

Assessment of resistance status of tomato progenies to *Ralstonia pseudosolanacearum* and *Ralstonia solanacearum* using analysis of GGE Biplot and REML/BLUP

ABSTRACT

The bacterial wilt caused by *Ralstonia pseudosolanacearum* and *Ralstonia solanacearum*, is among the bacterial diseases responsible for tomato fruit yields reduction in Brazil. The aim of this work was to assess of resistance status of tomato progenies to *Ralstonia pseudosolanacearum* and *Ralstonia solanacearum* using analysis of GGE biplot and REML/BLUP. Forty-three progenies resulting from the cross between Yoshimatsu and IPA-7 were assessed in the city of Recife, state of Pernambuco, Brazil, in 2016. We used a randomized block design with four replications applying the CCRMRS-74 and CCRMRS-185 isolates. The evaluations were performed considering the data of the days 10 and 20 after inoculation as different environments. The disease incidence and severity were evaluated with the help of a descriptive grade scale. The analysis of variance showed the existence of differentiated performances among the genotypes and among the bacteria, besides the presence of 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 in the identification of genotypes resistant of the two 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 showed more resistance to *Ralstonia solanacearum*. The selected individuals will continue the breeding program and will be used as source of variation of these bacterial species.

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

1. INTRODUCTION

The ninth largest producer of tomato, Brazil showed productivity of 1.45 million tons in 2016 [1;2]. Considered the causal agents of bacterial wilt in Brazil, *Ralstonia pseudosolanacearum* and *Ralstonia solanacearum* are the main pathogenic bacteria of tomato breeding. Bacterial wilt, considered as of worldwide importance due to economic risks and difficult control, causing up to 100% in damage, was reported in Brazil for the first bacterial wilt upon tobacco and potato cultures in the state of Rio Grande do Sul [3;4]. Currently, this disease has spread throughout the country, especially in the North and Northeast of Brazil [5].

Those bacteria enter the plant through injuries occurring in the root system; because of their colonization, there is accumulation of polysaccharides, which induces darkening and obstruction of the xylem. Consequently, water and nutrient translocation is limited, causing bacterial wilt without changing the green coloration [6]. The most recommended control measure is the use of cultivars resistant to those bacteria.

When considering the evaluation dates of the bacteria as different environments, the most resistant genotypes using the GGE biplot multivariate analysis and the REML/BLUP (Restriction Maximum Likelihood/Best Linear Unbiased Prediction). These methodologies prove to be efficient, robust and of easy understanding and interpretation of data, which provides 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 principal components (PC), so that the first component represents the proportion of performance obtained only from the genotype traits, and the second component shows the ratio of the performance that occurs due to the GxE interaction [11; 12].

Several works show the efficiency and superiority of the GGE biplot analysis for the indication of the genotypes [13; 14; 15; 16].

The genetic parameters make it possible to increase the efficiency in the estimate of population potential for the breeding, based on the mean and variance components. Those parameters are very useful for the breeder, since they make it possible to classify the genotypes chosen to be launched as commercial cultivars, besides allowing the prediction of gains, providing a foundation to establish 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 of resistance 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 F2 population obtained were used for the assessment of resistance status to *R. Pseudosolanacearum* and *R. solanacearum* species using the isolates obtained from the phyto bacteriology laboratory of the Federal Rural University of Pernambuco (UFRPE), CCRMRS-74 and CCRMRS-185 isolates, respectively.

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

Cultivars	Growth	Fruit format	Reaction	Company
Yoshimatsu	undetermined	Medium size, pluriloculated, red and not attractive to the market.	Resistant	INPA
IPA-7	determined	Medium size to large, red and well accepted in the market.	Susceptible	IPA
F ₁	undetermined	Medium size, red and round to oval fruit.	High resistance to <i>R. Pseudosolanacearum</i> and low resistance to <i>R. solanacearum</i> .	obtained at UFRPE*

*Universidade Federal Rural de Pernambuco.

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

A randomized block design with four replications was applied, in which each experimental unit was composed of four plants. The seeding was done in expanded polystyrene trays of 128 cells, with Basaplant® commercial substrate. Three seeds per cell were sown; after the emergence of the seedlings, the 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 from a mixture of soil and humus in the proportion of 3:1 in that order. Thirty days after seeding, the plants were inoculated by the root cutting method. With the help of a scalpel, a semicircular cut was made in the substrate near to the plant stem, and 15 ml of the bacterial suspension (5×10^8 CFU ml⁻¹) were placed in it [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 modified means of TZC (triphenyl tetrazolium tetrachloride) [19] for 48 h, at 30°C ± 2°C temperature. The suspension was transferred to agar nutritive-dextrose-yeast extract medium NYDA (10g dextrose, 3g meat extract, 5g yeast extract, 3g peptone and 18g agar l⁻¹), suspended in sterile distilled water (SDW). It was adjusted to 5x10⁸ CFU ml⁻¹ applying 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 were measured by means of descriptive grade 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 principal component analysis (PC) was employed to study the data collected, considering the data collection days, of each bacterium, as different environments. The first two PCs were used to join the genotypes; based on their values, the biplots were built by means of the Singular Value Decomposition (SVD), according to [21] the equation is:

$$Y_{ij} - \mu - \beta_j = \lambda_1 \xi_{i1} \eta_{1j} + \lambda_2 \xi_{i2} \eta_{2j} + \epsilon_{ij},$$

in which λ_1 and λ_2 are the greatest eigenvalues of the first and second principal components ACP1 and ACP2, respectively; ξ_{i1} and ξ_{i2} are the eigenvectors of the i-th genotype for ACP1 and ACP2, respectively; and η_{1j} and η_{2j} are the eigenvectors of the j-th environment for ACP1 and ACP2, respectively. The GGE biplot analysis was carried out with the help of the R software aided by the GGEbiplotGUI package of the R software [22].

To evaluate the effect of the GE interaction via 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 data vectors 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. This 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.

Given the uncertainty in the genotype classification, it is required a detailed study to select the genotypes that show resistance to either *R. Pseudosolanacearum* or *R. solanacearum* to continue in the breeding program for tomato breeding.

The biplot analysis of genotypes x environment described 84.8% of the total variation according to the first two principal components (PCs) (Figure 1). That result suggests that the graphic representations of the biplots proved to be extremely effective, since it explains almost all the proportion of the square sum of genotypes and of the GE interaction. That allows a clear interpretation of the results and a safe selection of the resistant genotypes by means of that methodology [27].

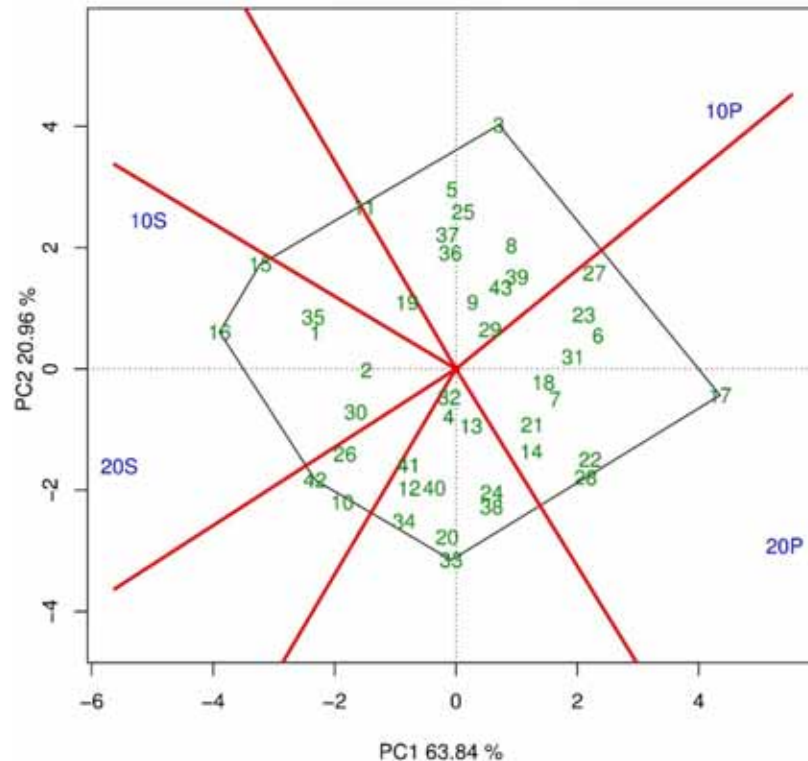


Figure 1. GGE biplot representing the “which-won-where” graph indicating the *Ralstonia pseudosolanacearum* e *Ralstonia solanacearum* resistance rankings of 43 genotypes. Environment 10S (10⁰ days after inoculation with *Ralstonia solanacearum*), 20S (20⁰ days after inoculation with *Ralstonia solanacearum*), 10P (10⁰ days after inoculation with *Ralstonia pseudosolanacearum*) e 20P (20⁰ days after inoculation with *Ralstonia pseudosolanacearum*).

The “which-won-where” biplot establishes a set of perpendicular lines, which divide it into several groups, and characterize the progenies in a way that the ones allocated in the vertex of the biplot are classified as of better performance and the ones that are in the polygons are the less responsive ones [28; 29].

The dates of the evaluations 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] this 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, *R. pseudosolanacearum*; 6, 7, 14, 18, 21, 22, 23, 27, 18 and

31, for the day 10, *R. pseudosolanacearum* also presented resistance; however, the grades of incidence were greater.

The genotypes that give rise to the vertices, but did not group any of the days of evaluation, 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*.

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 (Figure 2).

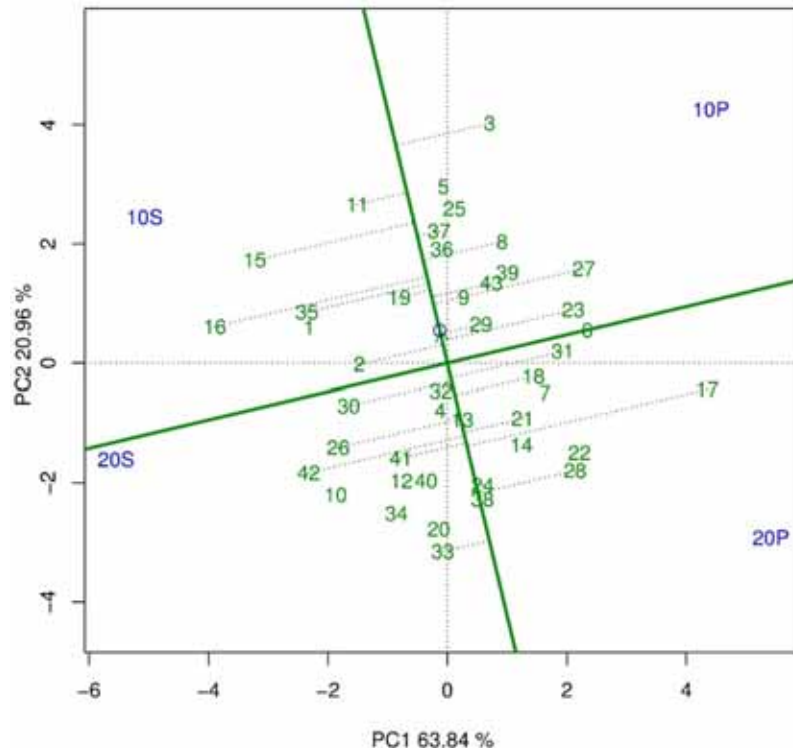


Figure 2. GGE biplot representing the means x stabilities indicating the yield rankings of 43 genotypes, and their respective incidence stabilities. Environment 10S (10⁰ days after inoculation with *Ralstonia solanacearum*), 20S (20⁰ days after inoculation with), 10P (10⁰ days after inoculation with *Ralstonia pseudosolanacearum*) e 20P (20⁰ days after inoculation with *Ralstonia pseudosolanacearum*).

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, and gave 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 showed 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 greater the projection formed by the genotype in relation to the axis, the more stable will be 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', and 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 (Figure 3). In this way, the genotypes 5 and 11 are the most wanted for the breeding program,

as they show good phenotypic resistance and stability, considered the closest ones to the ideal. In the same way, the genotypes 17, 20 and 33 were the ones that had the worst classification; this confirms they must be discarded.

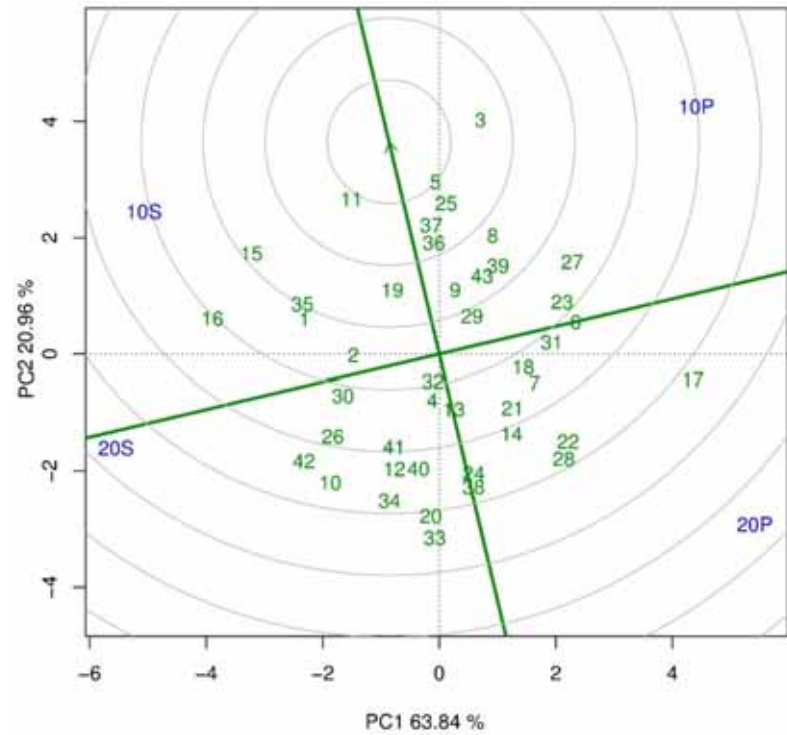


Figure 3. GGE biplot comparing 43 genotypes evaluated according to the estimate of an ideal genotype. Environment 10S (10⁰ days after inoculation with *Ralstonia solanacearum*), 20S (20⁰ days after inoculation with *Ralstonia solanacearum*), 10P (days after inoculation with *Ralstonia pseudosolanacearum*) e 20P (20⁰ days after inoculation with *Ralstonia pseudosolanacearum*).

The environments that produced smaller angles with the coordination of the average environment are considered the most representative ones, and the ones that show longer vectors are those that best describe the genotypes [34;35] (Figure 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 evaluated genotypes. The 20P (day 20 of evaluation of the *R. pseudosolanacearum* bacterium) proved to be less representative and distinguishing, and can be discarded [36; 37].

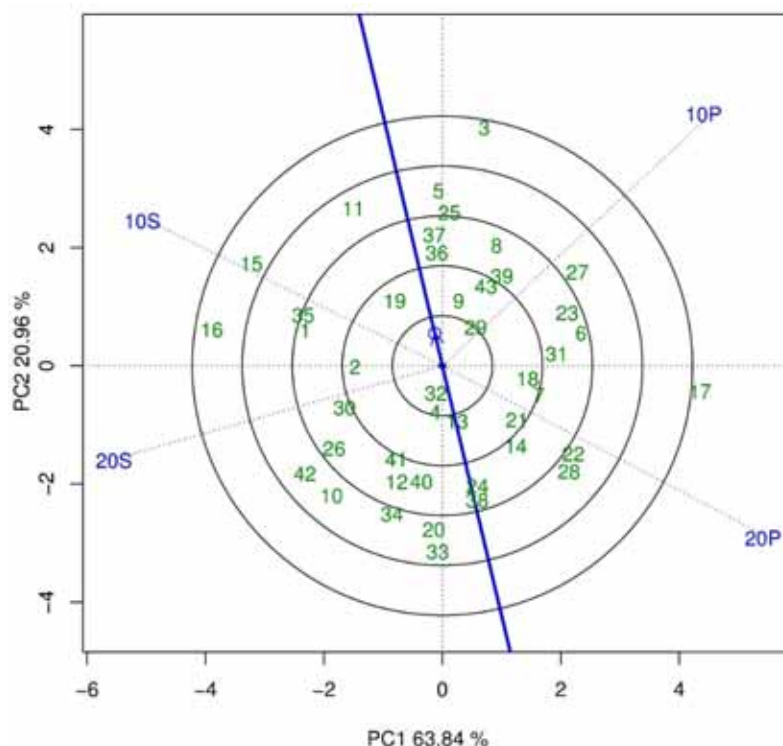


Figure 4. GGE biplot comparing 43 genotypes evaluated according to the discrimination and representativeness of environments for incidence. Environment 10S (10⁰ d days after inoculation with *Ralstonia solanacearum*), 20S (20⁰ days after inoculation with *Ralstonia solanacearum*), 10P (10⁰ days after inoculation with *Ralstonia pseudosolanacearum*) e 20P (20⁰ days after inoculation with *Ralstonia pseudosolanacearum*).

For a better result analysis, the REML/BLUP method was performed (Table 3). The genotypes 4, 6, 7, 14, 15, 17, 18, 34, 35 and 43 are among those ten that showed better values for the different environments and for the average 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.

Table 3. Genotypic values, adaptability and stability of genotypic values (MHPRVG) predicted by the REML / BLUP analysis for resistance to *R. pseudosolanacearum* e *R. solanacearum*, in 43 families F2: 3 of tomato Recife

Genotypes	<i>R. pseudosolanacearum</i>		<i>R. solanacearum</i>		Medium environment	MHPRVG
	10 ⁰ Day	20 ⁰ Day	10 ⁰ Day	20 ⁰ 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
Média geral	1.503	2.622	1.379	2.592	8.096	--

The REML/BLUP method combined with the GGE biplot, when classifying the genotypes resistant to *R. pseudosolanacearum* at the 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]. This 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 displayed resistance to *Ralstonia pseudosolanacearum*. Already the progenies 1, 2, 15, 16 and 35 showed resistance to *Ralstonia solanacearum*.

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