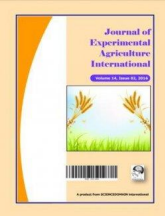

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Assessment of Resistance Status of Tomato Progenies to *Ralstonia pseudosolanacearum* and *Ralstonia solanacearum* Using Analysis of GGE Biplot and REML/BLUP

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Authors' contributions

This work was carried out in collaboration between all authors. Authors GAG and JLSCF designed the study and wrote the protocol. Authors TRAO and KDSC performed the statistical analysis and wrote the first draft of the manuscript. Authors AMMS, AMFS and PRS managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The bacterial wilt, caused by *Ralstonia pseudosolanacerum* and *Ralstonia solanacerum*, is among the bacterial diseases responsible for tomato fruit yield reduction in Brazil. The aim of this work was to assess the resistance status of tomato progenies to *Ralstonia pseudosolanacearum* and

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Ralstonia solanacearum using analysis of GGE biplot and REML/BLUP. In 2016, forty-three progenies derived from the cross between Yoshimatsu and IPA-7 were assessed in the Recife city, *Pernambuco* (PE), Brazil. It was used a randomized block design with four replications, applying the CCRMRS-74 and CCRMRS-185 isolates. The assessments were performed considering the data on the tenth and the twentieth days after inoculation as different environments. The incidence and severity of the disease were evaluated using a descriptive grading scale. The analysis of variance proved there are different performances between the genotypes and between the bacteria besides the interaction genotypes x bacteria. The selection of genotypes adapted to *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* are necessary to give continuity to the resistant tomato breeding program. There was agreement between the GGE biplot and REML/BLUP methods on the identification of genotypes resistant to both bacterial species evaluated. Genotypes 6, 7, 17, 18, 25, 27, and 31 showed resistance to *Ralstonia pseudosolanacearum*, and progenies 1, 2, 15, 16, and 35 demonstrated greater resistance to *Ralstonia solanacearum*. The individuals selected may continue the breeding program and be used as a source of variation of those bacterial species.

Keywords: Multivariate analysis; mixed models; interaction genotype x environment.

1. INTRODUCTION

The ninth largest producer of tomato, Brazil yielded 1.45 million tons of that fruit in 2016 [1,2]. However, bacterial wilt disease, caused by *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum*, has been a major problem of tomato production in that country. That disease, of worldwide importance for presenting economic risks and difficult control that may cause up to 100% in damage, was first reported upon tobacco and potato cultures in the state of Rio Grande do Sul, Brazil [3,4]. Currently, it has spread throughout the country, especially in the North and Northeast of Brazil [5].

Those bacteria enter the plant from injuries to the root system. Because of its colonization, there is an accumulation of polysaccharides, which induces darkening and obstruction of the xylem. Consequently, water and nutrient translocation are limited, causing bacterial wilt showing no change in the green color [6]. The most recommended control measure is the use of cultivars resistant to the bacteria.

When considering the evaluation dates of the bacteria as different environments, the most resistant genotypes can be selected by using the GGE biplot multivariate analysis and the REML/BLUP (Restriction Maximum Likelihood/Best Linear Unbiased Prediction). Those methodologies prove to be efficient, robust and of easy understanding and interpretation of data, which provide an efficient indication of the best genotypes [7,8,9,10].

The GGE biplot analysis combines the additive effect of genotype with the multiplicative effect of the GE interaction, building the biplot graphics from the main components (MC), in order that the first component represents the proportion of products obtained from the genotype traits, and the second component shows the ratio of the production that occurs due to the GxE interaction [11,12]. Many works show the efficiency and superiority of the GGE biplot analysis for the recommendation of genotypes [13,14,15,16].

The genetic parameters make it possible to increase the efficiency to estimate of population potential for breeding, based on the mean and variance components. Those parameters are useful for the breeder, since they make it possible to classify the possible genotypes to be launched as commercial cultivars, besides allowing the prediction of gains, providing the basis for establishing effective selection strategies, such as genetic variability and expression degree of a character from one generation to another [17].

Therefore, the aim of this work was to assess the resistant status of tomato progenies to *Ralstonia pseudosolanacearum* and *Ralstonia solanacearum* using analysis of GGE biplot and REML/BLUP.

2. MATERIALS AND METHODS

Crosses were made between the Yoshimatsu and IPA-7 genitors to obtain the F1 population (Table 1). Thirty-four (34) genotypes of the F_2 population obtained were used for the assessment of resistance status to *R. pseudosolanacearum* and *R. solanacearum* species using the isolates from the phytobacteriology laboratory of the *Universidade*

Table 1. Characteristics of cultivars used as parents. Recife / PE, 2016

Cultivars	Growth	Fruit format	Reaction	Company
Yoshimatsu	undetermined	Medium-sized, pluriloculated, red, and not attractive to the market.	Resistant	INPA
IPA-7	determined	Medium-sized to large, red, and well accepted in the market.	Susceptible	IPA
F_1	undetermined	Medium-sized, red, and round to oval fruit.	High resistance to *R. pseudosolanacearum* and low resistance to *R. solanacearum*.	obtained at UFRPE*

**Universidade federal rural de pernambuco*

Federal Rural de Pernambuco - UFRPE (Federal Rural University of Pernambuco), CCRMRS-74 and CCRMRS-185 isolates, respectively.

The experiments were conducted in 2016, in a greenhouse located at the Department of Agronomy, *UFRPE*, Recife-PE, lying between south latitude 8°01'02" and west longitude 34°56'41". The first experiment was carried out from September 28 to November 26, with an average temperature of 26.77°C and relative average humidity of 70.12%. The second one was performed from 26 October to 12 December, with an average temperature of 27.38°C and relative average humidity of 69.78%.

A randomized block design with four replications was used, in which each experimental unit was composed of four plants. Seeding was carried out in trays of expanded polystyrene of 128 cells, containing Basaplant® commercial substrate. Three seeds per cell were sown; after the emergence of the seedlings, thinning was performed, and only one plant per cell was left.

After 21 days of seeding, the seedlings were transplanted into 500-ml plastic pots filled with substrate based on a soil and humus mixture in the ratio of three to one, respectively. Thirty days after seeding, the plants were inoculated by the root cutting method. It was used a scalpel to make a semicircular cut in the substrate near the plant stem, in which 15 ml of the bacterial suspension ($5x10^8$CFU ml^{-1}) were placed [18].

After inoculation, the irrigations were done into plastic containers placed under the 500 ml-pots, in order to not drain the inoculum and keep the substrate moist.

2.1 Suspension Preparation

To prepare the inoculum suspension, the bacterial isolates applied were extracted in appropriate conservation. Subsequently, they were cultivated in a modified TZC medium (triphenyl tetrazolium tetrachloride) [19] for 48 h, at 30°C ± 2ºC temperature, and then transferred to nutritive-dextrose-yeast extract agar medium NYDA (10 g dextrose, 3 g meat extract, 5g yeast extract, 3 g peptone and 18 g agar l^{-1}), suspended in sterile distilled water (SDW). It was adjusted to $5x10^8$ CFU ml^{-1} using a photocolorimeter (Analyser 500 M, Brazil).

2.2 Genetics and Statistical Analyses

The assessments were performed using data from the days 10 and 20 after inoculation. The incidence and severity of the disease was measured by means of descriptive grading scale from 1 to 5, adapted from [20], in which: 1 = lack of symptoms; 2 = plants with up to one-third of wilted leaves; 3 = plants with up to two-thirds of wilted leaves; 4 = completely wilted plants; and 5 = dead plants.

The GGE biplot multivariate analysis was based on the information of phenotypic means. The main component analysis (MC) was employed to study the data collected, considering the data collection days of each bacterium as different environments. The first two MCs were used to group the genotypes; based on their values, the biplots were built by means of the Singular Value Decomposition (SVD). According to [21], the equation is as follows:

$$Yij - \mu - \beta j = \lambda 1 \xi i1 \eta 1j + \lambda 2 \xi i2 \eta 2j + \varepsilon ij,$$

in which λ1 and λ2 are the greatest eigenvalues of the first and second main components ACP1

and ACP2, respectively; ξi1 and ξi2 are the eigenvectors of the i-th genotype for ACP1 and ACP2, respectively; and η1j and η2j are the eigenvectors of the j-th environment for ACP1 and ACP2, respectively. The GGE biplot analysis was carried out with the R software aided by the GGEbiplotGUI package of the R software [22].

To evaluate the effect of the GE interaction using REML/BLUP, it was applied the statistical model 54 of the Selegen-REML/BLUP software [23], corresponding to:

$$y = Xb + Zg + Wc + e,$$

in which y, b, g, c, and e correspond, respectively, to the vectors of data of fixed effects (block means through the environments), genotype effects (random), effects of the interaction genotype x environment (random) and of random errors: X, Z and W represent incidence matrices for b, g, and c, respectively. That analysis was performed using the Selegen software [24].

3. RESULTS AND DISCUSSION

The coefficients of variation (CV%) show values ranging from medium (14.92) to high (30.00), according to [25]. Those values indicate good experimental accuracy and reliability of the assessed data.

The significant effect ($p \leq 0.01$) for genotypes and bacteria shows that there was genetic variability among the genotypes and different behavior among the bacteria, respectively (Table 2). Regarding the significance of the interaction genotypes x bacteria, it evidences different performance of the genotypes according to the species evaluated (*R. pseudosolanacearum* ou *R. solanacearum*); thus, the classifications of the tomato genotypes can be altered according to the species of bacterium considered. That is because different bacterial species may exhibit discrepant levels of virulence [26].

Table 2. Estimates of the mean squares of 34 progenies of tomatoes, evaluated in Recife

SV	DF	MS	
		10 DAI*	20 DAI*
Repetition	3	0.45	2.49
Genotypes	33	1.04**	3.07**
Bacterium	1	9.75**	0.83**
Genotypes x Bacterium	33	1.12**	4.44**
Error	1017	0.46	1.03
CV(%)		14.92	30.00

**DAI: days after inoculation*

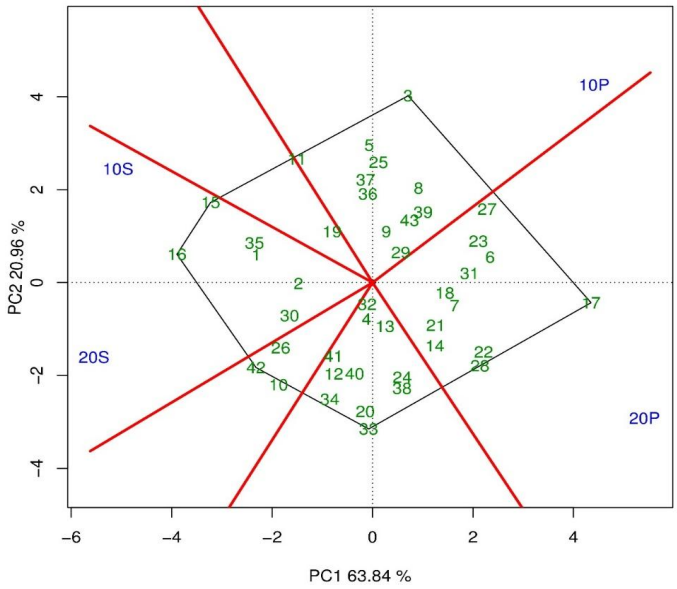

Fig. 1. GGE biplot representing the "which-won-where" graphic indicating the *Ralstonia pseudosolanacerum* and *Ralstonia solanacerum* resistance rankings of 43 genotypes. Environment 10S (10 days after inoculation with *Ralstonia solanacerum*), 20S (20 days after inoculation with *Ralstonia solanacerum*), 10P (10 days after inoculation with *Ralstonia pseudosolanacerum*) and 20P (20 days after inoculation with *Ralstonia pseudosolanacerum*)

Given the instability in the genotype classification, a detailed study is required to select the genotypes that show resistance to *R. pseudosolanacearum* and/or *R. solanacearum* to continue in the breeding program for tomato plant.

The biplot analysis of genotypes x environment described 84.8% of the total variation according to the first two main components (MCs) (Fig. 1). That result suggests that the graphic representations of the biplots proved to be extremely effective, since they explained almost all the proportion of the sum of squares of genotypes and of the GE interaction, providing a clear interpretation of results and a confident selection of the resistant genotypes by means of that methodology [27].

The "which-won-where" biplot establishes a set of perpendicular lines, which divide it into several groups, characterizing the progenies so that the ones allocated in the vertex of the biplot are classified as of better performance, and the ones that are into the polygons are the least responsive [28,29].

The evaluation dates of the bacteria resulted in three different groups so that the genotypes resistant to *R. solanacearum*, evaluated at the days 10 and 20, formed group 1. The genotypes that showed resistance to *R. pseudosolanacearum* at the days 10 and 20 formed groups 2 and 3, respectively.

The progenies 3, 17, 15, and 16 appeared at the vertices of the biplot and were considered more resistant to the bacteria. According to [30], that resistance is linked to defense mechanisms found in the roots, which is aimed at the colonization of bacteria, preventing them from multiplying rapidly by xylem.

The genotypes 1, 2, 30, and 35, at the days 10 and 20 of evaluation of *R. solanacearum*; 5, 8, 9, 11, 25, 29, 36, 37, 39, and 43, at the day 10 of assessment of *R. pseudosolanacearum*; 6, 7, 14, 18, 21, 22, 23, 27, 18, and 31, at the day 10 of analysis of *R. pseudosolanacearum* also presented resistance; however, the incidence scores were higher.

The genotypes that give rise to the vertices but did not group any of the evaluation days are considered susceptible to both bacteria. Therefore, they must be discarded from the breeding program, since they did not show resistance to *R. pseudosolanacearum* and *R. solanacearum*.

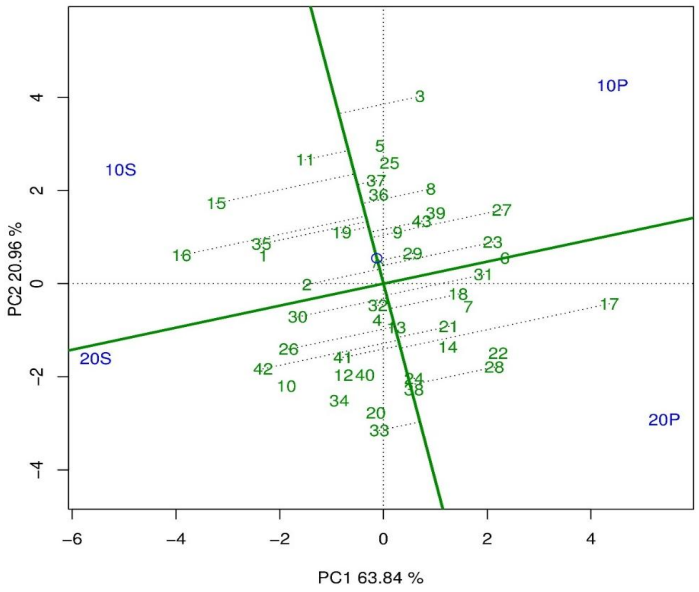

Fig. 2. GGE biplot representing the means x stabilities indicating the yield rankings of 43 genotypes and their respective incidence stabilities. Environment 10S (10 days after inoculation with *Ralstonia solanacerum*), 20S (20 days after inoculation with *Ralstonia solanacerum*), 10P (10 days after inoculation with *Ralstonia pseudosolanacerum*) and 20P (20 days after inoculation with *Ralstonia pseudosolanacerum*)

The severity and stability of the genotypes were assessed in the 'means x stabilities' biplot, in which the circle corresponds to the average environment coordination (AEC); the ideal environment is represented by the line that cuts the origin;and the arrow indicates the higher incidence of the bacterium. This graphic shows which genotypes demonstrate low stability, besides separating which ones had incidence above and below the average (Fig. 2).

Having said that, the genotypes 3, 5, 11, 25, 15, 37, 36, 8, 16, 35, 19, 1, 39, 43, 9, 27, 29, 23, and 2 showed severity below the overall mean, respectively, showing good resistance. The progeny 6 displayed severity close to the overall mean, and the genotypes 4, 7, 10, 12, 13, 14, 17, 18, 20, 21, 22, 24, 26, 28, 30, 31, 32, 33, 34, 38, 40, 41, and 42 presented high incidence, and can be discarded. Different levels of resistance to *R. solanacearum* and *R. pseudosolanacearum* were also found by [31,32,33].

Taking into consideration the stability, the higher the projection formed by the genotype in relation to the axis, the more unstable the genotype. Thus, the genotypes 37, 36, 19, 9, 29, and 5, besides having good resistance, were considered as the most stable, respectively.

The genotype considered 'ideal', applied as a reference to evaluate the other genotypes, is the one that presents longer vector length and zero GxA interaction, that is, the one that is nearer to the center (Fig. 3). In this way, the genotypes 5 and 11 are the most valuable for the breeding program, as they show good resistance and phenotypic stability, being considered the closest to the ideal. In the same way, the genotypes 17, 20, and 33 were the ones that had the worst classification; this confirms they have to be discarded.

The environments that made smaller angles with the coordination of the medium environment are considered the most representative, and those that show larger vectors are the ones that best describe the genotypes [34,35] (Fig. 4). Therefore, the 20S (day 20 of evaluation of the *R. solanacearum* bacterium) displayed good representativeness and discriminatory ability, thus, it provides reliable information and favors the selection of the genotypes evaluated. The 20P (day 20 of evaluation of the *R. pseudosolanacearum* bacterium) proved to be less representative and distinguishing and may be discarded [36,37].

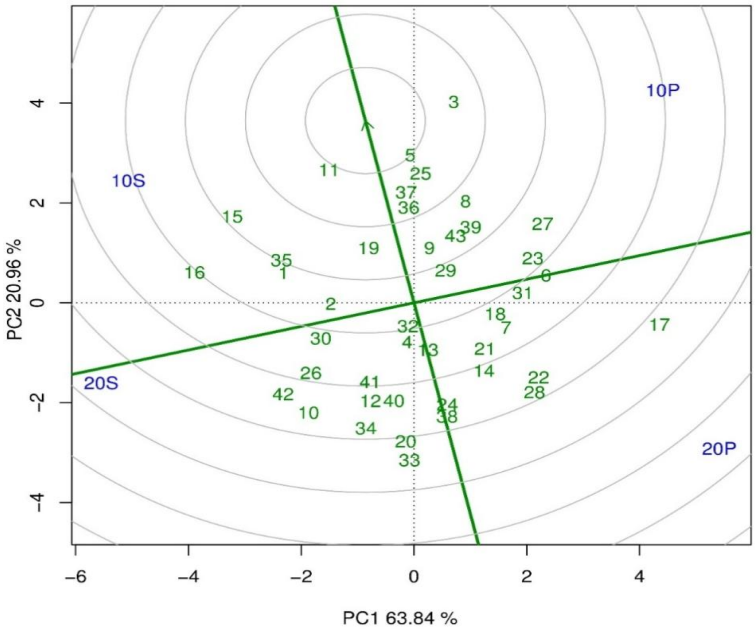

Fig. 3. GGE biplot comparing 43 genotypes evaluated according to the estimate of an ideal genotype. Environment 10S (10 days after inoculation with *Ralstonia solanacerum*), 20S (20 days after inoculation with *Ralstonia solanacerum*), 10P (10 days after inoculation with *Ralstonia pseudosolanacerum*) and 20P (20 days after inoculation with *Ralstonia pseudosolanacerum*)

Table 3. Genotypic values, adaptability, and stability of genotypic values (MHPRVG) predicted by the REML / BLUP analysis for resistance to *R. pseudosolanacearum and R. solanacearum* in 43 families $F_{2:3}$ of tomato Recife

Genotypes	R. *pseudosolanacearum*		R. *solanacearum*		Medium environment	MHPRVG
	10th Day	20th Day	10th Day	20th Day		
1	2.704	1.686	2.806	1.612	2.087	1.048
2	2.927	1.421	2.765	1.578	2.078	1.004
3	2.592	1.399	2.651	1.520	2.024	0.883
4	2.994	1.417	2.890	1.716	2.110	1.063
5	2.753	1.523	2.784	1.588	2.068	1.012
6	2.814	1.487	2.909	1.713	2.105	1.068
7	3.062	1.477	2.875	1.640	2.116	1.072
8	2.679	1.618	2.735	1.574	2.061	1.000
9	2.633	1.395	2.900	1.652	2.070	0.997
10	2.644	1.452	2.859	1.585	2.066	0.998
11	2.841	1.506	2.759	1.592	2.072	1.014
12	2.656	1.432	2.630	1.503	2.032	0.906
13	2.697	1.542	2.747	1.603	2.064	1.008
14	2.965	1.532	2.826	1.672	2.101	1.064
15	3.088	1.554	2.753	1.607	2.103	1.058
16	2.639	1.646	2.729	1.581	2.059	1.004
17	2.718	1.456	2.884	1.688	2.092	1.034
18	2.710	1.498	2.868	1.680	2.089	1.039
19	2.622	1.472	2.692	1.562	2.047	0.960
20	2.684	1.460	2.833	1.722	2.080	1.037
21	2.609	1.379	2.665	1.535	2.027	0.892
22	2.775	1.388	2.778	1.622	2.055	0.980
23	2.801	1.444	2.741	1.566	2.054	0.986
24	2.826	1.569	2.841	1.634	2.085	1.038
25	2.726	1.464	2.791	1.657	2.073	1.017
26	2.877	1.492	2.814	1.647	2.083	1.038
27	2.743	1.440	2.850	1.723	2.082	1.037
28	2.602	1.413	2.658	1.540	2.030	0.912
29	2.858	1.406	2.717	1.599	2.052	0.984
30	2.616	1.514	2.638	1.530	2.040	0.935
31	2.628	1.424	2.879	1.570	2.058	0.982
32	2.661	1.384	2.645	1.514	2.035	0.904
33	2.763	1.468	2.820	1.617	2.076	1.017
34	3.112	1.590	2.705	1.698	2.108	1.070
35	2.734	1.707	2.798	1.664	2.096	1.064
36	2.667	1.403	2.772	1.595	2.051	0.977
37	2.900	1.410	2.711	1.628	2.056	0.988
38	2.691	1.482	2.698	1.558	2.049	0.973
39	2.787	1.392	2.685	1.509	2.042	0.923
40	2.650	1.428	2.678	1.550	2.045	0.944
41	2.673	1.436	2.622	1.525	2.037	0.916
42	3.029	1.448	2.671	1.546	2.063	0.986
43	2.944	1.743	2.723	1.554	2.098	1.058
Overall Mean	1.503	2.622	1.379	2.592	8.096	--

For a better analysis of results, the REML/BLUP method was used (Table 3). The genotypes 4, 6, 7, 14, 15, 17, 18, 34, 35, and 43 are among the ten that showed better values for the different environments and for the medium environment. Besides, they are among the genotypes that obtained good resistance, adaptability, and stability, according to the harmonic mean method of relative performance of genetic value. From those, four (1, 15, 35, and 43) agreed with the results obtained by the GGE biplot method.

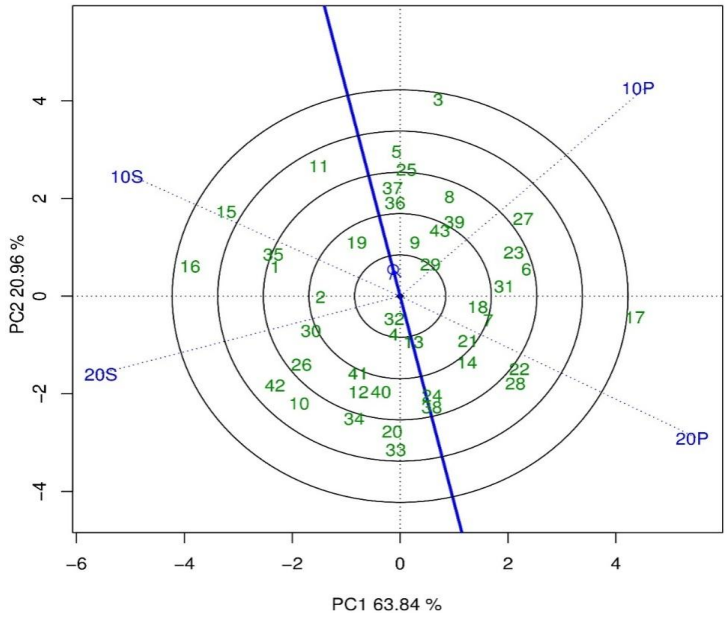

Fig. 4. GGE biplot comparing 43 genotypes evaluated according to the discrimination and representativeness of environments for incidence. Environment 10S (10 days after inoculation with *Ralstonia solanacerum*), 20S (20 days after inoculation with *Ralstonia solanacerum*), 10P (10 days after inoculation with *Ralstonia pseudosolanacerum*) and 20P (20 days after inoculation with *Ralstonia pseudosolanacerum*)

The REML/BLUP method agreed with the GGE biplot when classifying the genotypes resistant to R. pseudosolanacearum at days 10 (25) and 20 (6, 7, 17, 18, 27, and 31) after inoculation. Regarding the *R. solanacearum*, the genotypes 1, 15, 16, and 35 are among the resistant ones when assessed at the day 10 after inoculation, and the genotypes 2 and 15, at the day 20. These results allow the selection of resistant genotypes, thus avoiding the dissemination of these bacterial species [38].

The correspondence between the results obtained by the GGE biplot and REML/BLUP methods was also found by [39] and [40]. That equivalence between the results reveals that the application of different methodologies allows greater effectiveness and robustness to select the genotypes to be used in breeding programs.

4. CONCLUSION

Genotypes resistant to *Ralstonia solanacearum* and *Ralstonia pseudosolanacearum* were identified by the GGE biplot and REML/BLUP methods.

The genotypes 6, 7, 17, 18, 25, 27, and 31 demonstrated resistance to *Ralstonia pseudosolanacearum*. Moreover, the progenies 1, 2, 15, 16, and 35 showed resistance to *Ralstonia solanacearum*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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