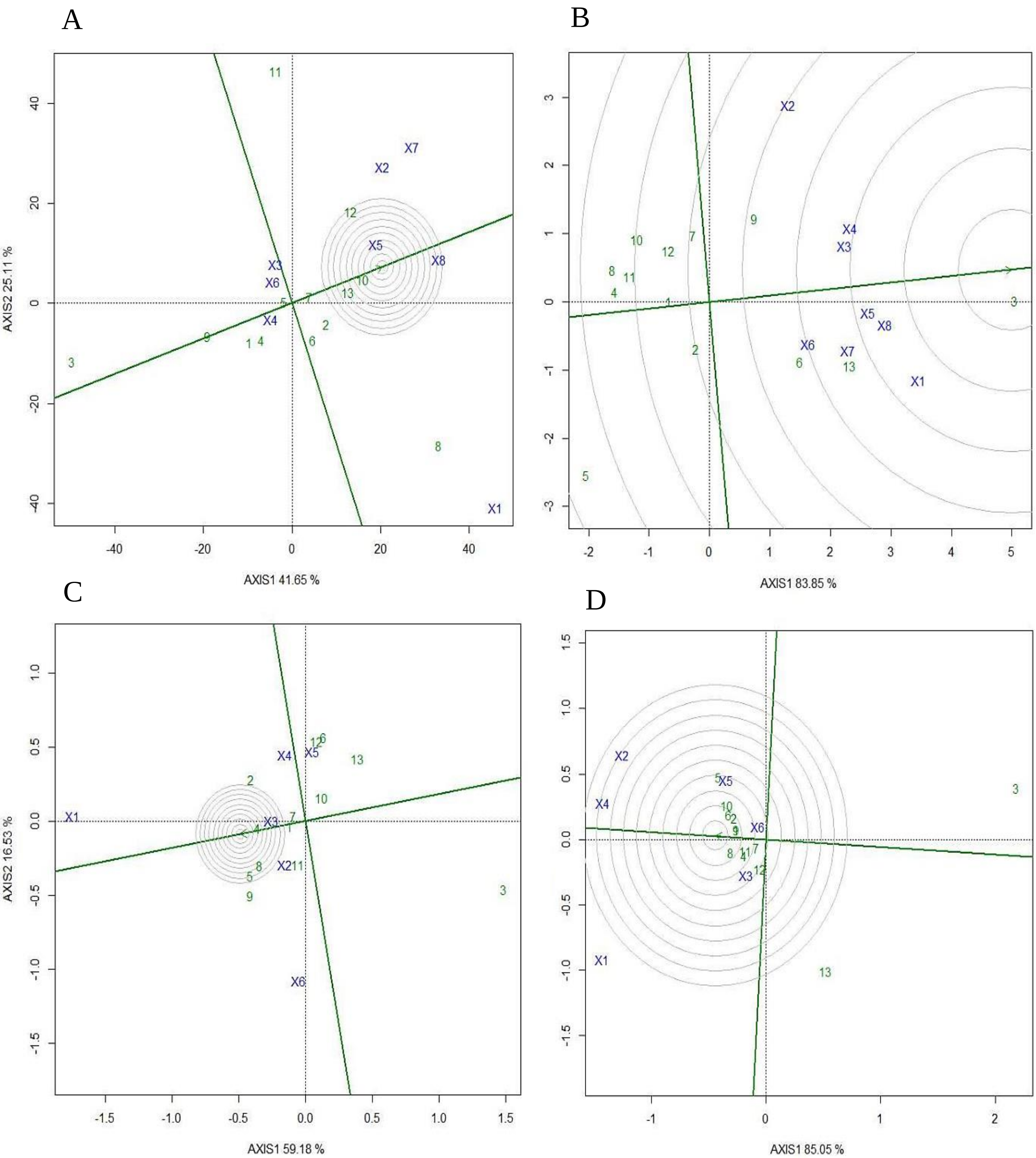

Figure 2. GGE biplot classifying genotypes according to an ideotype for A. grain yield[1]; B. the number of days for flowering[1]; C. severity for leaf blast[2]; D. severity of neck blast[2]. Lines: numbers 1 to 13 (green color). Environments: [1]alphanumeric designations X1, X2, X3, X4, X5, X6, X7, and X8 (blue color); [2]alphanumeric designations X1, X2, X3, X4, X5, and X6 (blue color).

4. Discussion

According to Resende and Duarte (2007), considering the precision and quality control in CUV experiments, these results help detect significant differences in the characters evaluated in this study. A field trial by Pimentel-Gomes (2009) showed that the coefficient of variation can be classified as low if it is lower than 10%, medium if it varies between 10-20%, high if it is between 20-30%, and very high when above 30%. Therefore, the coefficients of variation in this study, considering all environments, presented estimates below 20% for the evaluated characters, thus showing satisfactory precision in conducting the experiments.

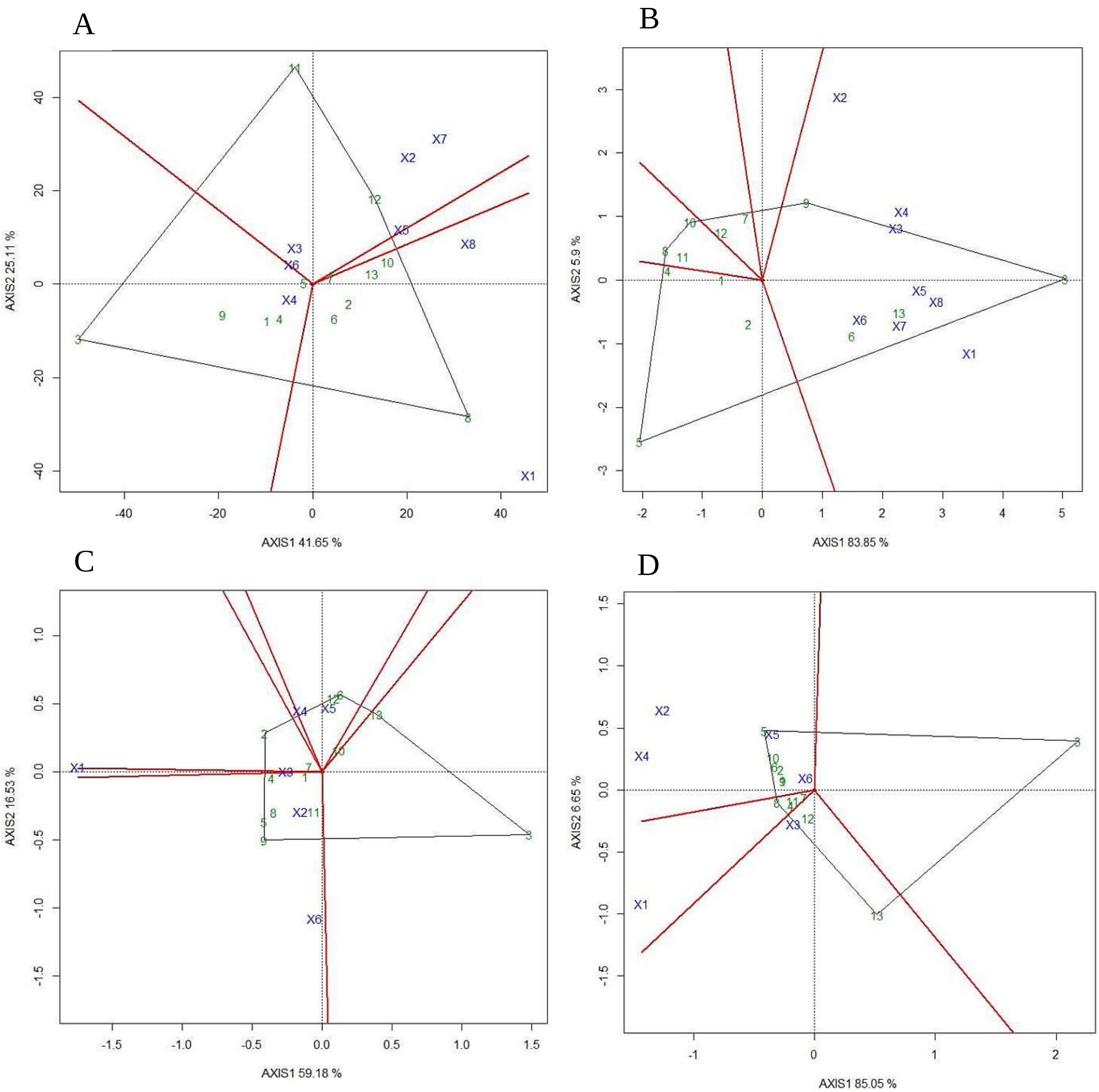

Figure 3. GGE biplot - "Who wins where" for A. grain yield[1]; B. the number of days for flowering[1]; C. leaf blast severity[2]; D. neck blast severity[2], which demonstrates the genotypes with the best performance and the environment. Lines: numbers 1 to 13 (green color). Environments: [1]alphanumeric designations X1, X2, X3, X4, X5, X6, X7, and X8 (blue color); [2]alphanumeric designations X1, X2, X3, X4, X5, and X6 (blue color).

It is noteworthy that the estimated productivity of the evaluated lines was higher than the Brazilian average for upland rice in the agricultural years of 2017/2018, about 2410 kg ha^{-1} (Conab 2018), indicating the success of the breeding program aimed at selecting genotypes for the grain yield character.

The genotype-environment interaction was significant for the evaluated field characteristics, indicating that the phenotypic behavior of lines was not equivalent in the eight environments evaluated. Therefore, it becomes challenging to suggest cultivars for different environments (Ramalho et al. 2012) because, under these circumstances, it is impossible to make a uniform recommendation for all locations without considerable damage to production. However, there are alternatives for breeders to minimize the intensity of the genotype-environment interaction, among which is the recommendation of genotypes with high phenotypic stability (Botelho et al. 2011).

The better behavior of the multiline compared to its constituent lines for grain yield and the number of days for flowering can be justified by the classification of competitive ability between the genotypes that can determine the efficiency of mixture performance. The literature reports the relationship between the ability to compete and some agronomic characteristics of rice. Akihama (1968) used two lines and their progenies to investigate competitive ability inheritance and the relationship with other characters. Selecting the ability to compete influenced the indirect choice of other agronomic traits. There are other studies on competitive ability in rice by Jennings and Herrera (1968) and Wolfe et al. (2000).

Mixing lines provided an above-average performance for most characteristics of grain yield and the number of days for flowering. This result is similar to that of Raboin et al. (2012), which presents the benefits of using a mixture of rice cultivars in southern Africa by reducing the damage from diseases and allowing a more stable production even under minimum planting cost conditions.

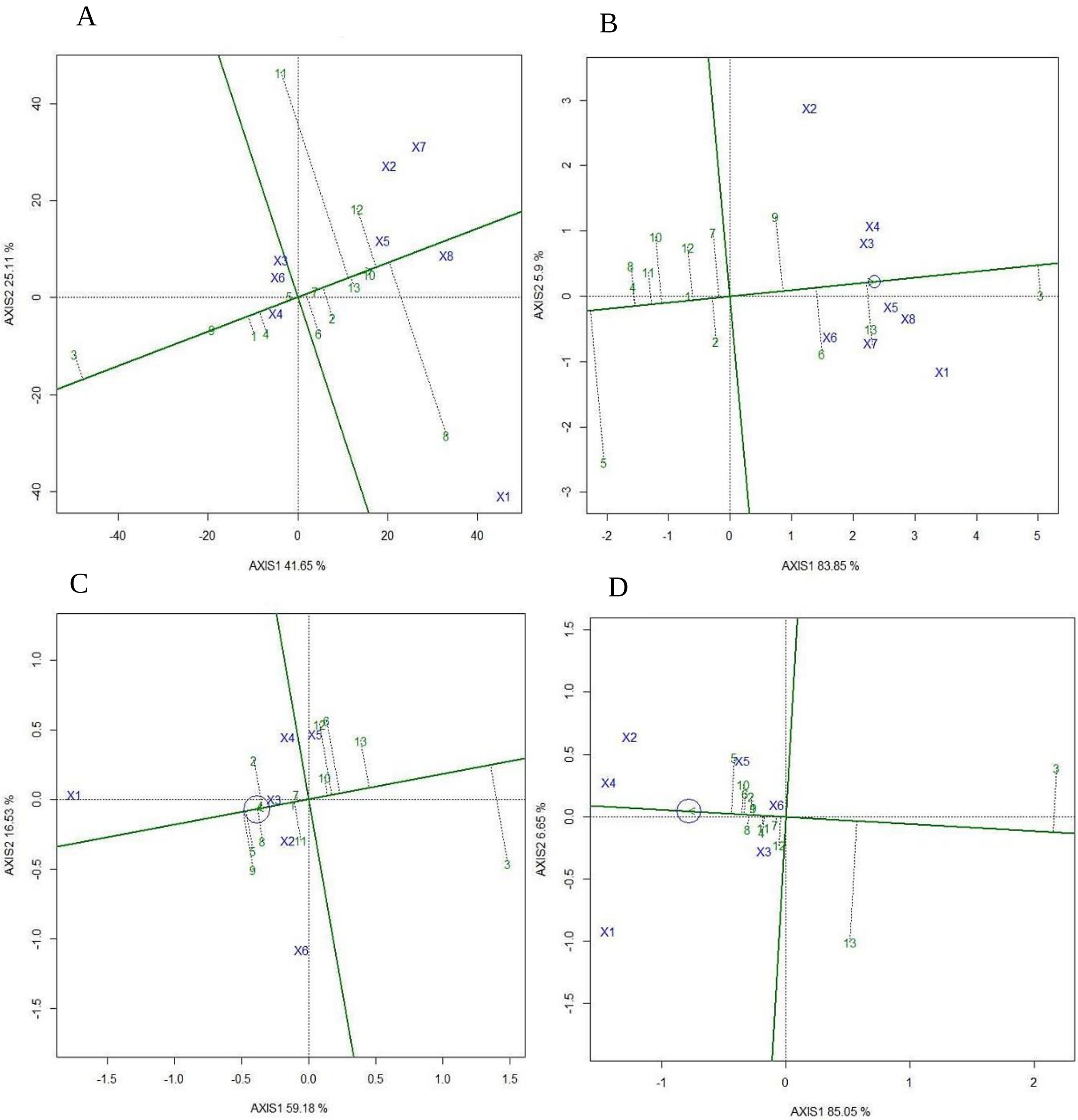

Figure 4. GGE biplot - "Average vs. Stability" for A. grain yield[1]; B. the number of days for flowering[1]; C. leaf blast severity[2]; D. neck blast severity[2], which shows the average performance regarding the evaluated characters and genotype stability. Lines: numbers 1 to 13 (green color). Environments: [1]alphanumeric designations X1, X2, X3, X4, X5, X6, X7, and X8 (blue color); [2]alphanumeric designations X1, X2, X3, X4, X5, and X6 (blue color).

The results of the leaf and neck blast incidence characters show that the multiline had some tolerance compared to these pathosystems, and its performance was close to the average among lines. This is a relevant report because it indicates the existence of genotypes with different resistance levels in the breeding program, in which the selection is initially successful for the characteristics in question (Morais Júnior et al. 2017; Cavatte et al. 2018). This can also be due to the high variability between rice genotypes regarding the reaction to blast, which depends on the prevalent population structure of the pathogen in the region (Santos et al. 2017). Rodríguez et al. (1998) showed high leaf blast variability in rice genotypes and concluded that the resistance level of some genotypes is low and requires complementary control measures. Prabhu and Filippi (2001) demonstrate the relevance of incorporating resistance genes in new genotypes against the most frequent breeds of the pathogen.

Other authors have discussed the beneficial effects of mixing lines (Browning 1969; Zhu 2000; Castro 2001). According to them, the disease level decreases through dilution and barrier effects, as previously mentioned. The dilution effect occurs due to a greater distance between susceptible plants, which reduces the speed of disease spread among plants. The barrier effect occurs due to resistant plants that work as a physical barrier, preventing the dispersion of pathogen spores. This effect is proportional to the number of resistant plants in the composition of the varietal mixture (Castro 2001). Zhu et al. (2005) used a genotype resistant to panicle blast and a susceptible one, obtaining satisfactory results for disease control, with a reduction of more than 90% of disease incidence in the susceptible genotype and 30-40% in the resistant genotype.

In China, Zhu et al. (2000) tested a variety resistant to blast and a susceptible one cultivated in monoculture and the mixture. The authors observed a 94% reduction in blast severity in mixtures than in separate genotype plantings. Nakajima et al. (1996) conducted a test on the island of Madagascar and verified the efficiency of the multiline composed of ten isogenic lines of the Sasanishiki rice genotype in suppressing rice blast. According to the authors, blast severity and the percentage of sick plants in line mixtures were lower than in isolated plantings. Although the results are satisfactory, few studies report the control of rice blast with multilines in Brazil. Therefore, selecting lines that constituted the varietal mixture was efficient, indicating a favorable selection of multiple characters based on the agronomic performance of the multiline in the field.

Our findings showed a solid genotypes-environment interaction for all characters, and this interaction was complex because the cultivar rankings changed when implanted in different locations. Complex interactions indicate the lack of coincidence in genotype rankings regarding environmental variations, which means that the behavior of some lines changes in a different environment, performing better in one place than another. The complex GxE interaction complicates breeding programs because it does not allow a perfect correlation between genotype and phenotype in different environments. This prevents breeders from recommending cultivars and selecting the best genotypes (Cruz and Castoldi 1991; Cruz et al. 2004).

The ‘Genotype Rankings’ graphs showed that the multiline (13) performed better than most used lines, indicating that it was close to the ideotype for grain yield and the number of days for flowering (Figures 2A and 2B). This result may relate to the high compensation capacity between the lines in the varietal mixture for the characters in question. This estimate also allows concluding that, when developing a multiline, a higher number of lines should be evaluated to identify the best ones for all characters and aid the decision on the mixture constituents (Botelho 2011). Gizlice et al. (1989) assessed soybean lines and their mixtures and found that the lines differed in the estimated parameters to evaluate the complementarity effect in the mixtures, inferring that the estimates of these compensation parameters can help identify lines to compose a multiline.

As for the severity of leaf and neck blast (Figures 2C and 2D), even when score estimates are lower than the most susceptible genotypes, an ideotype of resistance against such a pathogen was not considered, justifying that the ability for intraspecific competition between the lines in the mixture was not satisfactory for this character.

In Figure 3A (grain yield), genotypes 3, 8, and 11 form the polygon vertices. The environments were divided into three groups according to the red lines from the origin of the biplots. This division occurs according to the line group variations in a group of environments. The locations were grouped as follows: (i)

X1 and X8 (Lavras 2016/17 and Registro 2017/18), (ii) X4 (Lavras 17/18), and (iii) X2, X3, X5, X6, and X7 (Lambari 16/17, Patos 16/17, Lambari 17/18, Patos 17/18, Epamig 17/18). The genotypes at the vertices of the environment groups represent the best lines for that mega-environment. Line 3 (BRS MG Caçula) is the apex of the sector that comprehends the X4 environment, so this was the best genotype in such a location. For the sector that grouped the X1 and X8 environments, the genotype at the apex is 8 (CMG ERF 221-7), so it was the best for this mega-environment. For mega-environment (iii), the line representing the polygon vertex is 11 (CMG ERF 221-19), considered the best genotype for this group of environments. Therefore, all lines performed well for all characters in at least one location. The multiline, more specifically, presented satisfactory averages in mega-environment (i), which includes environments X1 and X8 (Lavras 16/17 and Registro 17/18).

In Figure 3B (the number of days for flowering), the eight environments were gathered into one group. Lines 3 (BRS MG Caçula) and 9 (CMG 1896) represented the polygon vertices of this single mega-environment. Genotypes 6 and 13 also showed satisfactory averages for the flowering cycle in at least one environment. Sectors in which none of the environments grouped genotypes 1, 2, 4, 5, 7, 8, 10, 11, and 12 obtained the lowest averages in one or more environments. Line 5 (CMG 2188) is among these genotypes, with the worst performance for early flowering.

The genotypes in the vertices of the polygon on the left are associated with below-average values, showing lower severity scores for leaf and neck blast (Figures 3C and 3D) and higher resistance to diseases in the mega-environment. The genotype corresponding to the vertex of the environmental group has the highest or lowest severity score estimate, depending on the position relative to the x-axis in its mega-environment. The sectors that did not group any environment include the genotypes with the most inconsistent average values for the evaluated characters in one or more locations. As the objective is to identify consistent genotypes, the treatments on the left deserve more attention in the breeding program. Therefore, lines 2 and 9 stood out regarding resistance to leaf blast and 5 and 8 for neck blast. Strain 3 (BRS MG Caçula) showed above-average estimates of disease severity scores and should be disregarded for resistance in the breeding program because it occupied both vertices on the right for leaf and neck blast.

In the “Average vs. Stability” graphs, genotypes can be classified according to the evaluated characters. The classification order was 8> 12> 10> 13> 11> 2> 7> 6> 5> 4> 1> 9> 3 for grain yield (Figure 4A). Genotype 8 (CMG ERF 221-7) was the most productive, but it had lower stability than genotype 12 (CMG ERF 221-29), which also showed an above-average production (second best). However, genotypes 5 (CMG 2188), 7 (CMG ERF 221-4), 9 (CMG 1896), and 13 (Multiline) were the most stable for grain yield, with emphasis on the multiline (genotype 13), which, combined with stability, obtained an above-average production. Genotype 11 (CMG ERF 221-19) contributed the most to the interaction, and despite presenting an above-average production and standing out in environments X2 (Lambari 2016/17) and X7 (Epamig 2017/18), did not perform well in other environments.

For the flowering cycle character (Figure 4B), line 3 (BRS MG Caçula) showed the best performance and stability, followed by lines 13 (Multiline), 6, and 9. Genotype 9 (CMG 1896) contributed the most to the interaction, with an above-average flowering cycle in the X2, X3, and X4 environments and not standing out in other locations.

According to Figures 4C and 4D, following the order established by the axis of the medium environment, lines 9 and 5 showed the highest leaf and neck blast resistance. Lines 3 (BRS MG Caçula) and 13 (Multiline) were the least resistant in both cases. Many genotypes showed good stability due to their higher proximity to the axis of the medium environment. This does not mean that these genotypes performed well for disease resistance but that their relative performance was consistent. As stability alone does not guarantee line performance, genotypes 7 and 8 stood out for leaf and neck blast, respectively, because they associated high stability with below-average severity scores. The least stable lines, those that most distanced themselves from the axis of the medium environment, are the most unpredictable or variable regarding resistance when changing locations. These genotypes also contributed the most to interaction estimates. In this case, they are not suitable alternatives to recommend a cultivar for a set of environments or regions. Following this criterion, the worst genotypes for recommendation are lines 3, 6, and 12 for resistance to leaf blast, and 3, 5, and 13 for neck blast, due to lower stability.

5. Conclusions

The multiline is an adequate strategy to provide higher phenotypic stability and reduce blast progress in the field. The choice of lines that constituted the varietal mixture was efficient for grain yield and the number of days for flowering, indicating a favorable selection according to the agronomic performance of the multiline in the field. The multiline performed above the average blast resistance compared to the most susceptible lines but with lower phenotypic stability for this character.

Authors' Contributions: CASTRO, D.G.: Conception and design, data acquisition, analysis, and interpretation, manuscript drafting, and final approval; MOURA, A.M.: Data analysis and interpretation; ALVES, N.B.: Manuscript drafting and final approval; TOMÉ, L.M.: Data acquisition, analysis, and interpretation, manuscript drafting, and final approval; BOTELHO, F.B.S.: Data acquisition, analysis, and interpretation; NETO, A.R.: Data acquisition, analysis, and interpretation; DE SOUZA, D.C.: Data acquisition, analysis, and interpretation; CASTRO, D.G.: Data analysis and interpretation, manuscript drafting, and final approval; BOTELHO, F.B.S.: Conception and design, data acquisition, analysis, and interpretation. All authors have read and approved the final version of the manuscript.

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