

Selection of female papaya progenies for resistance to phoma-spot using mixed models

Marcelo Vivas (^{1*}); Silvaldo Felipe da Silveira (²); Janieli Maganha Silva Vivas (²); Alexandre Pio Viana (¹); Antonio Teixeira do Amaral Junior (¹); Messias Gonzaga Pereira (¹)

(¹) Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Laboratório de Melhoramento Genético Vegetal, Avenida Alberto Lamego, 2000, 28013-602 Campos dos Goytacazes (RJ), Brasil.

(²) UENF, Laboratório de Entomologia e Fitopatologia, 28013-602 Campos dos Goytacazes (RJ), Brasil.

(*) Corresponding author: mrclvivas@hotmail.com

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Abstract

Landrace genotypes present sources of genes for adaptation, rusticity and resistance to diseases and environmental stress. This study evaluated the potential of landrace papaya genotypes in contributing to phoma-spot resistance genes. The experiment was a randomized block design with six replications consisted of 24 half-sib progenies of dioecious papaya - which segregated for sex. Only plants with female flowers were evaluated. We used mixed models to estimate variance components and the genetic value of landraces to incidence and severity of phoma-spot. Heritability estimates and selective accuracy demonstrated a favorable situation to the selection of resistant progenies, especially 'STA-02', 'STA-04', 'STA-05', 'STA-06', 'STA-07', 'STA-11', 'STA-12', 'STA-16', 'STA-21' and 'STA-22', which showed negative values of additive genetic effects for both incidence and severity of phoma-spot.

Key words: *Carica papaya*, genetic resistance, *Stagonosporopsis caricae*, genetic variability.

Seleção de progênies femininas de mamoeiro para resistência a mancha-de-phoma via modelos mistos

Resumo

Genótipos crioulos são importantes fontes de genes de adaptação, rusticidade e resistência de plantas a doenças bióticas e a estresse ambiental. Avaliou-se neste trabalho o potencial genético de contribuição de genótipos crioulos de mamoeiro na resistência a mancha-de-phoma. O experimento, implantado em blocos casualizados com seis repetições, foi composto de 24 progênies dioicas de meios-irmãos de mamoeiro que segregaram para o sexo, sendo avaliadas apenas as plantas com flores femininas. Foram estimados os componentes de variância e o valor genético de progênies para incidência e severidade de mancha-de-phoma via modelos mistos. As estimativas de herdabilidade e acurácia seletiva permitiram prever situação muito favorável à seleção de progênies resistentes, com destaque para as progênies STA-02, STA-04, STA-05, STA-06, STA-07, STA-11, STA-12, STA-16, STA-21 e STA-22, com valores negativos de efeitos genéticos aditivos tanto para severidade quanto para incidência da doença nas folhas.

Palavras-chave: *Carica papaya*, resistência genética, *Stagonosporopsis caricae*, variabilidade genética.

The papaya crop in Brazil is vulnerable to attack by numerous pathogens, including the fungus *Stagonosporopsis caricae* (Sydow & P. Sydow) Aveskamp, Gruyter & Verkley [= *Phoma caricae-papayae* (Tar) Punith.] (Aveskamp et al., 2010), which causes phoma-spot and stalk rot. In young leaves, necrotic and dark brown lesions grow and coalesce, reaching 3-5 centimeters, damaging large portion of the leaf area (Rezende and Martins, 2005). Under favorable weather conditions, in the absence of chemical control, the disease may cause the loss of all apical leaves.

One way to control these diseases is genetic resistance. Although there are breeding work aimed at achieving phoma-spot-resistant cultivars (Vivas et al., 2010; 2013a),

there are no commercial varieties with complete or cost-effective resistance (Vivas et al., 2010). In this way, there is a need to broaden the genetic basis of this crop as well as to intensify researches that focus on the genetic resistance of papaya to diseases in general. To increase the genetic basis, we can make use of landrace varieties. In this context, different studies have reported the use of landrace genotypes for increased genetic variability and frequency of genes for adaptation, rusticity and resistance to biotic and abiotic stress (Medeiros et al., 2008, Neitzke et al., 2009, Vivas et al., 2012; 2013b).

In papaya, there are reports of landrace progenies as a source of resistance to black-spot, caused by *Asperisporium*

caricae (Speg.) Maubl. (Vivas et al., 2012). It is believed that these can also contribute to resistance to other diseases, especially the phoma-spot, justifying the need to know the variability in such progenies. To know the variability, it is necessary to design efficient breeding strategies, which are of fundamental importance to estimate variance components (genetic parameters) and to predict genotypic values. However, the field experiment is vulnerable to unbalanced data due to various reasons. In the present study, data imbalance was caused by segregation of dioecious progenies into female and male flowers (Vivas et al., 2013b).

In the case of unbalanced data, the ANOVA leads to inaccurate estimates of variance components, thus making dubious prediction of genetic values. In these cases, the REML/BLUP (restricted maximum likelihood/best linear unbiased prediction) becomes a great procedure for genotypic assessment (Resende, 2006). In papaya, the use of mixed models is still little explored; there are reports of its use in estimating genetic parameters and genotypic values in segregating populations aiming at the selection of papaya individuals for morphoagronomic traits, such as fruit length and weight, total soluble solids and firmness of the fruit (Oliveira et al., 2012, Pinto et al., 2013) as well as the reduction of the physiological spot (Pinto et al., 2013). However, for disease-related characteristics this approach is pioneering. In this work, we estimated variance components and predicted genetic values of landrace progeny of papaya as to resistance to phoma-spot in leaves, from the field experiment, using the procedure BLUP/REML.

The experiment was conducted in June 2010, at the Água Limpa Farm, Municipality of Mimoso do Sul (Latitude 21°03'52"S, Longitude 41°21'59"W), Espírito Santo State, in a randomized block design with six replications of 24 dioecious papaya progeny that segregated for sex, as shown by Vivas et al. (2012). In June 2011, when the disease occurred naturally in the field, only plants with female flowers were evaluated for the incidence and severity of phoma-spot. Plants with male flowers were not selected by Vivas et al. (2013b) and eliminated to avoid contamination with unwanted pollen. Incidence (%) of leaves with symptoms of phoma-spot was calculated as

the ratio of number of leaves with symptoms by the total number of leaves per plant multiplied by one hundred. The severity of phoma-spot was visually estimated at the 5th fully expanded leaf with a diagrammatic scale adopted by Vivas et al. (2010).

With phenotypic data, we obtained, via Selegen-Reml/Blup software (Rezende, 2006), estimates of variance components (REML) software and predicted, via BLUP, the predicted additive genetic effect of parents and the predicted additive genetic effect for each individual, for each variable. We used the model 19: $y = \mathbf{Xr} + \mathbf{Za} + \mathbf{e}$. Where y is the data vector, r is the vector of repetition effects (assumed to be fixed) added to the overall mean, a is the vector of individual additive genetic effects (assumed to be random), and e is the vector of errors or residual (random). Uppercase letters represent the incidence matrices for these purposes. Aiming to assist in the selection of the best female progenies, predicted additive genetic effects for each parent were used to construct a scatter plot of data, considering the severity and incidence of phoma-spot, respectively, on the axes x and y . Subsequently, we inferred on parents by inference about the parents by graphical analysis of the scores distributed on the graph.

By analysis of variance components obtained by restricted maximum likelihood (REML), it was found for severity of phoma-spot in leaf that phenotypic variance was explained almost completely by additive variance. It was also observed that the estimated magnitudes of heritability based on the average of progenies were above 73% for the two variables under study (Table 1). This demonstrates a favorable situation for the selection of progenies that can contribute to the reduction of phoma-spot, given that much of the phenotypic variation observed in the field is due to the genetic component.

With regard to the quality of the experiment, Resende (2007) states that this should preferably be inferred based on accuracy. It is further understood that the selection process in breeding programs should aim accuracy values above 70%. In the present study, we observed estimates exceeding 85% for both traits evaluated (Table 1). As presented by Resende (2007), such accuracy estimates have a

Table 1. Estimates of variance components obtained for incidence of leaves with phoma-spot symptom and severity phoma-spot in leaf, through Restricted Maximum Likelihood (REML), Mimoso do Sul, 2011

Variance components	Incidence	Severity
Heritability on progeny mean	0.7344	0.7324
Accuracy of selection of progeny	0.8570	0.8558
Coefficient of individual additive genetic variation(%)	31.0501	91.2455
Coefficient of genetic variation among progenies (%)	15.5251	45.6227
Residual coefficient of variation (%)	22.8670	67.5442
Coefficient of relative variation	0.6789	0.6755
Standard deviation of the predicted genotypic value	4.1629	1.4355
Overall mean	52.0334	6.0828

high precision, facilitating the identification and selection of progenies with desirable characteristics. Graphical analysis from additive genetic values of progenies, for severity and incidence of phoma-spot, allowed the distribution of progenies into quadrants (Figure 1). Considering only the severity, the scores obtained with the additive genetic effects point 14 genotypes as candidates for selection, because they showed the lowest values. Moreover, for the incidence of leaves with phoma-spot symptoms, 13 genotypes showed negative values for predicted genetic effects, that is, tend to contribute to reduce the incidence of phoma-spot in leaf, for new generations.

Whereas the two estimates together, it has been found that the progenies 'STA-02', 'STA-04', 'STA-05', 'STA-06', 'STA-07', 'STA-11', 'STA-12', 'STA-16', 'STA-21' and 'STA-22', showed desirable values of additive genetic effects for both variables. Of these, the progenies 'STA-02', 'STA-06' and 'STA-11', showed the largest negative values of additive genetic effects. It is important to emphasize that selection from the additive genetic effect facilitates the achievement of superior plants, because we discarded plants with phenotypic values higher than the selected plants. Another point to be mentioned is the possibility of genetic gains for both the resistance to phoma-spot and black spot (Vivas et al., 2012, 2013b). These authors found

that the progenies 'STA-02', 'STA-04', 'STA-08', 'STA-13', 'STA-15' and 'STA-16' were the most promising for reducing the severity of black spot in papaya leaves and for increasing the number of leaves without symptoms of black spot. Vivas et al. (2013b), used the index of Mulamba and Mock (1978) to select the best 10 progenies and pointed beyond those described by Vivas et al. (2012), the progenies 'STA-05', 'STA-17', 'STA-22' and 'STA-24'. Thus, based on our results and the results presented in the aforementioned studies, it is concluded that the progenies 'STA-02', 'STA-04', 'STA-05', 'STA-16' and 'STA-22' have the potential to reduce both diseases: black spot and phoma-spot.

Considering the estimates of predicted additive genetic effects for the incidence of leaves with symptoms of phoma-spot and severity of black spot in papaya leaf, for the twenty best individuals within progenies and their respective blocks, we observed that 'STA-02 (2)', 'STA-02 (5)', 'STA-02 (6)', 'STA-04 (5)', 'STA-05 (5)', 'STA-06 (6)', 'STA-07 (5)', 'STA-11 (5)', 'STA-12 (1)', 'STA-22 (3)', 'STA-22 (4)' and 'STA-23 (2)', are promising, given the suitable values for both incidence of leaves with symptoms of phoma-spot and severity of black spot in leaves (Table 2). Of the above mentioned progenies, Vivas et al. (2013b) point out that 'STA-02' (2), 'STA-02' (6), 'STA-04' (5), 'STA-05' (5), 'STA-22' (3) have potential to reduce black spot.

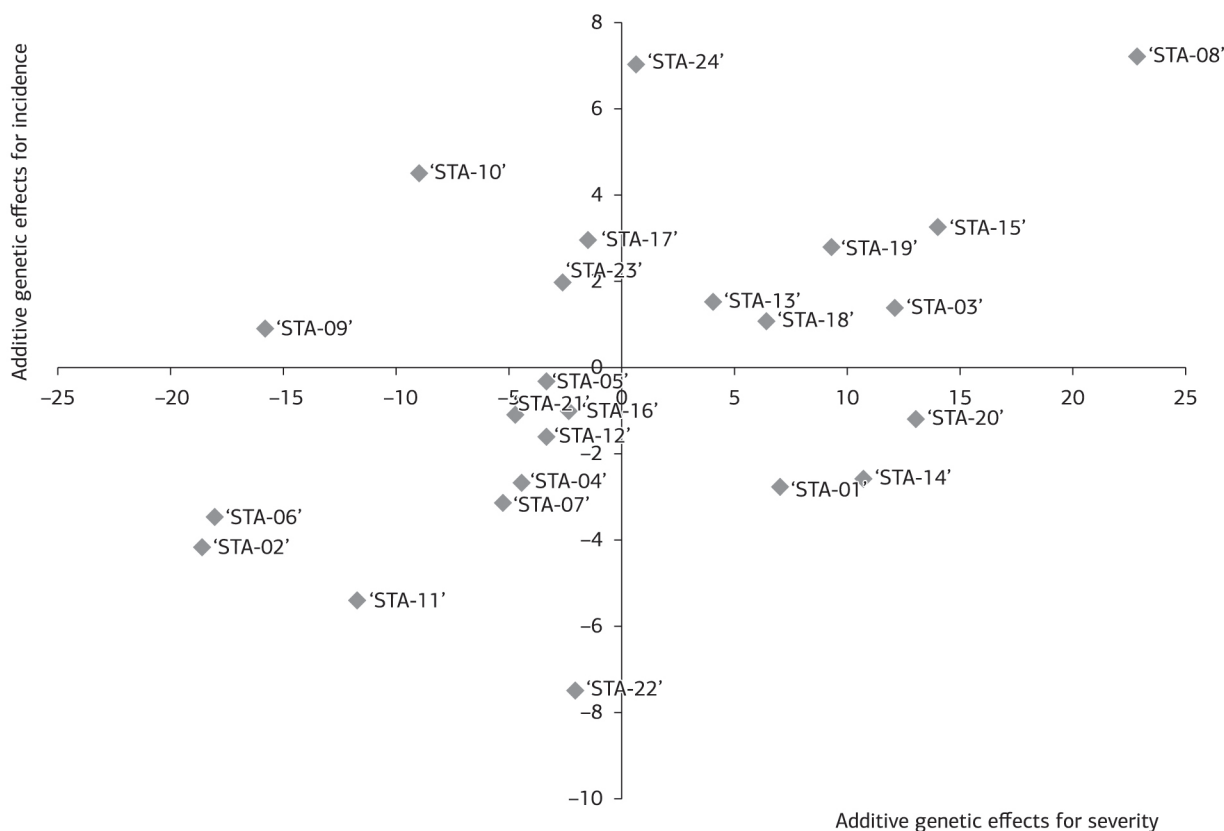


Figure 1. Scatter plot of scores in the quadrants discriminating the relative contribution of each parent to form the next generation.

Table 2. Estimates of phenotypic effects (f) and the predicted additive effects for incidence of leaves with phoma-spot symptoms and severity of phoma-spot in leaves for the twenty best progenies and their respective blocks, Mimoso do Sul, 2011

Order	Progeny (block)	Incidence of leaves		Progeny (block)	Severity in leaves	
		f	a		f	a
1	'STA-06' (6)	21.43	-45.82	'STA-18' (3)	0.00	-12.19
2	'STA-09' (1)	23.08	-31.64	'STA-22' (4)	0.00	-10.14
3	'STA-11' (5)	23.08	-29.39	'STA-11' (3)	1.00	-9.62
4	'STA-02' (5)	23.08	-28.08	'STA-22' (3)	2.00	-7.87
5	'STA-02' (2)	37.50	-22.73	'STA-03' (2)	0.00	-7.44
6	'STA-10' (2)	40.00	-21.11	'STA-05' (5)	2.00	-6.27
7	'STA-02' (6)	40.00	-20.13	'STA-06' (6)	0.00	-6.08
8	'STA-22' (4)	41.67	-15.40	'STA-02' (6)	0.00	-6.06
9	'STA-05' (5)	35.71	-13.66	'STA-07' (5)	2.00	-5.77
10	'STA-22' (3)	42.86	-11.95	'STA-04' (5)	2.00	-5.77
11	'STA-19' (2)	50.00	-10.77	'STA-22' (2)	1.00	-4.44
12	'STA-07' (5)	37.50	-10.68	'STA-14' (4)	5.00	-4.20
13	'STA-21' (4)	45.00	-9.94	'STA-01' (2)	2.00	-3.93
14	'STA-04' (5)	38.10	-9.92	'STA-11' (5)	3.00	-3.93
15	'STA-23' (2)	50.00	-8.59	'STA-02' (2)	2.00	-3.78
16	'STA-12' (1)	42.86	-6.68	'STA-23' (2)	3.00	-3.44
17	'STA-17' (3)	47.37	-5.81	'STA-12' (1)	0.00	-3.21
18	'STA-16' (1)	44.44	-4.68	'STA-01' (5)	4.00	-3.05
19	'STA-10' (5)	42.11	-3.60	'STA-02' (5)	4.00	-2.89
20	'STA-17' (6)	54.55	-3.56	'STA-06' (4)	6.00	-2.66

The progenies 'STA-16' (1) and 'STA-17' (6) are promising for reducing black spot (Vivas et al., 2013b) and have suitable values for reducing the incidence of leaves with phoma-spot symptoms.

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REFERENCES

- AVESKAMP, M.M.; GRUYTER, J.; WOUDEBERG, J.H.C.; VERKLEY, G.J.M.; CROUS, P.N. Highlights of the *Didymellaceae*: A polyphasic approach to characterise *Phoma* and related *pleosporalean* genera. *Studies in Mycology*, v.65, p.1-60, 2010. <http://dx.doi.org/10.3114/sim.2010.65.01>
- MEDEIROS, L.A.M.; BALARDIN, R.S.; COSTA, I.F.D.; GULART, C.A.; LENZ, G. Reação de germoplasma crioulo de feijoeiro (*Phaseolus vulgaris* L.) a *Colletotrichum lindemuthianum* Tropical Plant Pathology, v.33, p.273-280, 2008. <http://dx.doi.org/10.1590/S1982-56762008000400003>
- MULAMBA, N.N.; MOCK, J.J. Improvement of yield potential of the Eto Blanco maize (*Zea mays* L.) population by breeding for plant traits. *Egypt Journal of Genetics and Cytology*, v.7, p.40-51, 1978.
- NEITZKE, R.S.; BARBIERI, R.L.; HEIDEN, G.; BÜTTOW, M.V.; OLIVEIRA, C.S.; CORRÊA, L.B.; SCHWENGBER, J.E.; CARVALHO, F.I.F. Caracterização morfológica e dissimilaridade genética entre variedades crioulas de melão. *Horticultura Brasileira*, v.27, p.534-538, 2009. <http://dx.doi.org/10.1590/S0102-05362009000400021>
- OLIVEIRA, E.J.; FRAIFE FILHO, G.A.; FREITAS, J.P.X.; DANTAS, J.L.L.; RESENDE, M.D.V. Plant selection in F₂ segregating populations of papaya from commercial hybrids. *Crop Breeding and Applied Biotechnology*, v.12, p.191-198, 2012. <http://dx.doi.org/10.1590/S1984-70332012000300005>
- PINTO, F.O.; LUZ, L.N.; PEREIRA, M.G.; CARDOSO, D.L.; RAMOS, H.C.C. Metodologia dos modelos mistos para seleção combinada em progênies segregantes de mamoeiro. *Revista Brasileira de Ciências Agrárias*, v.8, p.211-217, 2013. <http://dx.doi.org/10.5039/agraria.v8i2a2409>
- REZENDE, J.A.M.; MARTINS, M.C. Doenças do mamoeiro (*Carica papaya* L.). In: KIMATI, H.; AMORIM, L.; REZENDE, J.A.M.; BERGAMIN FILHO, A.; CAMARGO, L.E.A. (Ed.). Manual de fitopatologia: Doenças das plantas cultivadas. São Paulo: Agronômica Ceres, 2005. p.435-443.
- RESENDE, M.D.V. O software Selegen Reml/Blup. Campo Grande: Embrapa Gado de Corte, 2006. 299p. (Embrapa Gado de Corte. Documentos)
- RESENDE, M.D.V. Matemática e Estatística na Análise de Experimentos e no Melhoramento Genético. Embrapa Florestas: Editora Colombo, 2007. 561p.
- VIVAS, M.; SILVEIRA, S.F.; TERRA, C.E.P.S.; PEREIRA, M.G. Reação de germoplasma e híbridos de mamoeiro à mancha-de-

phoma (*Phoma caricae-papayae*) em condições de campo. *Tropical Plant Pathology*, v.35, p.323-328, 2010. <http://dx.doi.org/10.1590/S1982-56762010000500009>

VIVAS, M.; SILVEIRA, S.F.; VIVAS, J.M.S.; PEREIRA, M.G. Patometria, parâmetros genéticos e reação de progênies de mamoeiro à pinta-preta. *Bragantia*, v.71, p.235-238, 2012. <http://dx.doi.org/10.1590/S0006-87052012005000021>

VIVAS, M.; SILVEIRA, S.F.; PEREIRA, M.G.; CARDOSO, D.L.; FERREGUETTI, G.A. Análise dialélica em mamoeiro para resistência a mancha-de-phoma. *Ciência Rural*, v.43, p.945-950, 2013a. <http://dx.doi.org/10.1590/S0103-84782013005000054>

VIVAS, M.; SILVEIRA, S.F.; VIVAS, J.M.S.; PEREIRA, M.G. Predição de ganhos genéticos e seleção de progênies de mamoeiro para resistência à pinta-preta. *Tropical Plant Pathology*, v.38, p.142-148, 2013b. <http://dx.doi.org/10.1590/S1982-56762013000200008>