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Resistance to multiple foliar diseases in papaya genotypes in Brazil

Marcelo Vivas [a], Silvaldo F. Silveira [a, *], Alexandre Pio-Viana [b], Antônio T. Amaral-Júnior [b], Geraldo A. Ferreguetti [c], Messias G. Pereira [b]

[a] Laboratory of Entomology and Phytopathology, Darcy Ribeiro North Fluminense State University (UENF), Campos dos Goytacazes, RJ CEP 28013-602, Brazil
[b] Laboratory of Plant Breeding, UENF, Campos dos Goytacazes, RJ, Brazil
[c] Caliman Agrícola SA, Linhares, ES, Brazil

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ABSTRACT

The absence of cultivars with satisfactory levels of genetic resistance justifies the search for genotypes incorporating disease-resistance genes in the papaya-breeding program. In this study, two field-plot experiments were carried out in the municipality of Linhares, Espirito Santo State, Brazil, to evaluate resistance to foliar diseases in a papaya germplasm collection. The variables incidence and severity of leaf diseases and black spot incidence and severity on fruits were considered for uni- and multivariate variance analyses. The Mahalanobis distance was calculated for each pair of genotypes, and the distance matrix was used for clustering methods. Papaya genotypes were grouped by the Tocher method and the hierarchical method of Un-weighted Pair Group with Arithmetic Mean (UPGMA) was used for dendrogram construction. Although no immune reaction was observed among the genotypes for any of the leaf diseases, the data analyses highlight the following genotypes as potential sources of resistance genes for use in a papaya breeding program: 'STZ 23 PL', 'Maradol', 'Maradol GL', 'JS 11', 'Americano', 'Caliman SG', 'Sekati', 'Sekati FLM', 'Waimanalo', 'Caliman AM', 'Papaya 46', 'Tailândia' and 'SH 12-06'. This is the first time multivariate analyses were employed to evaluate multiple disease resistance in a papaya crop.

1. Introduction

Papaya (*Carica papaya* L.) is one of the most cultivated and consumed tropical fruits in the world. Papaya fruits are excellent sources of calcium, pro-vitamin A and vitamin C (ascorbic acid) and are widely used in food diets based on their functional and digestive properties (Serrano and Cattaneo, 2010). The Brazilian contribution to global papaya production in 2012 was approximately 12.23%, equivalent to 1.51 million tonnes of fruits (FAO, 2014). The main Brazilian papaya production areas are located in the States of Bahia, Espírito Santo, Ceará and Rio Grande do Norte. However, papaya crop expansion in terms of cultivated area and yield has severe sanitary constraints. Viruses (*Papaya ringspot virus*, PRSV-P; *Papaya meleira virus*, PMeV) are the major cause of renewal of papaya plantations. This problem has partly been solved by the practice of roguing imposed by lawful means. The practice of legally obligated roguing has provided control of viruses and allowed the establishment of plantations in prime areas of papaya production in southeastern Brazil, including the northern Espírito Santo State and southern Bahia State. However, intensive cultivation of papaya crops in fixed areas like this has favoured some fungal foliar disease epidemics, causing severe losses in the field and postharvest (Alvarez and Nishijima, 1987; Rezende and Martins, 2005).

In Brazil, black spot [*Asperisporium caricae* (Speg.) Maubl] is considered the most important foliar disease of the papaya crop in the field (Ventura et al., 2003). The fungus induces numerous small necrotic lesions on leaves and fruits that are comparable to a rust attack in other hosts, resulting in severe reduction of the leaf photosynthetic area, leaf blight symptoms and premature leaf abscission. Lesions at the postharvest phase predispose the fruit to invasion by rot pathogens during ripening, and are responsible for the most severe losses in the papaya crop induced by black spot disease (Rezende and Martins, 2005). Another common disease in both commercial and domestic plantations is powdery mildew [*Streptopodium caricae* Liberato & Barreto (Liberato et al., 2004)]. The disease can cause premature defoliation and fruit epidermal lesions, restricting the growth of underlying tissues, causing the fruit to be deformed and without commercial value.

In the postharvest phase, the main diseases are anthracnose, caused by the "complex species" *Colletotrichum gloeosporioides*

* Corresponding author.
E-mail addresses: silvaldo@uenf.br, slipes1967@gmail.com (S.F. Silveira).

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Penz., and stalk rot, which is caused by different species of fungi (Alvarez and Nishijima, 1987), particularly *C. gloeosporioides* and *Stagonosporopsis caricae* (Sydow & P. Sydow) Aveskamp, Gruyter & Verkley (=*Phoma caricae-papayae* (Tar) Punith; =*Ascochyta caricae* Pat.) (Aveskamp et al., 2010; Liberato and Tatagiba, 2001; Rezende and Martins, 2005). In the absence of any control measures during the production and postharvest phases, the incidence of fruit with stalk rot reaches 100% (Ventura et al., 2003). In addition to stalk rot during the postharvest phase, *S. caricae* (=*P. caricae-papayae*) also causes phoma leaf spot. This disease induces extensive necrotic lesions on leaves and has resulted in changing production techniques as well as the active range of fungicides used to spray the papaya crop in Brazil (Vivas et al., 2010a, 2013a).

Fungicide spraying has been the primary measure of leaf-disease control in the papaya field because there are no genotypes with adequate levels of genetic resistance (Dianese et al., 2007; Vivas et al., 2010a, 2012c). However, the high cost, potential environmental damage and human contamination create a need for more friendly alternatives to control fungal diseases. Additionally, commercial barriers imposed by the presence of pesticide residues and the dependence of the papaya crop on conventional chemical control results in negative marketing for fruit export. Therefore, papaya crop breeding for disease resistance has emerged as a more sustainable and attractive strategy for the control of papaya leaf diseases. Despite the agronomic performance of Brazilian papaya hybrids in terms of quality and yield, genetic resistance to major diseases remains poorly comprehended. The Brazilian germplasm collections have not been explored for resistance to major diseases, and there is a need to expand the existing genetic basis (Vivas et al., 2010a, 2012c).

Multivariate statistical techniques are appropriate for studies in which several variables must be considered simultaneously, providing information and interpretations that are not possible with the use of univariate methods (Liberato et al., 1995). In phytopathology, multivariate techniques such as clustering methods have been used to determine the variability of specific pathogen isolates and to characterize genetic resistance to multiple diseases in crops (Anderson et al., 1990; Pataky et al., 1988; Vieira et al., 2009). Multivariate analysis is an important tool for the characterization of papaya accessions based on morphological characteristics (Quintal et al., 2012) and molecular markers (Oliveira et al., 2010). Although some studies have used cluster analysis to study plant resistance to several diseases simultaneously (Anderson et al., 1990; Pataky et al., 1988), this is the first such study for the papaya crop in Brazil.

2. Materials and methods

2.1. Experiments and experimental design

Two field-plot experiments were conducted in two distinct areas on a farm in the municipality of Linhares, Espírito Santo State, Brazil, at 21 m altitude. The location geographic coordinates were 19° 15′ latitude and 40° 10′ longitude. The local climate is characterized by average maximum temperature from 30.7 to 34 °C and average minimum temperature from 11.8 to 18.0 °C (INCAPER, 2014).

The first experiment was conducted during 2006–2007 (planted in the field in 2006 October) and 51 genotypes of the UENF-Caliman Germplasm Collection were evaluated in a randomized complete block design with two replications of 20 plants per plot, planted in double rows (10 plants in each row) with spacing of 2 × 1.8 m. Laterally, the treatments were 3.6 m from one another. For disease evaluation, only the four central plants of each row were considered. The second experiment, conducted during 2010–2012 (planted in the field in 2010 October) was set up and conducted in the same way as the first one and consisted of 59 genotypes. The experimental plots had 15 plants arranged in a single row, and the plants were spaced 2.0 × 3.6 m. Only the three central plants of each plot were evaluated in the second experiment. The eight extra genotypes of the second experiment were not included in the multivariate statistics analysis (below) because they are different from those of the first experiment.

Because the experiments also functioned as a germplasm bank collection and to ensure the production of fruits and seeds, fungicide sprays were performed monthly in both experiments. In the

Table 1
Averages of severity of black spot, phoma-spot and powdery mildew on leaf, and black-spot on fruit evaluated in papaya genotypes of heterotic group 'Formosa' and 'Solo' belonging to Uenf-Caliman Germplasm Collection, Linhares, Espírito Santo State, Brazil.

Heterotic group	Genotype	Powdery mildew		Phoma-spot		Black spot in leaf		Black spot in fruit	
Formosa	'Costa Rica'	2.19	a–c[a]	4.78	a–e	0.27	a–e	0.39	a–d
	'JS 11'	1.71	bc	2.29	b–e	0.20	c–e	0.26	a–d
	'JS 12'	0.74	c	3.72	b–e	0.20	c–e	0.05	cd
	'Cariflora'	5.07	a	9.43	a	0.10	e	0.07	cd
	'Tainung'	1.81	a–c	0.95	e	0.31	a–e	0.81	ab
	'Calimosa'	2.23	a–c	5.40	a–e	0.25	a–e	0.21	a–d
	'Americano'	1.52	bc	4.07	b–e	0.06	e	0.23	a–d
	'Mamão Bené'	2.40	a–c	2.11	b–e	0.05	e	0.13	cd
	'Papaya 42'	2.41	a–c	2.57	b–e	0.04	e	0.05	cd
	'Sekati FLM'	0.71	c	2.17	b–e	0.10	e	0.11	cd
	'SH 02-01'	1.73	a–c	3.14	b–e	0.15	de	0.16	cd
	'SH 04-02'	1.14	bc	4.98	a–e	0.04	e	0.28	a–d
	'SH 11-08'	1.11	bc	3.76	b–e	0.23	b–e	0.37	a–d
	'SH 12-06'	1.46	bc	2.53	b–e	0.04	e	0.83	a
	'SH 12-07'	0.82	c	2.75	b–e	0.10	e	0.12	cd
	'SH 14-05'	3.42	a–c	2.68	b–e	0.08	e	0.12	cd
	'SH 15-04'	1.62	bc	3.67	b–e	0.08	e	0.37	a–d
	'SH 50-09'	2.48	a–c	2.90	b–e	0.07	e	0.13	cd
	'Maradol GL'	1.42	bc	0.49	e	0.02	e	0.10	cd
	'Maradol'	0.35	c	0.47	e	0.04	e	0.07	cd
	'Sekati'	1.41	bc	2.40	b–e	0.07	e	0.23	a–d
	'Tailândia'	1.68	bc	2.86	b–e	0.13	e	0.47	a–d
Solo	'Caliman AM'	4.30	ab	2.68	b–e	0.12	e	0.18	b–d
	'Caliman G'	1.41	bc	2.93	b–e	0.21	c-e	0.13	cd
	'Caliman GB'	1.27	bc	4.46	a–e	0.11	e	0.12	cd
	'Caliman M5'	1.85	a–c	4.60	a–e	0.12	e	0.21	a–d
	'Caliman SG'	2.14	a–c	6.56	a–c	0.64	a	0.45	a–d
	'Diva'	1.15	bc	3.73	b–e	0.16	de	0.07	cd
	'Grampola'	0.62	c	1.49	c–e	0.06	e	0.39	a–d
	'Sunrise Solo'	1.11	bc	2.48	b–e	0.20	c-e	0.07	cd
	'Taiwan et'	1.79	a–c	4.42	a–e	0.15	de	0.13	cd
	'São Mateus'	1.23	bc	4.54	a–e	0.62	ab	0.48	a–d
	'Baixinho Super'	0.65	c	6.34	a–d	0.16	de	0.34	a–d
	'FMV'	2.00	a–c	5.18	a–e	0.22	c–e	0.20	a–d
	'Golden R'	1.50	bc	2.94	b–e	0.06	e	0.06	cd
	'Golden TF'	1.75	a–c	2.94	b–e	0.05	e	0.08	cd
	'Kapoho Solo PA'	2.07	a–c	4.08	a–e	0.10	e	0.07	cd
	'Kapoho Solo PV'	0.50	c	3.58	b–e	0.06	e	0.11	cd
	'Mamão Roxo'	1.61	bc	3.51	b–e	0.18	de	0.07	cd
	'Papaya 45'	1.81	a–c	2.86	b–e	0.07	e	0.10	cd
	'Papaya 46'	2.28	a–c	2.00	b–e	0.10	e	0.11	cd
	'STZ-03'	3.16	a–c	2.21	b–e	0.08	e	0.49	a–d
	'STZ 23 PL'	0.89	c	3.54	b–e	0.05	e	0.21	a–d
	'STZ-51'	0.55	c	2.85	b–e	0.19	c–e	0.69	a–c
	'STZ-63'	2.02	a–c	4.60	a–e	0.16	de	0.04	d
	'Sunrise Solo PT'	1.84	a–c	6.99	ab	0.11	e	0.13	cd
	'Waimanalo'	2.00	a–c	2.96	b–e	0.09	e	0.10	cd
	'Sunrise Solo 783'	0.88	c	3.45	b–e	0.53	a–d	0.42	a–d
	'BSA'	1.20	bc	3.51	b–e	0.06	e	0.15	cd
	'Sunrise Solo 72/12'	1.43	bc	1.03	De	0.58	a–c	0.08	cd
	'Sunrise Solo TJ'	1.43	bc	0.25	E	0.17	de	0.01	d

[a] Means calculated from data obtained from two field-plot experiments and two periods of evaluation for each experiment (May and August 2007 and December 2011 and February 2012). Means followed by the same letter within columns do not differ by the Tukey test at $P = 0.05$.