Trop. plant pathol. (2016) 41:380–389
DOI 10.1007/s40858-016-0109-1

ORIGINAL ARTICLE

Heterosis and genetic diversity for selection of papaya hybrids for resistance to black spot and phoma spot

Marcelo Vivas[1] · Helaine C. C. Ramos[2] · Pedro H. D. Santos[1] · Silvaldo F. Silveira[1] · Telma N. S. Pereira[2] · Antonio Teixeira do Amaral Jr.[2] · Messias G. Pereira[2]

Received: 3 February 2016 / Accepted: 26 October 2016 / Published online: 21 November 2016

Abstract In this work, two experiments were set up in order to select papaya hybrids and verify heterosis expression for black spot (*Asperisporium caricae*) and phoma spot (*Stagonoparopsis caricae*) resistance. Experiments were set up in randomized blocks with two replications. Black spot and phoma spot incidence and severity on leaves were evaluated, and fruit damaged area by black spot was estimated. These characteristics were used to estimate genetic distance with complement of Jaccard's Index. For black spot resistance, 77 % of hybrids showed heterosis for at least one of three evaluated characteristics and 58 % of hybrids showed heterobeltiosis effect. For phoma spot severity, there was heterosis manifestation in 56 % of hybrids, and 30 % of them showed heterobeltiosis effect. Therefore, heterosis was found in most of the hybrids, emphasizing the possibility of using hybridization to develop resistant genotypes. We observed that the best estimates of heterotic effects were obtained by crossing genetically distant individuals, indicating the existence of a positive relationship between genetic distance and hybrid performance. Together, the results highlight the hybrid 'Americano' x 'Waimanalo' as having a strong potential to reduce the intensity of these two diseases.

Keywords *Asperisporium caricae* · *Carica papaya* · *Stagonosporopsis caricae* · Genetic resistance · Hybrid vigor · Top cross

Section Editor: Rosana Rodrigues

✉ Marcelo Vivas
mrclvivas@hotmail.com

[1] Lab. de Entomologia e Fitopatologia, Centro de Ciências Tecnológicas e Agropecuárias, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ 28013-600, Brazil

[2] Lab. de Melhoramento Genético Vegetal, Centro de Ciências Tecnológicas e Agropecuárias, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, RJ 28013-600, Brazil

Introduction

Phoma spot, caused by *Stagonosporopsis caricae* (Sydow & P. Sydow) Aveskamp, Gruyter & Verkley [Syn. *Phoma caricae-papayae* (Tarr) Punith] (Aveskamp et al. 2010), is considered the second most important post-harvest disease of papaya in Brazil (Rezende and Martins 2005). The fungus is widespread in tropical regions (Hunter and Budderhagen 1972) and shows various symptoms. Another fungal disease that currently constitutes the main papaya disease is black spot caused by *Asperisporium caricae* (Speg.) Maubl (Rezende and Martins 2005). It causes severe losses by reducing the photosynthetic area of leaves and by depreciating the fruits for the market (Rezende and Martins 2005). The incidence and severity levels of these diseases can be so high that chemical control is mandatory to achieve profitable production at certain times of the year.

The main measure adopted to control these diseases is the use of fungicides (Dianese et al. 2007; Vivas et al. 2010). The selection of resistant genotypes is a sustainable alternative for disease control; however, in papaya, complete resistance has not been observed in elite genotypes (Vivas et al. 2010, 2012b). In field assessments, different levels of disease were observed (Vivas et al. 2015), probably due to a polygenic control of resistance. Crop improvement can be achieved by selection of resistant genotypes or by the best hybrid combinations, making use of hybrid vigour, also known as heterosis. Parteniani (2001) reports that breeders explored heterosis even before the concept was proposed. In addition, the term heterobeltiosis has been used to identify hybrids that present themselves better than the best parent (Vencovsky and Barriga 1992).

In the *Caricaceae* family, heterosis was observed in interspecific crosses of *Carica cauliflora* x *C. goudotiana* and *C. caulifora* x *C. monoecious* (Mekako and Nakasone 1975). In both crosses the characteristics of plant height, stem diameter, number and average fruit weight in the F_1 generation were significantly higher than among more vigorous parents. In papaya, Marin et al. (2006), studying heterotic effects among eight parents of the 'Solo' group and eight of the 'Formosa' group, observed the prevalence of heterobeltiosis in nine morphoragronomics characteristics, concluding that heterosis is a common effect in papaya hybrids. Martins et al. (2009) observed the early manifestation of heterosis in F_1 hybrids for physiological attributes of papaya seeds. Vivas et al. (2012a) reported that heterosis in papaya for resistance to black spot may provide significant genetic gains from both inter-and intra-heterotic crosses.

Considering the possibility of obtaining hybrids with lower levels of disease, the present study was conducted with the objectives of selecting resistant hybrids and determining the manifestation of heterosis or hybrid vigour for resistance to black spot (*A. caricae*) in leaves and fruits and phoma spot (*S. caricae*) in leaves of papaya hybrids.

Material and methods

Genetic material and experiment design

Two experiments were conducted during 2006–2007 (planted in the field in Oct 2006) at Caliman Agrícola S/A, in Linhares, Espírito Santo state. This region is a great papaya producer; therefore, inoculum is present all year, with no need to perform artificial inoculations. The first experiment consisted of a group of papaya hybrids from the cross of 20 elite genotypes, 13 of the 'Solo' and seven of the 'Formosa' group, and two testers, 'Sunrise Solo 72/12' ('Solo' group) and 'JS 12' ('Formosa' group). The second experiment involved 38 hybrids from the cross of 23 genotypes, 17 of 'Solo' and six of 'Formosa', with three testers of the 'Formosa' group: 'Americano', 'Maradol' and 'Sekati'.

A randomized block design was used with two replications; each replication had 20 plants, arranged in double rows (10 plants in each row) in 2.0 x 1.8 m spacing. All cultural practices were applied as recommended for the crop. Fungicide sprays were made weekly or biweekly. Until 60 days before the first evaluation, the following active ingredients were used: pyraclostrobin (Apr 16, 23 and May 21) and chlorothalonil (Apr 30). Before the second evaluation, the following active ingredients were sprayed: azoxystrobin (Jul 10 and Aug 17), chlorothalonil (Aug 03), lime sulfur (Aug 10) and methyl thiophanate (Jul 27).

Evaluation of traits related to resistance

Two evaluations were conducted, the first one in late May and the second one in late Aug 2007. The plot where the evaluations were performed consisted of four rows, and these were used to calculate the average per plot. The incidence and severity of black-spot and phoma-spot were evaluated. The incidence of leaves with disease symptoms was obtained as the percentage ratio between the number of leaves with symptoms and the total number of leaves. Black spot severity data in leaves was obtained using the diagrammatic scale described by Vivas et al. (2012b), evaluating the leaf with axils attached to the first open flower. Phoma spot severity was evaluated by the willing leaf immediately below the leaf with the first open flower, with the aid of the diagrammatic scale adopted by Vivas et al. (2010). Since there was no scale to estimate the severity in fruit, black spot lesions were counted on the surface of the fruit at stage 1 of ripening. Subsequently, the number of black spot lesions on the fruit were converted into injured surface area (PPF). The counts were transformed into percentage by multiplying the number of lesions by the mean values estimated via Quant, corresponding to 0.26 %.

Statistical analysis

Statistical analysis was performed with SAS (SAS Institute 1992) and GENES (Cruz 2013) softwares, estimating the average of the hybrids and their parents. Subsequently, heterosis (Average Parent Heterosis - APH) and heterobeltiosis (Heterosis of Superior Parent- HSP) were estimated.

APH was estimated as:

$$\left(\frac{HDA-PDA}{PDA}\right)*100$$

where HDA = hybrid disease average, and PDA = parent disease average.

HSP was estimated as:

$$\left(\frac{HDA-SPA}{SPA}\right)*100$$

where HDA = hybrid disease average, and SPA = superior parent average (Falconer 1989).

Mahalanobis distances (D2) were calculated for all pairs of genotypes using all disease variables in the calculations. Based on the genetic distance matrix, the genotypes were grouped and a dendrogram constructed using the grouping method by average distance (UPGMA). The fit between the distance matrix and the dendrogram was estimated by the cophenetic correlation coefficient (CCC) developed by Sokal and Rohlf (1962). All data analyses were performed with the Genes program (Cruz 2013).

Results and discussion

Resistance to black spot

In first experiment, a tendency was observed for hybrids with the tester 'Sunrise Solo 72/12' (SS 72/12) to generate lower levels heterosis and heterobeltiosis, on average, for all variables related to *A. caricae* resistance. For severity of black spot the average of hybrid heterosis derived from crosses with 'SS 72/12' was negative (Table 1). In the second experiment, the estimates of the lowest averages were not obtained for a specific tester, however, there was a tendency for lower average of heterosis in hybrids obtained with 'Americano' (Table 2).

The hybrid combinations 'Caliman M5', 'Diva', 'Sunrise Solo' and 'Caliman G' x 'JS 12' showed negative estimates of heterosis for black spot severity (Table 1). Ide et al. (2009) reported that hybrids from crosses using 'Diva', 'Sunrise Solo' and 'Caliman G' with 'JS 12' presented a higher insertion of the first fruit as well as a lower number of total and commercial fruit, generating lower yield. Therefore, it should be considered that these features will be transferred along with resistance to the hybrids. In this sense, the combination 'JS 12' x 'Caliman M5' is highlighted, since this hybrid had the lowest estimated heterosis for incidence of black spot in leaves (APH = 0.17).

All combinations involving the tester 'SS 72/12', except the one with 'Maradol GL', showed negative heterosis estimates for black spot severity in leaves. The combinations 'SS 72/12' x 'Mamão Roxo' and 'SS 72/12' x 'Maradol' also showed negative estimates of heterobeltiosis (Table 1). For these two hybrids, Ide et al. (2009) reported that the first has higher insertion of the first fruit, greater number of fruits and less productivity, while the second has the opposite for these same traits. The combination 'SS 72/12' x 'Costa Rica' should also be considered, since this hybrid was superior to its parents average. In other words, the estimate of heterosis for severity of black spot in fruit was negative and of high magnitude.

Table 1 Mean hybrid disease averages (HDA), estimates of average parent heterosis (APH) and heterobeltiosis or heterosis of superior parent (HSP) for resistance to black spot in papaya hybrids derived from crosses between 'JS 12' and 'SS 72/12'

Tester	Parent	Black spot incidence in leaves			Black spot severity in leaves			Black spot incidence in fruit		
		HDA	APH	HSP	HDA	APH	HSP	HDA	APH	HSP
JS 12	Caliman M5	77.28	0.17	1.33	0.05	−6.80	77.78	0.48	154.11	164.64
	Taiwan ET	79.96	1.96	2.49	0.04	40.98	59.26	0.51	139.44	181.77
	Diva	82.32	4.14	5.50	0.04	−2.38	51.85	0.55	379.13	1024.49
	Grampola	82.86	2.95	6.20	0.04	1.41	33.33	0.50	218.85	278.03
	Sunrise Solo	80.26	2.10	2.87	0.03	−1.49	22.22	0.48	223.08	309.32
	Caliman GB	82.88	5.91	6.23	0.05	57.38	77.78	0.84	337.70	361.88
	Caliman SG	80.60	1.59	3.30	0.05	44.62	74.07	0.32	115.65	180.53
	Caliman G	82.20	2.89	5.35	0.05	−16.81	74.07	0.66	388.56	635.56
	Kapoho Solo PA	82.05	6.46	7.79	0.05	64.29	70.37	0.67	337.01	429.92
	BSA	82.31	12.76	21.09	0.06	110.34	125.93	0.34	163.28	349.33
	São Mateus	84.18	5.62	7.89	0.05	44.93	85.19	0.72	524.14	1319.61
	Kapoho Solo PV	80.62	2.91	3.33	0.04	29.41	62.96	0.29	150.21	444.44
	Sunrise Solo PT	82.12	4.68	5.25	0.05	104.08	127.27	0.36	209.01	592.31
	Waimanalo	79.60	7.05	12.61	0.05	66.67	85.19	0.36	82.83	100.00
	Maradol	77.62	0.80	2.14	0.04	18.03	33.33	0.38	191.25	367.07
SS 72/12	Costa Rica	81.63	2.32	2.71	0.04	−29.41	33.33	0.10	−32.65	75.00
	Tailândia	81.68	7.60	13.86	0.05	−1.10	66.67	0.22	12.98	296.43
	Mamão Bené	82.79	3.86	4.35	0.03	−7.46	34.78	0.17	135.37	208.93
	Mamão Roxo	80.38	1.37	2.41	0.02	−56.41	−37.04	0.10	51.47	83.93
	Maradol	81.95	5.01	7.83	0.03	−35.90	−7.41	0.43	517.39	660.71
	Maradol GL	80.74	1.69	2.59	0.03	6.45	83.33	0.26	506.98	770.00
	Sekati	83.07	3.01	3.72	0.05	−9.57	92.59	0.14	144.07	157.14

Table 2 Mean hybrid disease averages (HDA), estimates of average parent heterosis (APH) and heterobeltiosis or heterosis of superior parent (HSP) for resistance to black spot in papaya hybrids derived from crosses between 'Americano', 'Sekati' and 'Maradol'

Tester	Parent	Black spot incidence in leaves			Black spot severity in leaves			Black spot incidence in fruit		
		HDA	APH	HSP	HDA	APH	HSP	HDA	APH	HSP
Americano	Caliman M5	81.13	3.69	6.38	0.03	−78.31	−64.47	0.21	21.76	43.75
	S Solo 783	81.93	4.01	5.95	0.03	−78.23	−64.00	0.25	18.27	70.83
	Costa Rica	81.49	2.06	2.53	0.04	−68.83	−37.93	0.21	10.29	45.14
	Taiwan et	82.58	3.86	4.77	0.03	−73.91	−20.59	0.43	122.11	200.00
	Diva	78.40	−2.17	−2.08	0.03	−77.39	−54.39	0.27	181.87	455.10
	Grampola	79.71	−2.29	−0.62	0.02	−81.57	−54.55	0.32	132.61	122.92
	Sunrise Solo	80.49	0.99	1.63	0.03	−76.53	−37.50	0.08	−39.69	−33.05
	Caliman AM	75.04	−6.55	−6.45	0.02	−76.59	−25.00	0.16	21.05	31.97
	Caliman GB	78.96	−0.49	0.60	0.02	−76.81	−29.41	0.20	13.62	36.11
	Caliman SG	79.46	−1.21	−0.94	0.03	−67.77	−10.53	0.32	149.03	183.19
	Caliman G	78.85	−2.65	−1.70	0.04	−72.97	−59.30	0.23	99.15	158.89
	SS 72/12	81.32	1.46	1.54	0.02	−83.41	−59.09	0.13	26.00	125.00
	BSA	83.06	12.09	22.18	0.02	−79.41	−32.26	0.15	36.99	100.00
	S Solo TJ	84.27	4.68	4.31	0.04	−63.13	−9.09	0.42	321.89	643.86
	São Mateus	79.57	−1.51	−0.80	0.03	−72.09	−28.57	0.07	−29.23	35.29
	K Solo PV	80.50	1.34	2.34	0.03	−72.90	−29.27	0.34	239.39	522.22
	S Solo PT	79.37	−0.21	0.63	0.03	−72.31	22.73	0.22	124.49	323.08
	Mamão Roxo	81.06	2.16	3.28	0.03	−68.12	−2.94	0.21	87.50	162.50
	Sekati	81.06	0.44	1.05	0.03	−74.59	−56.34	0.05	−50.49	−17.74
	BSuper	81.37	1.08	1.45	0.03	−74.87	−3.85	0.30	164.63	256.47
	STZ-52	82.36	1.53	2.68	0.04	−60.19	24.24	0.25	89.31	110.17
	Waimanalo	62.96	−16.56	−10.94	0.01	−92.23	−75.76	0.08	−58.22	−47.92
Sekati	Caliman G	80.60	−1.09	−0.74	0.03	−64.33	−60.56	0.10	28.95	58.06
	Caliman AM	81.06	0.33	−0.17	0.07	26.21	103.13	0.07	−23.91	12.90
	Caliman SG	81.55	0.78	1.12	0.04	−19.27	15.79	0.06	−32.57	−4.84
	Caliman GB	79.88	0.05	1.77	0.03	−40.95	−8.82	0.11	−15.59	79.03
	Sunrise Solo	80.28	0.10	1.37	0.04	−31.53	−5.00	0.07	−22.22	12.90
	Diva	83.09	3.05	3.78	0.03	−46.88	−40.35	0.12	119.82	148.98
	Caliman M5	79.91	1.49	4.78	0.05	−30.61	−28.17	0.04	−72.09	−41.94
	SS 72/12	82.59	2.42	3.13	0.05	−20.00	4.55	0.48	715.25	758.93
	JS 12	77.69	−2.41	−0.42	0.07	48.98	170.37	0.15	23.46	141.94
Maradol	Caliman G	73.23	34.47	137.18	0.03	−48.33	−8.82	0.11	25.58	31.71
	Caliman AM	80.75	41.00	161.53	0.02	−39.39	−37.50	0.16	54.90	92.68
	Caliman SG	79.17	42.21	156.40	0.04	16.67	23.53	0.47	376.92	467.07
	Sunrise Solo	77.89	44.93	152.26	0.02	−35.14	−29.41	0.25	151.00	206.10
	Diva	83.54	54.69	170.58	0.03	−34.07	−11.76	0.68	930.53	1277.55
	SS 72/12	77.80	45.67	151.96	0.03	−17.95	−5.88	0.14	102.90	150.00
	Caliman M5	82.74	60.88	167.97	0.06	14.55	85.29	0.22	54.68	162.20
	S Solo PT	82.66	45.74	167.73	0.02	−17.86	4.55	0.25	274.63	382.69
	JS 12	67.32	44.30	118.03	0.04	27.87	44.44	0.22	63.50	162.20

In the second experiment, derived from crosses with the testers 'Americano', 'Sekati' and 'Maradol', the hybrid 'Americano' x 'Waimanalo' showed negative estimates of heterosis and heterobeltiosis for the three characteristics related to black spot resistance (Table 2). Vivas et al. (2011) reported that this hybrid had the highest effect of specific combining ability for severity of black spot in leaves and fruit. Also regarding the tester 'Americano' with the genotypes

'Diva', 'Grampola', 'Caliman AM', 'Caliman SG', 'Caliman G' and 'São Mateus', the hybrids had negative estimates of heterosis and heterobeltiosis for incidence and severity of black spot in leaves. Cattaneo (2001), working with RAPD and AFLP to study the genetic diversity of 22 genotypes of papaya, observed that the genotypes 'Caliman G', 'Caliman SG', 'Caliman GB', 'Grampola' and 'Sunrise Solo TJ' clustered in one group. This result suggests that 'Caliman SG', 'Caliman G' and 'Caliman GB' are genetically very close, since they behaved similarly when combined with the genotypes 'Americano', 'Maradol' and 'Sekati'.

The testers 'Americano', 'Sekati', 'Maradol' (experiment 2) and 'SS 72/12' (experiment 1) showed that they can contribute to the reduction of black spot on papaya, since most hybrids showed negative heterosis and heterobeltiosis estimates. These genotypes could be used in crosses to generate hybrids with lower severity of black spot combined with distinct with morpho-agronomic characters, since 'Americano' and 'Maradol' produce fruit with higher weight, 'Sekati' produces fruit with intermediate weight, and 'SS 72/12' produces fruit with lower weight.

Most of the hybrids derived from crosses with 'Sekati' showed negative heterosis estimates for black spot incidence in fruit. 'Caliman SG' and 'Caliman M5' x 'Sekati' also had negative estimates of heterobeltiosis, ie, the hybrids had averages better than their parents' average (Table 2). Most hybrids also had estimates of negative heterosis for black spot severity in leaves. However, this is not reflected in the estimates of heterosis and heterobeltiosis for black spot incidence. Since severity refers to the percentage of diseased leaf area and incidence refers to the proportion of leaves with disease symptoms, it is possible that the genes responsible for these traits are not the same.

Hybrids with 'Maradol' showed negative heterosis estimates for black spot severity in leaves, and positive estimates for incidence in leaves and fruit. These hybrids showed higher average than those of their parents, which is not desirable in the case of disease. One possible explanation is that the genes responsible for severity and incidence are not the same, as mentioned above for crosses involving 'Sekati'. Also noteworthy is that 'Maradol' is one of the most resistant genotypes to black spot (Dianese et al. 2007), which makes better parent averages difficult to be achieved. The genotypes 'Caliman SG', 'Caliman M5' and 'JS 12' should not be used in crosses with 'Maradol', since such hybrids showed positive heterosis estimates for black spot severity in leaves.

According to Allard (1999), the expected effect of heterosis exploration is the increase in productivity, however, several other agronomic traits are also maximized. One of the attributes that can be potentiated is resistance to disease, by developing hybrids with lower average than the parents' average. On this regard, considering the resistance of hybrids to black spot, a heterosis effect was found in 27, 86, 100, 100 and 44 % of hybrids derived from crosses with the genitors 'JS 12', 'SS 72/12', 'Americano', 'Sekati' and 'Maradol', respectively, for at least one of the evaluated characteristics. About 58 % of the hybrids showed heterobeltiosis effect for at least one of those evaluated characteristics. These results confirm the possibility of heterosis exploration to produce hybrids that add desirable agronomic characteristics besides disease resistance.

Resistance to phoma spot

In the first experiment the average values were close; however there was a slight tendency for crosses with 'JS 12' to have lower average heterosis and heterobeltiosis for phoma spot severity (Table 3). Possibly, this genotype may contribute to the reduction of leaf phoma spot, and concomitantly reduce stalk rot in post-harvest. Moreover, the control of phoma inoculum in leaves in the field can reduce the incidence of stalk rot in post-harvest. Suzuki et al. (2007) reported gains of up to 24 % in reduction of stalk rot when the sanitization was performed, with removal of senescent and sick leaves.

In the second experiment, considering the overall average for each tester, negative heterosis estimates for the severity of phoma spot were observed for 'Sekati' and 'Maradol', and for heterobeltiosis for 'Sekati' (Table 4). Possibly, these genotypes carry genes that confer resistance to phoma spot, as reported by Vivas et al. (2010) when evaluating the germplasm collection of UENF/CALIMAN. These authors observed that these two genotypes were more resistant in terms of disease severity and were in the intermediate group for incidence of phoma spot in leaves.

Analyzing each tester in particular for the incidence of phoma spot, negative estimates of heterosis and heterobeltiosis were not observed, especially in the first experiment. However, the severity estimates were negative for the two testers evaluated. The genotypes 'Diva', 'Sunrise Solo' and 'Sunrise Solo PT' when combined with 'JS 12' gave rise to hybrids with negative estimates of heterosis and heterobeltiosis. Of these, the hybrids 'JS 12' x 'Diva' and 'JS 12' x 'Sunrise Solo' also had negative estimates of heterosis for resistance to black spot. Thus, these combinations can be used to reduce the intensity of the two diseases. However, it must be emphasized that these hybrids had higher insertion for the first fruit, lower number of commercial fruit and lower production (Ide et al. 2009).

Crosses involving 'Mamão Roxo' and 'Sekati' x 'SS 72/12' had negative estimates of heterosis and heterobeltiosis simultaneously to phoma spot severity (Table 3). Vivas et al. (2010) did not identify 'Mamão Roxo' as resistant to phoma spot, therefore it is suggested that the heterosis observed in the hybrid is derived largely from alleles inherited from 'SS 72/12'. Also with regard to this tester, the combination 'SS 72/12' x 'Maradol' stands out by presenting negative estimate of heterosis for phoma spot severity, once again indicating that

Table 3 Mean hybrid disease averages (HDA), estimates of average parent heterosis (APH) and heterobeltiosis or heterosis of superior parent (HSP) for resistance to phoma spot in papaya hybrids from the cross between ‘JS 12’ and ‘SS 72/12’

Tester	Parent	Phoma spot incidence in leaves			Phoma spot severity in leaves		
		HDA	APH	HSP	HDA	APH	HSP
JS 12	Caliman M5	76.49	13.82	22.53	3.99	−27.12	2.57
	Taiwan ET	80.20	16.09	28.47	5.75	25.67	47.89
	Diva	81.72	17.11	30.90	3.66	−19.21	−5.97
	Grampola	82.14	24.37	31.57	4.68	−23.07	20.40
	Sunrise Solo	78.85	13.43	26.31	3.88	−32.36	−0.33
	Caliman GB	79.59	11.12	27.50	5.08	−27.48	30.68
	Caliman SG	82.46	15.41	32.08	7.04	−25.88	81.17
	Caliman G	83.22	18.48	33.30	5.38	−14.43	38.25
	Kapoho Solo PA	83.18	25.02	33.24	5.96	−6.26	53.19
	BSA	80.20	29.69	30.94	5.26	26.07	35.19
	São Mateus	77.54	7.29	24.21	4.69	19.02	20.73
	Kapoho Solo PV	80.22	13.59	28.50	4.83	−8.48	24.25
	Sunrise Solo PT	81.74	12.75	30.93	3.54	−27.58	−8.85
	Waimanalo	81.31	29.72	30.25	6.69	30.48	72.02
	Maradol	79.52	70.45	157.54	5.85	147.36	594.77
SS 72/12	Costa Rica	80.79	7.16	7.92	8.71	43.52	49.13
	Tailândia	80.14	18.13	34.12	4.23	0.24	63.00
	Mamão Bené	80.63	12.96	20.66	7.36	35.87	47.26
	Mamão Roxo	79.71	2.20	4.98	5.51	−13.40	−5.57
	Maradol	75.60	41.56	144.84	2.56	−23.26	204.39
	Maradol GL	75.32	18.97	48.61	3.69	2.30	166.91
	Sekati	83.63	19.94	31.65	3.87	−24.71	−12.86

the genotypes ‘Sekati’ and ‘Maradol’ possess resistance genes for this disease.

From all the testers evaluated in the second experiment, only for ‘Maradol’ there were no hybrid combinations which presented negative estimates of heterosis and heterobeltiosis for phoma spot severity (Table 4). Based on these results, two conclusions could be reached. The first refers to the fact that ‘Maradol’ is a genotype with low estimates of phoma spot severity, as reported by Vivas et al. (2010). The second is that 77 % of the hybrids obtained with this tester had negative estimates of heterosis for phoma spot severity, inferring that this genotype has lower average of severity and transmits this trait to its hybrids. Therefore, in the future, it can be used to direct hibridizations that add disease resistance and desirable morpho-agronomical traits.

For the ‘Sekati’ tester, 89 % of hybrids had negative heterosis estimates for phoma spot severity. The hybrids ‘Caliman G’, ‘Caliman AM’, ‘Caliman SG’ and ‘Diva’ x ‘Sekati’ also had negative estimates of heterobeltiosis. The genotypes ‘Caliman G’, ‘Caliman SG’, ‘Caliman GB’, ‘Grampola’ and ‘Sunrise Solo TJ’ were clustered in the same group based on molecular diversity (Cattaneo 2001). Therefore, it is suggested that ‘Caliman SG’, ‘Caliman G’ and ‘Caliman GB’ are genetically very close and may possess common alleles, as they behaved similarly when combined with ‘Sekati’. However, Vivas et al. (2010), analyzing the behavior of genotypes per se, observed that the group showed the highest disease severity. Therefore, it is suggested that the genotype that carries resistance gene in this case is ‘Sekati’.

For the hybrids with ‘Americano’, 36 % of them had negative estimate of heterosis for phoma spot severity. The combination ‘Americano x Waimanalo’ was the only one to produce negative estimates of heterosis for phoma spot incidence in experiment 2. Considering only the severity of phoma spot, the genotypes ‘Caliman M5’, ‘Sunrise Solo’, ‘Caliman AM’, ‘Mamão Roxo’ and ‘Waimanalo’ x ‘Americano’ resulted in hybrids with negative estimates of heterosis and heterobeltiosis (Table 4).

In general, considering the severity of phoma spot in leaves, heterosis was observed in 67, 43, 36, 89 and 78 % of the hybrids obtained with the parents ‘JS 12’, ‘SS 72/12’, ‘Americano’, ‘Sekati’ and ‘Maradol’, respectively. Considering the average of the higher parent, 30 % of the

Table 4 Mean hybrid disease averages (HDA), estimates of average parent heterosis (APH) and heterobeltiosis or heterosis of superior parent (HSP) for resistance to phoma spot in papaya hybrids from the cross between 'Americano', 'Sekati' and 'Maradol'

Tester	Parent	Phoma spot incidence in leaves			Phoma spot severity in leaves		
		HDA	APH	HSP	HDA	APH	HSP
Americano	Caliman M5	79.80	30.00	57.10	5.39	−13.39	−0.11
	Sunrise Solo 783	83.73	31.70	64.84	5.40	−37.75	0.00
	Costa Rica	82.58	31.43	62.57	5.88	0.48	8.80
	Taiwan et	79.21	25.19	55.94	5.67	6.33	7.71
	Diva	79.07	23.61	55.66	7.36	39.28	42.48
	Grampola	77.85	29.26	53.25	9.06	32.49	67.83
	Sunrise Solo	83.38	30.88	64.14	5.36	−17.30	−0.69
	Caliman AM	81.17	20.74	59.79	5.15	−38.37	−4.63
	Caliman GB	80.92	22.96	59.30	7.44	−4.10	37.85
	Caliman SG	79.56	21.22	56.62	7.61	−25.79	40.98
	Caliman G	81.73	26.86	60.89	7.31	3.91	35.43
	SS 72/12	79.87	26.05	57.24	6.58	17.01	21.76
	BSA	75.37	34.54	48.37	5.22	5.97	17.28
	Sunrise Solo TJ	84.61	30.91	66.56	6.23	12.28	15.28
	São Mateus	76.89	15.70	51.37	4.77	1.47	19.23
	Kapoho Solo PV	79.31	22.38	56.14	8.34	38.27	54.52
	Sunrise Solo PT	77.88	16.79	53.31	6.61	17.04	22.46
	Mamão Roxo	78.75	20.36	55.03	5.08	−17.34	−5.91
	Sekati	73.50	28.59	44.70	7.75	57.52	74.55
	Baixinho Super	76.10	28.10	49.80	6.84	27.33	27.93
	STZ-52	78.77	21.36	55.07	6.39	2.97	18.41
	Waimanalo	54.30	−4.52	6.89	2.91	−50.59	−46.19
Sekati	Caliman G	76.56	8.16	20.52	4.01	−38.80	−9.62
	Caliman AM	80.13	8.89	26.15	1.85	−76.51	−58.33
	Caliman SG	78.73	9.36	23.94	3.72	−61.97	−16.24
	Caliman GB	77.70	7.65	22.32	4.68	−35.72	5.43
	Sunrise Solo	78.21	11.62	23.12	4.64	−22.76	4.46
	Diva	79.78	13.43	25.58	4.03	−16.05	−9.21
	Caliman M5	77.76	14.76	22.40	5.36	−6.82	20.63
	SS 72/12	80.74	15.79	27.10	4.46	−13.29	0.36
	JS 12	74.95	19.01	20.05	5.07	21.73	14.17
Maradol	Caliman G	73.23	34.47	137.18	2.66	−44.18	215.44
	Caliman AM	80.75	41.00	161.53	3.10	−48.99	268.17
	Caliman SG	79.17	42.21	156.40	2.89	−63.73	243.71
	Sunrise Solo	77.89	44.93	152.26	3.93	−6.53	366.86
	Diva	83.54	54.69	170.58	3.72	23.86	341.69
	SS 72/12	77.80	45.67	151.96	4.34	30.06	415.91
	Caliman M5	82.74	60.88	167.97	3.53	−10.58	319.36
	Sunrise Solo PT	82.66	45.74	167.73	3.29	−2.46	290.50
	JS 12	67.32	44.30	118.03	2.32	−1.95	175.42

hybrids exceeded the average. The majority of hybrids produced by crossing with 'Sekati' showed negative magnitudes of heterosis and heterobeltiosis, which confirms the possibility of using this genotype to produce hybrids with resistance to the phoma spot.

This study can guide breeding programs for the establishment of hybrids with lower levels of black spot in leaves and fruit and of phoma spot in leaves, as well as provide information on quality traits of plant and fruit. Similarly, as observed for morpho-agronomic characters (Marin et al. 2006) and physiological quality of seeds (Martins et al. 2009) in papaya, heterosis is a common effect for traits associated with resistance to papaya fungal diseases. Recently, Vivas et al. (2012b, 2013) pointed out that heterosis in papaya for resistance to black spot and to phoma spot may provide significant genetic gains, thereby contributing to the reduction of chemical control in the crop. It was possible to identify papaya hybrids with potential for reducing the incidence and severity of black spot and of phoma spot, especially the hybrids 'Americano' x 'São Mateus' and 'Americano' x 'Waimanalo'. The first has potential to reduce the incidence and severity of black spot in leaves and the severity of black spot in fruit (Table 2), while the second has the potential to reduce the intensity of both diseases (Tables 2 and 4).

Genetic diversity for disease traits

We observed significant effects of genotype variation for all variables analyzed in this study, confirming the genetic variability of hybrids and genotypes under study. The variables measured in this study were also used to estimate the genetic dissimilarity between the analyzed genotypes, whose distances are represented in Fig. 1. Analyzing the dendrogram branches, we can see that most genotypes showed low genetic variability average for the variables related to black spot and phoma spot resistance. However, there are genotypes with clear distinction concerning the mentioned features.

By adopting a 0,20 dissimilarity cutoff it is possible to observe the formation of three groups, one of them (group I) comprising the majority of genotypes from the 'Solo' and 'Formosa' groups. Based on the results reported by Vivas et al. (2010, 2015), group I genotypes are characterized for presenting intermediate to low resistance to the diseases analyzed, justifying the grouping observed.

The second group consists of only one genotype ('Caliman SG'), indicating the distinction of this genotype in relation to other genetic materials in terms of resistance to the two diseases. Results reported by Vivas et al. (2010, 2015) show that 'Caliman SG' has high values for incidence and severity of both phoma spot and black spot. This fact may have contributed to its allocation in a unitary group, showing its distinction compared to other genotypes.

The third group, formed by 'Maradol', 'Maradol GL' and 'Americano', presented greater distance to the other genotypes, indicating the predominance of traits that distinguish them. According to the results presented by Vivas et al. (2010), among the various germplasm bank genotypes analyzed, 'Maradol', 'Maradol GL' and 'Americano' had low values for incidence and severity of phoma spot. Regarding black spot, 'Maradol' and 'Maradol GL' genotypes stand out because they have low values for disease incidence and severity in fruits and leaves (Vivas et al. 2015). In general, group III

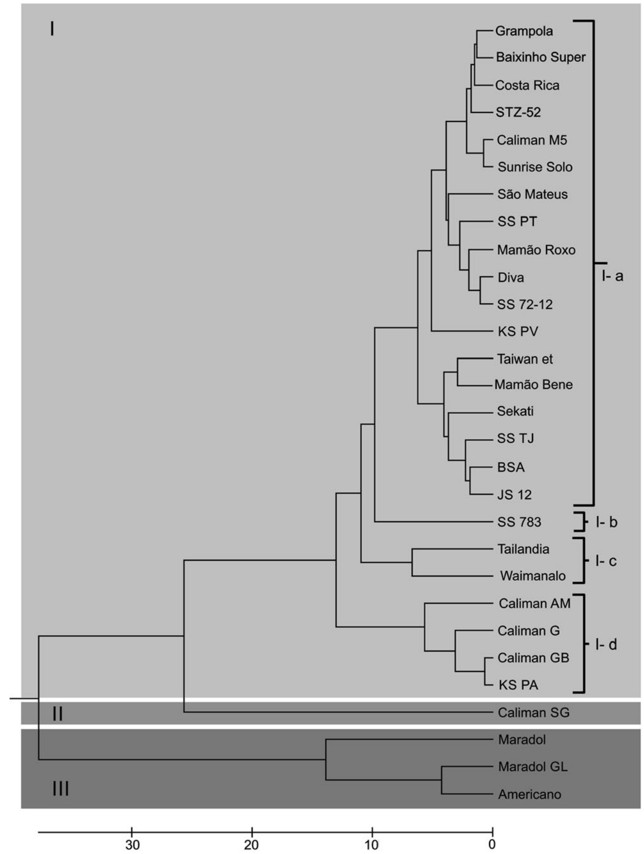

Fig. 1 Dendrogram resulting from the analysis of 29 papaya genotypes obtained by the UPGMA clustering method and using Mahalanobis distance as a measure of genetic distance based on incidence and severity of black spot and phoma spot diseases

consists of highly resistant genotypes to the diseases under study.

Regarding the testers used in the experiments, 'Americano' and 'Maradol' were placed in the most divergent group (group III), whereas the other testers ('Sekati', 'JS12' and 'SS 72/12') were allocated to the group with greater similarity (group I). For resistance to phoma spot, testers allocated in group I have average values around 65 and 70 % higher for incidence and severity, respectively, compared to 'Americano' and 'Maradol' (Vivas et al. 2010). Also with respect to testers allocated in group I, there is a greater similarity between 'Sekati' and 'JS12', while 'SS72/12' was allocated on a different subgroup. This genetic distance pattern between genotypes corroborates the results found by Vivas et al. (2010) for phoma spot resistance.

According to quantitative genetics theory, for any degree of dominance higher than zero heterosis will be due to allele frequency between the parents, and there is a positive correlation between genetic divergence and heterosis (Falconer 1989). Thus, it is suggested that genotypes with a genetic distance that justifies allelic complementation result in hybrid vigour. In this sense, when analyzing the hybrid combinations that have low heterosis and heterobeltiosis values for the three characteristics related to black spot resistance, it is clear that the intersection that showed the best result ('Americano' x 'Waimanalo') also had long genetic distance, which may have contributed to greater complementarity and positive allelic heterosis expression. This result is very encouraging since it enables the development of hybrids with minor injuries on leaves and fruit, resulting in improved photosynthetic capacity and lower post-harvest losses. In addition, the cross between resistant and genetically distant genotypes increases the chance of identifying hybrids with superior segregation for the development of new cultivars.

For phoma spot, 'Sekati' stood out for resulting in a greater number of hybrid combinations with negative values of heterosis and heterobeltiosis. The crosses with the best results were obtained with 'Caliman G', 'Caliman AM', 'Caliman SG' and 'Diva', all of which showed intermediate genetic distance in relation to 'Sekati' (Fig. 1). Despite the good results with 'Sekati', the best result for phoma spot was also obtained by crossing 'Americano' x 'Waimanalo', with negative estimates of heterosis and heterobeltiosis for severity and negative heterobeltiosis for incidence. As mentioned for resistance to black spot, the 'Americano' and 'Waimanalo' genotypes present great genetic distance and are allocated to different groups based on genetic dissimilarity. Although distant in relation to the diseases analyzed in this study, the two genotypes were highlighted as potential sources of resistance genes to foliar diseases by Vivas et al. (2015). Together, these results support the hypothesis that the genotypes that have genes and/or different alleles for resistance to the diseases analyzed here have a greater chance of making good complementary alleles, resulting in heterosis. However, for the inclusion of these genotypes in breeding programs it is necessary to consider their performance for agronomic traits.

Some studies have shown that genetic divergence is not always associated with heterosis (Paterniani et al. 2008; Mohammadi et al. 2008; Guimarães et al. 2009). This may be true when the genomic regions accessed to estimate the genetic distance (either by molecular markers or by agronomic traits) have no relationship to the characters used to investigate the heterotic effect. Bernardo (1992) suggests that the low heritability of the trait, high variation in allele frequencies of parenting, different levels of dominance among the hybrids, inadequate genomic coverage and analysis of diversity based on randomly dispersed genomic regions (not linked to QTLs) are possible reasons for the low correlations found between hybrid performance and genetic diversity.

Particularly in relation to disease resistance, what is sought are negative values and high magnitude of heterosis, which means that the hybrid should have lower values than the parents' average for disease incidence and severity. Drawing a parallel between genetic distance and expression of heterosis, our study showed that the best results were obtained by crossing genetically distant genotypes regarding their resistance to black spot and phoma spot. However, it was also possible to verify the occurrence of heterosis among genetically close genotypes, for example, 'SS 72/12' x 'Mamão-Roxo', which showed negative heterosis and heterobeltiosis estimates for incidence of black spot and phoma spot, but showed unsatisfactory results for severity of the two diseases. Another example is 'Sekati' x 'Caliman M5', which had a negative heterobeltiosis estimate for severity of black spot in fruit.

Although we did not conduct specific correlation analysis, the results obtained showed a significant relationship between genetic distance and heterosis for disease resistance. This is in accordance with the hypothesis that genotypes with different alleles tend to have higher genetic distance, which increases the chance of obtaining good allelic complementarity to the measured features, resulting in a significant heterotic effect. Moreover, our results indicate that the presence of a few different alleles decreases the genetic distance; however, it does not prevent the expression of heterosis for a smaller number of specific characteristics.

In conclusion, it was possible to identify papaya hybrids with potential for reducing the incidence and severity of black spot and phoma spot, especially the hybrid 'Americano x Waimanalo', which has the potential to reduce the intensity of both diseases. It was also found that the best estimates of heterotic effects were observed in more genetically distant genotypes, highlighting these materials as promising for the development of resistant cultivars, as well as for the development of segregating populations with potential for superior segregating identification. These results suggest that genetic distance based on phenotypic traits can predict hybrid performance despite the existence of an environmental component in its expression.

Acknowledgments The authors wish to thank Financiadora de Estudos e Projetos (FINEP), Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ), Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF) and Caliman Agrícola S/A for financial and logistical support.

References

Allard RW (1999) Principles of Plant Breeding, 2nd ed. Wiley, New York

Aveskamp MM, Gruyter J, Woudenberg JHC, Verkley GTM, Crous PW (2010) Highlights of the *Didymellaceae*: a polyphasic approach to characterise *Phoma* and related pleosporalean genera. Stud Mycol 65:1–60

Bernardo R (1992) Relationship between single-cross performance and molecular marker heterozygosity. Theor Appl Genet 83:628–634

Cattaneo LF (2001) Avaliação da divergência genética e análise de gerações em mamoeiro (*Carica papaya* L.). D.Sc. Thesis, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes

Cruz CD (2013) GENES - a software package for analysis in experimental statistics and quantitative genetics. Acta Sci Agron 35:271–276

Dianese AC, Blum LEB, Dutro JB, Lopes LF, Sena MC, Freitas LF, Yamanishi OK (2007) Reação de genótipos de mamoeiro à varíola e à podridão-do-pé. Fitopatol Bras 32:419–423

Falconer DS (1989) Introduction to Quantitative Genetics, 3rd ed. Wiley, New York

Guimarães CT, Schuster I, Magalhães JV, Souza Júnior CL (2009) Marcadores moleculares no melhoramento. In: Borém A, Caixeta ET (eds) Marcadores Moleculares, 2nd ed. Editora UFV, Viçosa, pp 129–176

Hunter JE, Buddenhagen IW (1972) Incidence, epidemiology and control of fruit diseases of papaya in Hawaii. Trop Agric 46:61–71

Ide CD, Pereira MG, Viana AP, Pereira TNS (2009) Use of testers for combining ability and selection of papaya hybrids. Crop Breed Appl Biotechnol 9:60–66

Marin SLD, Pereira MG, Amaral Júnior AT, Martelleto LAP, Ide CD (2006) Heterosis in papaya hybrids from partial diallel of ‘Solo’ and ‘Formosa’ parents. Crop Breed Appl Biotechnol 6:24–29

Martins GN, Pereira MG, Silva RF, Oliveira ACS, Silva F (2009) Efeito do pólen nas características físicas e fisiológicas de sementes de mamão. Revista Brasileira de Sementes 31:19–26

Mekako HU, Nakasone HY (1975) Interespecific hybridization among six *Carica* species. J Am Soc Hortic Sci 100:237–242

Mohammadi SA, Prasanna BM, Sudan C, Singh NN (2008) SSR heterogenic patterns of maize parental lines and prediction of hybrid performance. Biotechnol Biotechnol 22:541–547

Paterniani MEAGZ (2001) Use of heterosis in maize breeding: history, methods and perspectives - a review. Crop Breed Appl Biotechnol 1: 159–178

Paterniani MEAGZ, Guimarães PS, Lüders RR, Gallo PB, Souza AP, Laborda PR, Oliveira KM (2008) Capacidade combinatória, divergência genética entre linhagens de milho e correlação com heterose. Bragantia 67:639–648

Rezende JAM, Martins MC (2005) Doenças do mamoeiro (*Carica papaya* L.). In: Kimati H, Amorim L, Rezende JAM, Bergamin Filho A, Camargo LEA (eds) Manual de Fitopatologia: Doenças das Plantas Cultivadas. Ceres, São Paulo, pp 435–443

SAS Institute (1992) Statistical Analyses System. Version 6.12. SAS Institute, Cary

Sokal RR, Rohlf FJ (1962) The comparison of dendrograms by objective methods. Taxon 11:33–40

Suzuki MS, Zambolim L, Liberato JR (2007) Progresso de doenças fúngicas e correlação com variáveis climáticas em mamoeiro. Summa Phytopathol 33:167–177

Vencovsky R, Barriga P (1992) Genética Biométrica no Fitomelhoramento. Sociedade Brasileira de Genética, Ribeirão Preto

Vivas M, Silveira SF, Terra CEPS, Pereira MG (2010) Reação de germoplasma e híbridos de mamoeiro à mancha-de-phoma (*Phoma caricae-papayae*) em condições de campo. Trop Plant Pathol 35:323–328

Vivas M, Silveira SF, Terra CEPS, Pereira MG (2011) Testers for combining ability and selection of papaya hybrids resistant to fungal diseases. Crop Breed Appl Biotechnol 11:36–42

Vivas M, Silveira SF, Cardoso DL, Pereira MG, Santos PHD, Ferreguetti GA (2012a) Capacidade combinatória e heterose para resistência à pinta-preta em mamoeiro por meio de análise dialélica. Trop Plant Pathol 37:326–332

Vivas M, Silveira SF, Vivas JMS, Pereira MG (2012b) Patometria, parâmetros genéticos e reação de progênies de mamoeiro à pinta-preta. Bragantia 71:235–238

Vivas M, Silveira SF, Pereira MG, Cardoso DL, Ferreguetti GA (2013) Análise dialélica em mamoeiro para resistência a mancha-de-phoma. Ciênc Rural 43:945–950

Vivas M, Silveira SF, Viana AP, Amaral Júnior AT, Ferreguetti GA, Pereira MG (2015) Resistance to multiple foliar diseases in papaya genotypes in Brazil. Crop Prot 71:138–143