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Efficiency of circulant diallels via mixed models in the selection of papaya genotypes resistant to foliar fungal diseases

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ABSTRACT. Diallel crossing methods provide information regarding the performance of genitors between themselves and their hybrid combinations. However, with a large number of parents, the number of hybrid combinations that can be obtained and evaluated become limited. One option regarding the number of parents involved is the adoption of circulant diallels. However, information is lacking regarding diallel analysis using mixed models. This study aimed to evaluate the efficacy of the method of linear mixed models to estimate, for variable resistance to foliar fungal diseases, components of general and specific combining ability in a circulant table with different s values. Subsequently, 50 diallels were simulated for each s value, and the correlations and estimates of the combining abilities of the different diallel combinations were analyzed. The circulant diallel method using mixed modeling was effective in the classification of genitors regarding their combining abilities relative to the complete diallels. The numbers of crosses in which each genitor(s) will compose the circulant diallel and the estimated heritability affect the combining ability estimates. With three crosses per parent, it is possible to obtain good concordance (correlation above 0.8) between the combining ability estimates.

Key words: *Carica papaya*; Diallel cross; Combining ability; Quantitative genetics

INTRODUCTION

Papaya (*Carica papaya* L.) is a semi-perennial species and an important crop from economic and social points of view. Papaya crops require a workforce all year. During the production cycle, papaya is susceptible to fungal leaf diseases, including black spot [*Asperisporium caricae* (Speg.) Maubl.], phoma spot [*Stagonosporopsis caricae* (Sydow & P. Sydow) Aveskamp, Gruyter & Verkley] and powdery mildew (*Streptopodium caricae* Liberato & R.W. Barreto). Because no genotype is resistant to these diseases (Dianese et al., 2007; Vivas et al., 2010, 2012c), spraying with fungicides has become the primary control measure. However, pesticide residues create an obstacle for papaya export from Brazil. Thus, disease resistant varieties originating from genetic improvements have emerged as a viable alternative for sustainable papaya production due to their lower environmental impact.

To meet market needs for papaya cultivars with desirable agronomic traits, such as high productivity, high soluble solid content and disease resistance, the breeder can use several resources and methods. One strategy commonly used for this purpose is direct crossing. Direct crossing aims to recombine the present genetic variability to create improved forms (Bernardo and Bohn, 2007). Diallel crosses are often used in genetic improvement programs to provide information regarding the responses of genitors between each other and between their hybrid combinations (Cruz and Vencovsky, 1989).

The main concern with diallel crosses is the limited number of hybrid combinations obtained and evaluated when a large number of genitors are studied. When dealing with a plant the size of papaya, this concern is more evident because experiments occupy large areas and are more expensive to establish and perform. An option that offers greater flexibility for diallel crossing with a large number of involved genitors is the circulant diallel method. This type of diallel method was proposed a few decades ago (Kempthorne and Curnow, 1961) and enables the involvement of a much greater number of parents. However, circulant diallels have rarely been used. Thus, although some studies have indicated that circulant diallel methods are more efficient relative to complete diallel methods (Veiga et al., 2000), information regarding diallel analysis via mixed models, especially for fruit trees, is lacking.

Diallel analysis via mixed models is more interesting, because pedigree information can be included and untested simple hybrids predicted with the use of the best linear unbiased predictor (BLUP) proposed by Henderson (1974). Resende (2007) claims that major developments in analytical methods have occurred in plant improvement due to the development of this method. In addition, the same author states that this method is excellent and that the most accurate in different experimental scenarios. However, information regarding diallel analysis in fruit trees is lacking.

Thus, this study aimed to evaluate the efficiency of the linear mixed models method in estimating the general combining ability (GCA) and specific combining ability (SCA) in a circulant table with different s sizes.

MATERIAL AND METHODS

Data were used from experiments carried out in a complete diallel scheme at Caliman Agrícola S/A in Linhares, Espírito Santo, Brazil. Twenty-eight hybrid F_1s from eight genitors were evaluated in a randomized complete block design with four replications. Four genitors were from the 'Formosa' group ('Maradol', 'JS12-N', 'JS12-4', and 'Sekati'), and four were from the 'Solo' group ('Waimanalo', 'Golden', 'Sunrise Solo 72-12', and 'São Mateus'). Each plot consisted of 10 plants (two rows of five plants each) spaced at 2.0 x 1.8 m. A lateral spacing of 3.6 m was used between treatments.

Each plot consisted of three central plants chosen at random. The chosen plants were analyzed for the severity of phoma spot, powdery mildew and black spot on leaves using a diagrammatic scale (Santos et al., 2011; Vivas et al., 2010, 2012c). The severity of phoma spot on leaves was estimated on a leaf located below the axil of the petiole and attached to a newly opened flower. The severity of powdery mildew and black spot on leaves were estimated on a leaf with the axil of the petiole attached to a newly opened flower.

We obtained the predicted additive genetic effects of genitors and the specific combining abilities of the crosses using the Selegen-Reml/Blup software (Resende, 2006) with the estimates of variance component (REML Single). The following model was used:

$$y = Xb + Zg + Ws + Tp + \varepsilon \ ,$$

where y, b, g, s, p, and ε correspond to the observed data vector, block effects vector (assumed to be fixed), GCA effects of the genitors involved in the cross vector (assumed to be random), SCA effects of the cross vector (assumed to be random), plot effect vector (assumed to be random) and random error, respectively. X, Z, W, and T are incidence matrices for b, g, s, and p, respectively.

Distributions and variance structures associated with the model terms are listed as follows.

$$\begin{aligned}
&y\big|b,V \sim N(Xb,\ V)\\
&g\ \big|I\sigma_g^2\ \sim N(0,I\sigma_g^2\ )\\
&s\big|\sigma_s^2 \sim N(0,I\sigma_s^2)\\
&p\big|\sigma_p^2 \sim N(0,I\sigma_p^2)\\
&\varepsilon\big|\sigma_\varepsilon^2 \sim N(0,I\sigma_\varepsilon^2)\\
&V = Z\ \sigma_g^2 Z\ ' + WI\sigma_s^2 W' + TI\sigma_p^2 T' + I\sigma_e^2
\end{aligned}$$

The effects of GCA and SCA were estimated as follows:

$\hat{c}_g^2 = \dfrac{\hat{\sigma}_g^2}{\hat{\sigma}_y^2}$: Estimation of the coefficient of determination for the GCA effects of the involved genitors.

$\hat{c}_s^2 = \dfrac{\hat{\sigma}_s^2}{\hat{\sigma}_y^2}$: Estimation of the coefficient of determination for the SCA effects of the crosses.

In addition to analyzing the complete diallel with the F_1s, we analyzed 50 circulant diallels (for each s value) with the genitors randomized to obtain 50 simulations from each situation. The efficiency of the circulant diallel method compared with the complete diallel method was quantified by a Pearson correlation between the estimated combining ability values obtained in the complete and circulant diallel simulations for each s value.

$$Corr(\hat{c}^2_{complete}, \hat{c}^2_{circulaing}) = \frac{\hat{\sigma}_{com,cir}}{\hat{\sigma}_{com}, \hat{\sigma}_{cir}}$$

Because 50 diallels were simulated for each s value, it was possible to identify the mean correlation and standard deviation estimates associated with each situation.

RESULTS AND DISCUSSION

Among the three diseases, the additive genetic variance was greater than the dominance variance. In addition, the genetic variance (additive and dominance) was greater than the residual variance for black spot severity (Table 1). Consequently, greater heritability estimates were obtained for black spot severity (Table 1). In addition, moderate heritability estimates were obtained for early blight severity and creole progeny of papaya trees (Vivas et al., 2012c). By estimating GCA (predicted additive genetic effect), the genitor ‘Maradol’ from the ‘Formosa’ group and the genitors ‘Waimanalo’, ‘Golden’ and ‘São Mateus’ from the ‘Solo’ group were identified as promising for obtaining papaya tree populations with low disease severity (Table 2). The results obtained here using mixed modeling partially resemble the results obtained with Griffing (1956) analysis by Vivas et al. (2012a,b, 2013). A high correlation between these two methods for GCA was previously reported for bean plants (Baldissera et al., 2012).

Table 1. Estimated variance components for the severity on papaya leaves of black spot (SBS), powdery mildew (SPM) and phoma spot (SPS) using the restricted maximum likelihood (REML).

Components of variance	SBS	SPM	SPS
Additive genetic variance	0.051	0.024	0.353
Genetic dominance variance	0.002	0.011	0.248
Residual variance	0.024	0.100	0.931
Individual phenotypic variance	0.077	0.135	1.532
Narrow sense heritability	0.67 ± 0.31	0.18 ± 0.16	0.23 ± 0.18
Overall mean	0.47	0.68	0.97

Table 2. Estimated general combining ability (predicted additive genetic effects) of the genitors using the best linear unbiased predictor (BLUP) for the severity of black spot (SBS), powdery mildew (SPM) and phoma spot (SPS) on papaya leaves.

Genitors	SBS	SPM	SPS
‘Maradol’	0.011	-0.173	-0.556
‘JS 12-N’	0.102	0.059	0.168
‘JS 12-4’	0.163	0.074	0.209
‘Sekati’	0.349	0.069	-0.358
‘Waimanalo’	-0.327	-0.127	0.655
‘Golden’	-0.057	-0.001	-0.034
‘Sunrise Solo 72/12’	-0.171	0.137	0.343
‘São Mateus’	-0.069	-0.038	-0.428

The combinations of ‘Maradol x Waimanalo’, ‘Maradol x Sunrise Solo 72/12’, ‘Maradol x São Mateus’, ‘JS 12-N x Golden’, ‘JS 12-4 x Waimanalo’, ‘Sekati x Waimanalo’, ‘Sekati x Golden’, and ‘Sekati x São Mateus’ resulted in negative estimates of SCA for the three variables studied (Table 3). From these results, it was inferred that superior genotypes could be obtained by exploring the hybrid and using directed crossing, such as diallel crossing. However, although the diallel appears to be an interesting method because it provides information on the response of genitors between themselves and their hybrid combinations (Cruz and Vencovsky, 1989), this method is limited when working with a large number of genitors.

Table 3. Estimated specific combining ability effects of the crosses obtained using the best linear unbiased predictor (BLUP) for the severity of black spot (SBS), powdery mildew (SPM) and phoma spot (SPS) on the leaves of papaya hybrids resulting from diallel crosses.

Crosses	SBS	SPM	SPS
‘Maradol x JS 12-N’	0.008	0.011	-0.247
‘Maradol x JS 12-4’	-0.018	0.026	-0.099
‘Maradol x Sekati’	0.020	-0.011	-0.127
‘Maradol x Waimanalo’	-0.005	-0.092	-0.212
‘Maradol x Golden’	0.008	0.014	0.139
‘Maradol x Sunrise Solo 72/12’	-0.006	-0.073	-0.168
‘Maradol x São Mateus’	-0.006	-0.033	-0.067
‘JS 12-N x JS 12-4’	0.003	-0.031	0.009
‘JS 12-N x Sekati’	0.018	0.135	-0.152
‘JS 12-N x Waimanalo’	0.004	-0.037	1.089
‘JS 12-N x Golden’	-0.007	-0.058	-0.222
‘JS 12-N x Sunrise Solo 72/12’	-0.016	0.064	-0.356
‘JS 12-N x São Mateus’	-0.004	-0.030	0.116
‘JS 12-4 x Sekati’	0.027	0.072	0.361
‘JS 12-4 x Waimanalo’	-0.013	-0.010	-0.264
‘JS 12-4 x Golden’	0.010	0.017	0.053
‘JS 12-4 x Sunrise Solo 72/12’	-0.003	-0.022	0.371
‘JS 12-4 x São Mateus’	0.004	0.017	-0.137
‘Sekati x Waimanalo’	-0.014	-0.045	-0.389
‘Sekati x Golden’	-0.028	-0.068	-0.262
‘Sekati x Sunrise Solo 72/12’	0.012	0.028	0.115
‘Sekati x São Mateus’	-0.014	-0.048	-0.050
‘Waimanalo x Golden’	-0.003	0.018	0.196
‘Waimanalo x Sunrise Solo 72/12’	-0.001	0.008	0.662
‘Waimanalo x São Mateus’	0.010	0.041	-0.161
‘Golden x Sunrise Solo 72/12’	0.006	0.088	0.103
‘Golden x São Mateus’	0.010	-0.013	-0.056
‘Sunrise Solo 72/12 x São Mateus’	-0.004	0.032	-0.246

Among the diallel analysis methods, analysis using circulant diallels (Kempthorne and Curnow, 1961) provided more flexibility for diallel crossing, significantly reduced the number of crosses in which each genitor was involved and enabled the participation of a greater number of genitors. For the three diseases studied, the correlation means increased proportionally with the number of crosses for each genitor based on the estimated GCA (additive effects of the genitors) between the circulant and complete diallels (Table 4). For black spot severity, only four crosses were observed per genitor. Thus, it was possible to estimate the mean correlation (near 0.95) and reduced standard deviation for the correlations between the additive estimated effects of the different simulated diallels (Table 4).

Table 4. Mean and standard deviation (SD) of the correlation estimates between general combining ability (additive effects) estimates obtained in the circulant diallel with different numbers of crosses for each genitor(s), obtained for the severity of black spot (SBS), powdery mildew (SPM) and phoma spot (SPS) on papaya leaves using 50 diallels simulated.

Variable	No. of crosses by genitor	Mean (SD)			
		S = 4	S = 5	S = 6	Complete
SBS	3	0.92 (0.07)	0.89 (0.08)	0.88 (0.10)	0.88 (0.10)
	4		0.96 (0.03)	0.94 (0.05)	0.94 (0.05)
	5			0.98 (0.02)	0.97 (0.02)
	6				0.99 (0.01)
SPM	3	0.87 (0.17)	0.81 (0.16)	0.76 (0.16)	0.76 (0.16)
	4		0.91 (0.09)	0.87 (0.10)	0.85 (0.11)
	5			0.94 (0.06)	0.90 (0.08)
	6				0.96 (0.04)
SPS	3	0.83 (0.21)	0.77 (0.21)	0.75 (0.21)	0.70 (0.23)
	4		0.91 (0.11)	0.88 (0.11)	0.82 (0.14)
	5			0.96 (0.05)	0.91 (0.09)
	6				0.95 (0.06)

To estimate the severity of powdery mildew and phoma spot, the number of crosses in which each genitor was involved were increased to estimate the mean correlation above 90% among the diallels simulated (Table 4). The discrepancy between the mean correlation estimates between different simulated diallels for the severity of powdery mildew, phoma spot and black spot could be attributed to the heritability value (Table 1). Thus, the severity of powdery mildew and phoma spot expressed heritability estimates by approximately 20%. However, for the severity of black spot, these estimates were nearly 70%. These results agreed with those obtained by Veiga et al. (2000), in which the s value was dependent on heritability for hybrid selection, which can be smaller or larger than h^2.

From the results obtained in the present study, it is possible to use mixed modeling as a viable alternative for obtaining reliable genitor inferences without conducting all planned crosses. Cruz et al. (2012) claimed that diallel analysis provides useful parameter estimates in selecting genitors for hybridization and in the understanding of the genetic effects involved in the determination of traits, leading to knowledge of the genetic relationships between the genitors involved in the cross. However, this method has been rarely used in fruit trees (especially papaya) (Subhadrabandhu and Nontaswatsri, 1997; Marin et al., 2006a,b; Vivas et al., 2012a,b, 2013). Veiga et al. (2000) identified the number of crosses required for evaluating the potential of the genitors as a primary constraint of using diallel crosses. This study suggested that the number of crosses per genitor could be reduced.

The diallel cross method provides relative information regarding the responses of the parents between each other and reports their responses in combinations with other genitors for hybrid formation (Cruz and Vencovsky, 1989). Here, we determined correlations of SCA estimates between all diallels and the simulated diallels. From the means and standard deviations of the correlation estimates, high correlations of SCA estimates were observed between the complete diallel and simulated diallels with different numbers of crosses per genitor (Table 5). For the severity of powdery mildew and phoma spot, three crosses per genitor were sufficient for expressing a correlation of more than 0.9. However, for the severity of black spot, four crosses per genitor were sufficient for obtaining a mean correlation of more than 0.9 (Table 3). Veiga et al. (2000) obtained mean correlations of nearly 0.8 for diallels that were simulated with three crosses per genitor. With 11 crosses per genitor, the mean correlation values were higher and more stable. In contrast, an increase in the mean and a decrease in the standard deviation were observed in this study as the number of crosses per genitor increased. However, with four crosses per genitor, the mean correlation estimates were above 0.9 for the three variables studied (Table 5). Ferreira et al. (2004) evaluated the yield of maize cobs in diallel and observed that a 30% reduction in the number of crosses per genitor did not affect the choice of the best parents and hybrids (GCA and SCA effects).

Table 5. Mean and standard deviation (SD) of correlation estimates among the specific combining ability estimates obtained using the circulant diallel method with different numbers of crosses for each genitor(s), obtained for the severity of black spot (SBS), powdery mildew (SPM) and phoma spot (SPS) on papaya leaves using 50 diallels simulated.

Variable	No. of crosses by genitor	Mean (SD)			
		S = 4	S = 5	S = 6	Complete
SBS	3	0.91 (0.06)	0.86 (0.10)	0.84 (0.11)	0.83 (0.11)
	4		0.95 (0.04)	0.92 (0.04)	0.91 (0.05)
	5			0.96 (0.03)	0.94 (0.05)
	6				0.97 (0.02)
SPM	3	0.97 (0.04)	0.95 (0.06)	0.94 (0.06)	0.94 (0.05)
	4		0.98 (0.03)	0.97 (0.03)	0.96 (0.03)
	5			0.98 (0.02)	0.97 (0.02)
	6				0.99 (0.02)
SPS	3	0.97 (0.04)	0.95 (0.05)	0.94 (0.05)	0.93 (0.05)
	4		0.98 (0.03)	0.97 (0.03)	0.96 (0.03)
	5			0.98 (0.02)	0.97 (0.02)
	6				0.98 (0.02)

From the data obtained in the present study, the efficiency of using mixed models for estimating the combining ability effects with a small number of crosses per genitor is evident. Veiga et al. (2000) reported that using a small number of crosses per genitor was a problem due to the small number of hybrids evaluated. To mitigate this problem, crosses can be directed in the presence of each genitor, because SCA increases as the divergence between the genitors increases. In addition, the possibility of using biotechnology and statistical procedures for genetics to estimate the SCA values for hybrids that were not evaluated should be considered (Bernardo, 1994, 1995).

Overall, the circulant diallels method using mixed modeling was more efficient than the complete diallel method for classifying genitors regarding their combining abilities. The number of crosses in which each genitor(s) composes the circulant diallel and the heritability estimations affect the combining ability estimations. However, with three crosses per genitor, it is possible to obtain good agreement (correlation above 0.8) between the combining ability estimates.

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