

## Genetic parameters and performance of papaya genotypes to black spot resistance (*Asperisporium caricae*)

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### Abstract

Estimation of genetic parameters such as genetic variability of germplasm allows inferring genotype-environmental interaction for a given variable. The information is important for the process of choosing the variables to be applied to the superior genotype selection. This study aimed at evaluating characteristics related to genetic resistance of papaya to black spot during time testing, as well as estimating genetic parameters associated with some characteristics. The experiment was carried out in RCBD design at Agua Limpa farm, Espírito Santo state, Brazil, using six genotypes: 'STZ-03', 'SS-PT', 'Golden' ('Solo' group) 'Maradol' ('Formosa' group) 'STA-04' 'STA-10' (landraces), and four repetitions. The 6 treatments were arranged in single row, spacing 2 m between rows and 1.5 m within plants. Nine evaluations were performed during 9 months. We quantified plants on a monthly basis for the characters such as symptom appearance of black spot (FS) on leaves; the incidence of leaves with black spot symptoms (IBS); the severity of black spot on the fifth leaf (SBS5F) and on the leaf with axil attached to the first open flower (SBSFO). By means of the evaluation values, we built a Boxplot graphic to characterize the magnitude of the variables and to describe the dispersion of the data set throughout the evaluations. Analysis of variance, genetic parameter estimate and comparative test of mean were also conducted. The Boxplot graphic allowed classification and magnitude of the variables and described the dispersion of the data set during evaluations. The results showed that SBS5F and the SBSFO were the characteristics that generated reliable results to select genotypes in all evaluations. They showed high H<sup>2</sup> (Coefficient of genotypic determination), CVg (Coefficient of genotypic variance), CVr (Coefficient of relative variance) and AS (Selective accuracy). The months July, August, September and October showed higher representativeness to evaluate attributes related to resistance to black spot in papaya leaves.

**Keywords:** Boxplot; *Carica papaya*; Foliar disease; Genetic resistance; Landraces.

**Abbreviations:** AS\_Selective accuracy; Cve\_Coefficient of experimental variance; CVg\_Coefficient of genotypic variance; CVr\_Coefficient of relative variance; FS\_First symptoms of black spot; H<sup>2</sup>\_Coefficient of genotypic determination; IBS\_Incidence of leaves with black spot symptoms; SBS5F\_Severity of black spot on the fifth leaf; SBSFO\_Severity of black spot on the leaf with axil attached to the first open flower;  $\sigma^2_g$ \_Genotypic variance;  $\sigma^2_p$ \_Phenotypic variance estimate and  $\sigma^2_r$ \_Residual variance.

### Introduction

Brazil presents high potential to increase fresh fruit production, which is the sector that employs 27% of agricultural labor force in Brazil (Treichel et al., 2016). Among the fruits produced, the papaya tree (*Carica papaya* L.), generated US\$ 43 million dollars of export value in 2016, including 37.9 thousand tons of exported fruit (Kist et al., 2017). The States of Bahia (723,582 tons) and Espírito Santo (361,270 tons) are considered the largest producers, providing 74% of national production (IBGE, 2015). In order to have a successful and profitable production of papaya, there is the need for having a perception of expanding

production capacity focusing on quality and sustainability of the environment, food safety and social responsibility. Moreover, a great challenge is to escape phytosanitary limitations (Amorim et al., 2016). Another issue is the restricted genetic diversity of commercial crops, which increases the vulnerability of culture to pest attack and diseases such as black spot (Moraes et al., 2011). The *Asperisporium caricae* (Speg.) Maubl. black spot is one of the fungal diseases that has expressive consequences in terms of photosynthetic area and recurring damages to production and papaya fruit quality (Oliveira et al., 2000; Martelleto et

al., 2009; Souza et al., 2014). The development of resistant cultivars is a strategic measure to sustain and increase competitiveness of papaya agribusiness, as well as to follow the trend of the conscious market concerning the food security, considering the large exportation of papaya from Brazil to the European and American markets. We need to take into account both the fruit quality and the requirement of increasingly smaller pesticide concentrations in food (Costa and Pacova, 2003; Bragoto et al., 2017). In this sense, it is essential to be aware of the genetic variability of the genotypes to set plans for improvement strategies (Vivas et al., 2014; Vivas et al., 2015; Vivas et al. 2016; Poltronieri et al., 2017). Accordingly, the use of genetic parameter estimates is relevant to show the variability of genetic materials (Cardoso et al., 2009; Vivas et al., 2012; Vivas et al., 2017). Furthermore, the genetic parameter estimates allow deducing the environmental influence on a given variable. That information is important in the variable choice process to be used in the selection of superior genotypes. For black spot on papaya leaves, researchers observed coefficient values of genotypic determination from 3.81 to 69.51 (Vivas et al., 2012). However, that was a particular evaluation, thus there is a need for evaluations at different times of the year. In order to allow evaluation, we need to know the change in the estimates of parameters throughout the epidemic (of time) once the disease has the indication of three factors: environment, pathogen and host (Amorim et al., 2016). Therefore, this research aimed at evaluating attributes related to genetic resistance to papaya black spot through time, as well as estimating genetic parameters associated with the characteristics evaluated. The contrast of the ways of estimating repeatability coefficients for the evaluated characteristics paves the next steps to the breeding programs in papaya.

## Results and Discussion

### Descriptive analysis

The Boxplot graphic (Figure 1) is formed by a box built parallel to the axis of the data scale, which starts in the first quartile and ends in the third quartile, and where a line is drawn in the position of the median. The box is common to all variants and is used to represent 50% centrals of data distribution (Bussab and Morettin, 2017). We applied the Boxplot graphic to describe the dispersion of the data set during the evaluations, and to characterize the variable magnitude in the different evaluations. We noted slight dispersion of leaf variable starting black spot symptoms (FS) in the evaluations between February and July (Figure 1A). We also noticed greater changes in median values between March and April; June and July; July and August; and August and September, showing that, as months pass, the disease evolves infecting new leaves (Figure 1A). For incidence of black spot symptoms on leaf (IBS), we perceived the variance tendencies were similar to what was seen in FS. That indicates a variance in the number of leaves with black spot as months pass (Figure 1B). The black spot severity estimated on the fifth leaf (SBS5F) and on the leaf with axil attached to the first open flower (SBSFO) presented small variance in the mean interval values, pointing that the environment has little influence on the estimates of those characteristics, where the most part of the variance comes

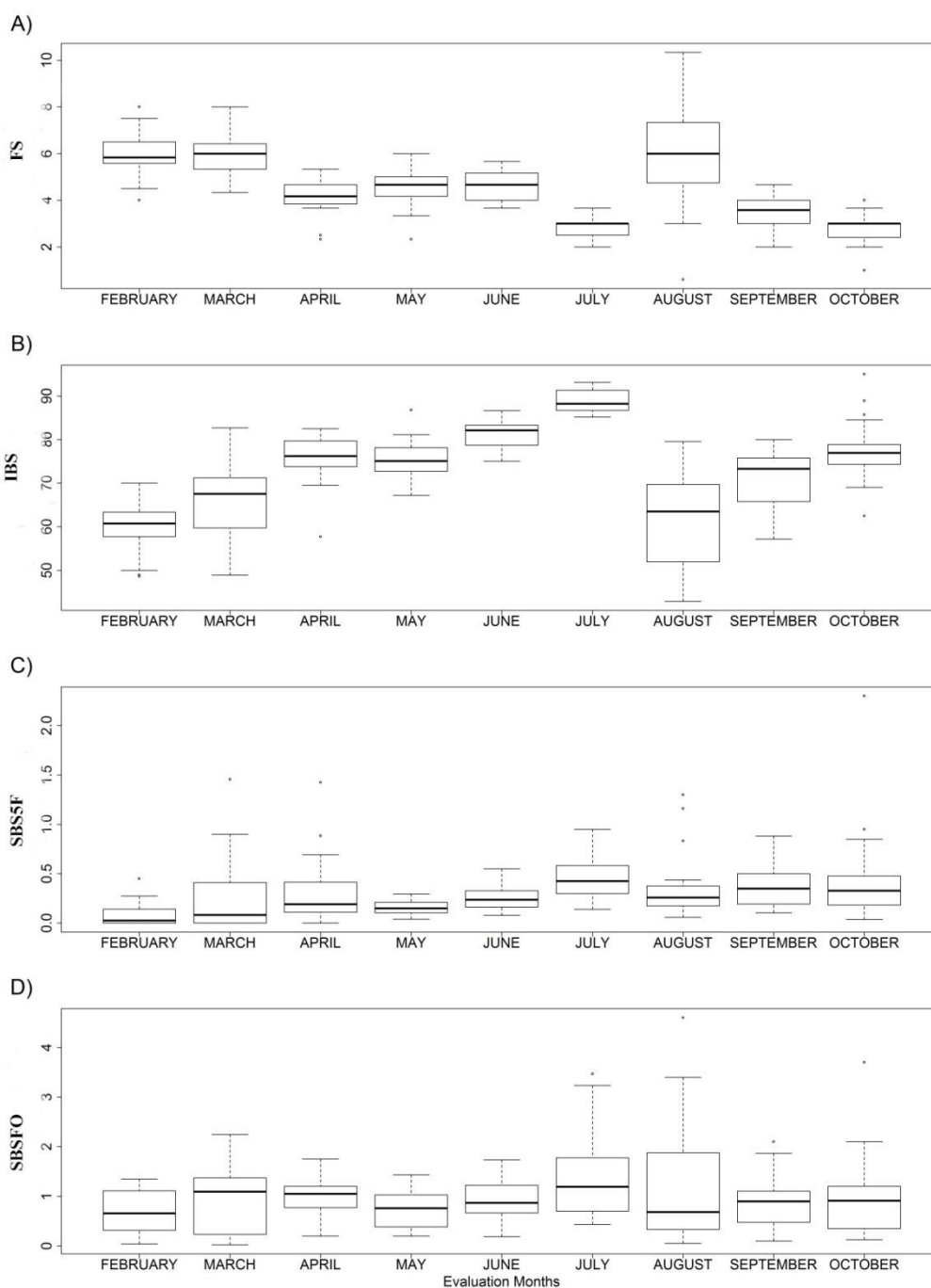
from the genotypes (Figure 1C and D). We also observed outliers, which are a common variant of this kind of graphic. The outliers are the atypical values or values far from the majority of data points. Such values were expected in this research because of the heterogeneity of the genetic constitution of the treatments. The genetic diversity of genotypes on the dendograms was reported by Cardoso et al. (2009); Vivas et al. (2015) and Silva et al., (2017). In this study, we observed that genotypes 'STZ-03', 'SS-PT', 'Maradol' and 'Golden' are distant from each other. Moreover, the genotypes 'STA-04' and 'STA-10' are progenies of half-siblings, from the landraces genetic material, presenting high variability in the treatments. The outliers may also be due to the existence of genotype performance for resistance to black spot. Vivas et al. (2012) described a different performance for severity to black spot among genotypes 'Maradol', 'STA-04', 'STA-10' and 'Golden', being the first three genotypes classified as resistant and the last one, as susceptible.

### Analysis of variance, genetic parameters and tests of averages

Before proceeding to the variance analysis, we conducted test for normality. The Shapiro-Wilk test showed that most of the variances analyzed presented normal distribution (data not shown). The exception was the evaluation conducted in October for (SBSS5F). Therefore, evaluation was eliminated from the variance analysis and other results. Variances in the treatment significances were noted throughout evaluations, in particular, for leaf starting black spot symptoms (FS) and incidence of leaves with black spot symptoms (Tables 1 and 2). The variances noticed to the source of genotypic variance in the different months of evaluation, suggest differentiated performance of the genotypes in response to environmental changes (Cruz et al., 2012). That information allows concluding which months are better to conduct genotype selection; however, there is the need to estimate some genetic parameters to make reliable selection of genotypes possible. The coefficients of experimental variance ( $CVe$ ) oscillated from 10.45 to 34.65, and the  $CVg$  oscillated from 10.67 to 12.75 (Table 1). Those values can be considered as satisfactory, as Vivas et al., (2012) found a  $CVe$  of 21.45% and  $CVg$  of 7.51%. From the same characteristic, it can be noticed that the coefficient of relative variance ( $CVr$ ) was greater than 1 (Table 2) only in February and March. The  $CVr$  with estimate values close to 1 recommends that the genetic control of the characteristic is high, showing slightly influenced by the environment. It is no coincidence that those months presented the greatest estimates of heritability (Table 2). Nevertheless, only the coefficient of experimental variance did not show the quality of the experiment. Recently, some works have been adopted the selective accuracy (Storck and Silva, 2014; Campos et al., 2016). The selective accuracy was presented greater than 81% for this characteristic. Values of selective accuracy greater than 70% are considered acceptable for a field experiment (Resende e Duarte, 2007). Although that variance presented relevant estimates for the genetic parameters estimated, only four out of nine evaluations presented significant effect for the genotypes (Table 2). This demonstrates that FS did not present stable to discriminate

**Table 1.** Characteristics of the genotypes used.

Identification	Origin	Main characteristics
Golden	Company Caliman SA	Elite genotype
Sunrise Solo PT	Company Caliman SA	Elite genotype
STZ-03	Company Caliman SA	Elite genotype
Maradol	Company Caliman SA	Elite genotype
STA-04	Landraces	Source of resistance
STA-10	Landraces	Source of resistance



**Fig 1.** The Boxplot values in different months for (A) the first symptoms of black spot on leaf - FS; (B) Incidence of leaves with black spot symptoms - IBS; (C) Severity of black spot on the fifth leaf - SBS5F; and (D) Severity of black spot on the leaf with axil attached to the first open flower - SBSFO.

**Table 2.** Summary of variance analysis, genetic parameters and test for mean comparison presented for different evaluations of Leaf to start black spot symptom (FS).

FV	GL	Mean Squares								
		FEB.	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEPT	OCT.
Block	3	0.20	1.06	0.34	0.37	0.10	0.05	1.19	0.45	0.14
Genotype	5	2.70**	2.50**	1.42*	0.96 <sup>ns</sup>	0.74 <sup>ns</sup>	0.23 <sup>ns</sup>	8.04 <sup>ns</sup>	0.87*	0.86 <sup>ns</sup>
Residue	15	0.39	0.43	0.39	0.64	0.27	0.26	4.40	0.30	0.39
CVe(%)		10.45	11.01	14.75	17.78	11.15	17.62	34.65	15.35	22.47
Genetic Parameter Estimates										
CVg(%)		12.75	12.10	11.98	--	--	--	--	10.67	--
CVr		1.22	1.10	0.81	--	--	--	--	0.69	--
$\sigma^2_P$		0.68	0.62	0.35	--	--	--	--	0.22	--
$\sigma^2_G$		0.58	0.52	0.26	--	--	--	--	0.14	--
$\sigma^2_r$		0.39	0.43	0.39	--	--	--	--	0.30	--
H <sup>2</sup>		85.62	82.85	72.52	--	--	--	--	65.88	--
AS		0.93	0.91	0.85	--	--	--	--	0.81	--
Test for Mean Comparisons										
Golden		4.96b	4.94b	3.71ab	3.75	4.00	2.63	4.57	3.00a	2.42
STZ-03		5.54b	5.17b	4.09ab	4.17	4.96	3.25	8.29	3.42a	2.75
SS-PT		6.34ab	7.08a	4.67ab	4.92	4.33	3.00	4.72	3.83a	2.88
Maradol		7.38a	6.04ab	5.04a	4.42	5.00	2.83	6.84	3.13a	2.13
STA-10		5.83b	6.08ab	4.42ab	4.92	5.08	2.88	6.42	4.25a	3.46
STA-04		5.75b	6.37ab	3.46b	4.92	4.67	2.63	5.50	3.67a	3.00
Overall Mean		5.97	5.95	4.23	4.51	4.67	2.87	6.05	3.55	2.77

(CVe) – Coefficient of experimental variance, (CVg) – Coefficient of genotypic variance, (CVr) – Coefficient of relative variance, ( $\sigma^2_P$ ) – Phenotypic variance estimate, ( $\sigma^2_G$ ) – Genotypic variance, ( $\sigma^2_r$ ) – Residual variance, (H<sup>2</sup>) – Coefficient of genotypic determination and (AS) – Selective accuracy.

**Table 3.** Summary of variance analysis, genetic parameters and test for mean comparison presented for different evaluations of black spot Incidence on leaf (IBS).

FV	GL	Mean Squares								
		FEB	MAR.	APR.	MAY	JUN.	JUL.	AUG.	SEPT	OCT.
Block	3	27.13	21.72	12.86	22.69	11.82	0.73	43.31	98.47	55.86
Genotype	5	49.43 <sup>ns</sup>	285.8**	13.14 <sup>ns</sup>	20.50 <sup>ns</sup>	19.37 <sup>ns</sup>	13.52 <sup>ns</sup>	355.6**	70.85*	97.22*
Residue	15	22.45	14.98	34.98	16.92	6.84	5.05	54.71	21.52	25.23
CVe(%)		7.92	5.79	7.77	5.46	3.22	2.53	11.91	6.51	6.47
Genetic Parameter Estimates										
CVg(%)		--	12.32	--	--	--	--	13.97	4.93	5.46
CVr		--	2.13	--	--	--	--	1.17	0.76	0.84
$\sigma^2_P$		--	71.46	--	--	--	--	88.92	17.71	24.31
$\sigma^2_G$		--	67.71	--	--	--	--	75.24	12.33	18.00
$\sigma^2_r$		--	14.98	--	--	--	--	54.71	21.52	25.23
H <sup>2</sup>		--	94.76	--	--	--	--	84.62	69.63	74.05
AS		--	0.97	--	--	--	--	0.92	0.83	0.86
Test for Mean Comparisons										
Golden		57.07	71.33b	74.66	77.11	82.40	89.51	60.34ab	74.2ab	80.85ab
STZ-03		61.90	68.56bc	75.45	73.84	78.73	86.09	50.61b	73.6ab	78.08ab
SS-PT		59.08	56.79d	76.08	73.66	81.78	87.29	62.37ab	63.86b	70.09b
Maradol		65.41	80.33a	79.57	79.06	83.54	89.73	53.47b	74.64a	84.57a
STA-10		55.57	60.89cd	76.17	73.67	78.48	90.11	71.64a	68.6ab	75.24ab
STA-04		59.92	62.8bcd	74.73	74.46	83.09	90.86	74.10a	72.2ab	77.18ab
Overall Mean		59.82	66.79	76.11	75.30	81.34	88.93	62.09	71.21	77.67

(CVe) – Coefficient of experimental variance, (CVg) – Coefficient of genotypic variance, (CVr) – Coefficient of relative variance, ( $\sigma^2_P$ ) – Phenotypic variance estimate, ( $\sigma^2_G$ ) – Genotypic variance, ( $\sigma^2_r$ ) – Residual variance, (H<sup>2</sup>) – Coefficient of genotypic determination and (AS) – Selective accuracy.

**Table 4.** Summary of variance analysis, genetic parameters and test for mean comparison presented for different evaluations of black spot Severity estimated on the fifth leaf (SBS5F).

FV	GL	Mean Squares								
		FEV	MAR	ABR	MAI	JUN	JUL	AGO	SET	OUT
Block	3	0.004	0.034	0.035	0.000	0.005	0.076	0.267	0.101	--
Genotype	5	0.043**	0.501**	0.285*	0.02**	0.040**	0.124**	0.171*	0.09**	--
Residue	15	0.006	0.038	0.066	0.001	0.009	0.018	0.044	0.019	--
<b>CVe(%)</b>		<b>97.61</b>	<b>76.48</b>	<b>83.34</b>	<b>20.37</b>	<b>37.49</b>	<b>29.56</b>	<b>60.64</b>	<b>37.34</b>	<b>70.83</b>
Genetic Parameter Estimates										
CVg(%)		116.59	133.66	75.82	44.63	35.99	35.37	51.29	36.81	--
CVr		1.197	1.748	0.910	2.236	0.959	1.195	0.846	0.987	--
$\sigma^2_P$		0.011	0.125	0.071	0.005	0.010	0.031	0.043	0.023	--
$\sigma^2_G$		0.009	0.116	0.055	0.005	0.008	0.026	0.032	0.019	--
$\sigma^2_r$		0.006	0.038	0.066	0.001	0.009	0.018	0.044	0.019	--
H <sup>2</sup>		85.09	92.43	76.8	95.05	78.66	85.13	74.11	79.54	--
AS		0.923	0.961	0.876	0.976	0.887	0.923	0.861	0.892	--
Genotypes										
Test for Mean Comparisons										
Golden		0.25a	0.90a	0.48ab	0.22ab	0.38a	0.54a	0.26ab	0.59a	0.52
STZ-03		0.09ab	0.41b	0.31ab	0.13cd	0.17b	0.41ab	0.16ab	0.41ab	0.37
SS-PT		0.00b	0.01b	0.15b	0.16bc	0.35ab	0.61a	0.62a	0.36ab	0.21
Maradol		0.00b	0.01b	0.01b	0.09d	0.18ab	0.35ab	0.54ab	0.46ab	1.21
STA-10		0.00b	0.17b	0.16ab	0.09cd	0.15b	0.19b	0.12b	0.17b	0.05
STA-04		0.15ab	0.03b	0.75a	0.27a	0.27ab	0.65a	0.39ab	0.23b	0.33
<b>Overall Mean</b>		<b>0.08</b>	<b>0.25</b>	<b>0.31</b>	<b>0.16</b>	<b>0.25</b>	<b>0.46</b>	<b>0.35</b>	<b>0.37</b>	<b>0.45</b>

(CVe) – Coefficient of experimental variance, (CVg) – Coefficient of genotypic variance, (CVr) – Coefficient of relative variance, ( $\sigma^2_P$ ) – Phenotypic variance estimate, ( $\sigma^2_G$ ) – Genotypic variance, ( $\sigma^2_r$ ) – Residual variance, (H<sup>2</sup>) – Coefficient of genotypic determination and (AS) – Selective accuracy.

**Table 5.** Summary of variance analysis, genetic parameters and test for mean comparison presented for different evaluations of black spot Severity estimated on the leaf with axil attached to the first open flower (SBSFO).

FV	GL	Mean Squares								
		FEV	MAR	ABR	MAI	JUN	JUL	AGO	SET	OUT
Block	3	0.10	0.02	0.03	0.16	0.27	0.51	1.80	0.17	0.27
Genotype	5	0.63**	1.49**	0.36**	0.34**	0.43**	2.27**	4.50**	0.88**	2.02**
Residue	15	0.08	0.15	0.05	0.07	0.07	0.30	0.34	0.06	0.21
<b>CVe(%)</b>		<b>39.75</b>	<b>42.07</b>	<b>22.62</b>	<b>34.00</b>	<b>29.58</b>	<b>40.07</b>	<b>49.28</b>	<b>28.34</b>	<b>49.12</b>
Genetic Parameter Estimates										
CVg(%)		53.35	62.77	27.97	34.33	32.5	51.42	85.63	51.11	71.66
CVr		1.34	1.49	1.24	1.01	1.10	1.28	1.74	1.80	1.46
$\sigma^2_P$		0.16	0.37	0.09	0.08	0.11	0.57	1.13	0.22	0.50
$\sigma^2_G$		0.14	0.34	0.08	0.07	0.09	0.49	1.04	0.20	0.45
$\sigma^2_r$		0.08	0.15	0.05	0.07	0.07	0.30	0.34	0.06	0.21
H <sup>2</sup>		87.81	89.9	85.95	80.31	82.84	86.82	92.35	92.86	89.49
AS		0.94	0.95	0.93	0.90	0.91	0.93	0.96	0.96	0.95
Genotypes										
Test for Mean Comparisons										
Golden		1.08ab	1.39ab	1.12ab	1.01 <sup>a</sup>	1.19a	2.37a	2.07ab	1.58a	1.18ab
STZ-03		1.24a	1.35ab	1.27a	0.71ab	1.11a	1.32ab	0.50c	0.97bc	0.98b
SS-PT		0.16c	0.36c	0.68bc	0.48ab	0.98a	0.84b	0.88bc	0.79bcd	0.56b
Maradol		0.67abc	1.61a	1.27a	1.05a	1.18a	2.20a	2.92a	1.19ab	2.19 <sup>a</sup>
STA-10		0.57bc	0.13c	0.57c	0.36b	0.34b	0.55b	0.30c	0.29d	0.15b
STA-04		0.49bc	0.70bc	1.08abc	0.93ab	0.74ab	0.92b	0.48c	0.49cd	0.58b
<b>Overall Mean</b>		<b>0.7</b>	<b>0.92</b>	<b>1</b>	<b>0.76</b>	<b>0.92</b>	<b>1.36</b>	<b>1.19</b>	<b>0.88</b>	<b>0.94</b>

(CVe) – Coefficient of experimental variance, (CVg) – Coefficient of genotypic variance, (CVr) – Coefficient of relative variance, ( $\sigma^2_P$ ) – Phenotypic variance estimate, ( $\sigma^2_G$ ) – Genotypic variance, ( $\sigma^2_r$ ) – Residual variance, (H<sup>2</sup>) – Coefficient of genotypic determination and (AS) – Selective accuracy.

the genotypes, even though the performance of the genotypes throughout evaluations did not suffer great alterations in its ranking (Table 2), mainly 'STZ-03' and 'Maradol', as more resistant, and 'STA-04', in the intermediate group. Similar to what was noticed in FS, it can be seen that variance presented itself unstable during the evaluations, when analyzing the incidence of leaves with black spot symptoms (IBS). Among the nine evaluations conducted, a significant effect of genotype was observed in only four of them, confirming that particular evaluations would not be enough to combine all genetic variance existent for that characteristic (Table 3). Despite the fact that the IBS presented the lowest estimates of coefficient of experimental variance and selective accuracy above 80% (Table 3), we observed that this characteristic was not effective in revealing the real potential of the genotypes, since the estimates of  $CV_r$  presented values greater than 1 in only two evaluations (Table 3). There were alterations on the genotype ranking for IBS, which oscillated among the extracts of high incidence and low incidence, especially 'STZ-03' and 'Maradol' (Table 3). In contrast to leaf starting black spot symptom and incidence of leaves with black spot symptoms (Tables 2 and 3), greater magnitudes in the coefficients of experimental variance were observed (Tables 4 and 5) after analyzing the severities of black spot (estimate on the fifth leaf - SBS5F, and on the leaf with axil attached to the first open flower - SBSFO). Those magnitudes may be attributed to the means of the characteristics, which varied from 0.08 to 0.46 for SBS5F (Table 4) and from 0.88 to 1.36 for SBSFO (Table 5). High magnitudes of  $CV_e$  did not express necessarily low experimental accuracy, varying parameter from characteristic to characteristic. Resende and Duarte (2007) pointed out the selective accuracy as an indicator to infer the quality of experiment in a program for genetic improvement. In this study, the SBS5F and SBSFO presented the highest estimates of selective accuracy (Tables 3 and 4). For SBS5F, high values for genotypic coefficient of determination ( $H^2$ ) were found, varying from 74% to 95% (Table 4). It is not a coincidence that the high values estimated for  $CV_r$  (Table 4) validated the  $H^2$  and indicated that the coefficient of genetic variance was greater than the coefficient of experimental variance. For the severity of black spot estimated in the fifth leaf, there was alteration in severity ranking, indicating environment variation under this characteristic. As an example, the genotype 'Maradol' appeared in low severity in the evaluations conducted in February, March and April, and in July and August. It was occurred in high severity. Another example is the genotype 'STA-04', which appeared in low severity in the evaluations in March and September. However, in April, May and July, it appeared having high severity (Table 4). The severity of black spot estimated on the leaf with axil attached to the first open flower (SBSFO) presented the greatest magnitudes for the estimate genetic parameters, with  $CV_r$  greater than 1 and  $H^2$  greater than 90% (Table 5). That result calls for the possibility of obtaining better results when practicing the selection for this variance, as it presents lower environmental influence and greater dispersion of the estimated values (Figure 1), besides presenting high estimates of AS during the different times of the year evaluated. Therefore, considering the estimates of genetic parameters and the dispersion of the experimental units in the Boxplot, we observed that the SBSFO was superior to the

other variances. It was noted that although there is alteration in the genotype ranking throughout the evaluations, there is a tendency for the genotypes to occupy the smallest positions. Except for May and August, the genotypes 'Maradol', 'STA-04' and 'STA-10' were always at the lowest positions in ranking (Table 5).

## Materials and Methods

### Plant materials

We studied six genotypes of papaya in the present study: Golden', 'Sunrise Solo PT' and 'STZ-03', considered lineages of 'Solo'; 'Maradol', lineage of 'Formosa'; 'STA-04' and 'STA-10' progeny of half-siblings of landraces papaya (Table 1).

### Experimental conditions and design

The experiment was set up in randomized block design with four repetitions at Agua Limpa farm, Mimoso do Sul municipality, (Latitude 21° 03'52" S, Longitude 41°21'59"W) in the Espírito Santo State. The six genotypes were considered as treatments. One month after sowing the seeds in a greenhouse, the seedlings were taken to the field. The treatments were arranged in a single row, in spacing of 2 m between rows and 1.5 m between plants. Considering papaya plants are semi-perennial and that there is a possibility of loss of plants throughout the evaluations, each experimental plot was initially composed of three plants of each treatment. The fertilization was conducted according to suggestion by Marin et al. (1995). The monthly evaluations were started when the plants were four-month-old and finished nine months later. It was not necessary to execute artificial inoculation with *A. caricae* in papaya plants once the inoculum is in the environment all year long (Suzuki et al., 2007).

### Characteristics evaluated

Four months after the installation of experiment, we started monthly variable evaluations, quantifying in which leaf the first symptoms of black spot (FS) appeared. To estimate that value, we counted the apex to the base which was the leaf that presented the first symptoms of the disease, considering as the first leaf the youngest fully expanded one (leaves whose central lobe had a length equal to or less than the length of the petiole).

The incidence (%) of leaves with black spot symptoms (IBS), was obtained from the relation between the number of leaves infected and the total number of leaves per plant. The severity of black spot on the fifth leaf (SBS5F) and on the leaf with axil attached to the first open flower (SBSFO) were determined based on the methodology adopted by Vivas et al. (2011), with the aid of a diagrammatic scale (0.2, 1.6, 3.5, 5.4, 7.6 and 12.8% of injured leaf area).

### Statistical analysis

The data of each characteristic were plotted in the Boxplot graphic throughout time using the base package of R. statistical program to help visualizing the disease evolution over the evaluation periods (months). Data of each evaluation was submitted to the normality test (Shapiro-Wilk). When the normal distribution of data was found, they

were submitted to analysis of individual variance for each characteristic throughout evaluation time. The significant effect of the source of variance genotype was obtained by the variance analysis. It was estimated based on the expressions:  $H^2 = \frac{\phi_G}{\sigma_p^2}$ , in which  $H^2$  is the coefficient of genotypic determination based on the genotype mean;  $\phi_G = \frac{MSG-MSE}{r}$ ,  $\phi_G$  is the value of the quadratic component of the genotypic variable;  $MSG$  is the mean square of the genotype,  $MSE$  is the mean square error,  $r$  is the number of repetitions,  $\sigma_p^2 = \frac{MSG}{r}$ , in which  $\sigma_p^2$  is the mean phenotypic variance. The selective accuracy (AS) and the coefficient of relative variance (Cv<sub>r</sub>) were estimated according to the methodology of (Resende, 2007):  $AS = \sqrt{1 - 1/F_c}$  and  $Cv_r = \sqrt{(F_c - 1)/b}$ , in which  $F_c$  is the value of the F-test the effect associated to the genotype with the variance analysis and  $b$  is the number of repetitions, which in this case are blocks. Lastly, the genotype means were compared by the Turkey's test (probability of 0.05). The variance analyses and the mean test were obtained from the Genes computational application (Cruz, 2013).

## Conclusion

In conclusion, the SBS5F and the SBSFO were the characteristics which generated better results to select genotypes in all evaluations with high magnitudes of  $H^2$ , CV<sub>g</sub>, CV<sub>r</sub> and AS. Confirming that in the estimation of the genetic components, these characteristics show less influence of the environment. July, August, September and October were the months with greater representativeness to evaluate attributes related to resistance to black spot in papaya leaves. One evaluation for black spot resistance is not enough; therefore, four evaluation periods, on the months of July, August, September and October, are recommended based on the  $H^2$ , CV<sub>g</sub>, CV<sub>r</sub> and AS magnitudes.

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