

# Development of superior lines of papaya from the Formosa group using the pedigree method and REML/Blup procedure

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**ABSTRACT:** Developing superior lines based on a simultaneous response from many traits of agronomic importance is a fundamental strategy to expand papaya cultivar supply, mainly the much-needed cultivars belonging to group Formosa. Our goal was to associate the pedigree method with REML/Blup procedure to select plants of progenies  $F_{2,3}$  with superior agronomic performance. The variance components and genetic parameters were estimated using REML approach, and the genetic values were predicted using Blup methodology. The combined selection was performed using the IG2 selection index based on weights associated with agronomic values to indicate the best progenies and lines. The heritability estimates of progeny mean were high in most traits and evidenced significant genetic variability between progenies. The narrow-sense individual heritability was moderate in traits such as commercial fruits and soluble solid contents, which indicates a

favorable condition for selections within progenies. The index was consistent in the selection of six superior progenies according to the assessed traits. This index also enabled promising genetic gains in traits of great interest such as yield and pulp thickness. Although the progenies are derived from self generations, they proved to be superior in traits of significant importance such as yield, commercial fruits, and pulp thickness when compared to the Tainung-01 hybrid, which is a worldwide-planted cultivar used as a reference to Formosa representatives. Six lines were selected within the superior progenies to continue the Formosa group-lines development program. The association of the pedigree method with REML/Blup procedure can be useful to assist breeders in an autogamous breeding program.

**Key words:** *Carica papaya*, combined selection, segregating populations.

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Received: Sep. 20, 2018 – Accepted: Feb. 22, 2019



## INTRODUCTION

Brazil is the second largest papaya producer in the world; it produced 1.6 million tons in 2014, which represented 12.7% of world production (FAO 2017). However, although papaya is important to the agribusiness in the country, the commercial plantations use a reduced number of cultivars, resulting in a narrow genetic base (Dias et al. 2011) and, consequently, in-plant vulnerability to biotic and abiotic stress. Commercial plantations of large-fruit Formosa-standard papaya strongly rely on Taiwan's hybrid seed imports; however, it increases the risks of having new pests and diseases affecting the crops, besides the high cost of the seeds. Thus, it is necessary broadening the genetic base in the crop, as well as developing cultivars with desirable agronomic traits, tolerance against abiotic stress and resistance against the main pests and diseases in order to meet the requirements of the internal and external markets (Dantas and Lima 2001).

The development of cultivars depends on the availability of genetic variability and the favorable simultaneous genotype response to most traits of agronomic importance (Ramos et al. 2014a). The genetic variability found in segregating papaya generations may be used to identify transgressive genotypes, as well as to develop lines in order to obtain pure-line cultivars. In the papaya crop, studies carried out in segregating populations showed a wide genetic variability for several traits of economic importance, thus allowing the selection of superior genotypes (Karunakaran et al. 2010; Oliveira et al. 2012; Ramos et al. 2014a; Cortes et al. 2018). Different methods may conduct these segregating generations. In the papaya, the backcrossing method was used to conduct segregating generations to transfer genomic regions that control hermaphroditism (Silva et al. 2007) and tolerance to physiological disturb (Pinto et al. 2013). The single seed descent method was used to obtain recombinant inbred lines from the Formosa group (Cortes et al. 2018). The pedigree method was used for the advanced and conduction of segregating populations derived from backcrossing in order to select recombinant inbred lines to obtain hybrids (Ramos et al. 2014a; Barros et al. 2017).

Moreover, the recombinant inbred lines may be used as parents in hybridization programs to the exploitation of heterosis. It is possible to obtain papaya lines and hybrids because papaya plants can be subjected to selfing without significant inbreeding depression (Dantas and Lima

2001). The development of genotypes with commercial hybrid-like traits allows saving the money used to import seeds and helps to prevent phytosanitary issues. The development of inbred lines – mainly those belonging to group Formosa – enables small- and medium-sized farmers to produce their seeds at low cost, as well as to reuse them in subsequent crops.

The successful selection of superior lines depends on the effectiveness of breeding methods. The pedigree method is mostly used in the genetic improvement of autogamous plants to develop superior lines (Ramalho et al. 2013). Several cultivars of autogamous plants species were developed using this method, for example, soybean (Miranda et al. 2003), castor bean (Savy Filho et al. 2007), wheat (Scheeren et al. 2007), and beans (Carbonell et al. 2010). One of its main advantages is that it allows knowing the genealogy of the selected lines. The REML/Blup procedure has significantly increased the efficiency of the method, mainly when the relationship matrix is applied to estimate genetic values (Ramalho et al. 2013). The REML/Blup procedure has been successfully used to select superior papaya genotypes (Oliveira et al. 2012; Pinto et al. 2013; Ramos et al. 2014b; Cortes et al. 2018). Also, this strategy has effectively increased the genetic gains in long-cycle autogamous plants, as well as speed up the development of lines (Ramalho et al. 2013).

Thus, the current study aimed to select superior lines among  $F_{2,3}$  progenies belonging to group Formosa via pedigree method using the REML/Blup procedure.

## MATERIALS AND METHODS

### Development of population

Thirty progenies  $F_{2,3}$  were used in this study. These progenies were derived from the selection of 30 individuals of the  $F_2$  generation from crosses between Sekati and JS12 lines (Cortes 2017b<sup>3</sup>). Both parents are endogamous lines belonging to the same heterotic group, Formosa group, however, contrasting for agronomic and sensorial traits. The Sekati parent produces large fruits, excellent pulp firmness, and median soluble solid contents.

<sup>3</sup>Cortes, D. F. M. (2017b). Desenvolvimento de linhagens de mamoeiro assistido por imagens digitais (Ph.D. Thesis). Campos dos Goytacazes: Universidade Estadual do Norte Fluminense Darcy Ribeiro. In Portuguese.

On the other hand, the JS12 parent diverges from Sekati in the last two traits, since it presents moderate pulp firmness and high soluble solid contents. Thus, the Sekati parent was used as pulp firmness source, whereas the JS12 parent was used as a flavor source. According to studies about the inheritance resistance to fungal diseases, the Sekati parent stood out for having phoma spot, black spot, and powdery mildew resistant alleles (Vivas et al. 2013).

The  $F_2$  generation was obtained by selfing of  $F_1$  plants derived from crosses between Sekati and JS12 parents. A population of 294 plants derived from the selfing of  $F_1$  plants was tested in the field. IG2 (Ramos et al. 2014a) selection index based on weights associated with agronomic values was used to select 30 superior individuals (Cortes 2017b).

### Phenotyping of $F_{2,3}$ progenies

The experiment was conducted under field conditions in Linhares County, Espírito Santo State, Brazil (19°06' and 19°18' S; 39°45' and 40°19' W; altitude 30 m). The 30  $F_{2,3}$  progenies previously selected the parents and a commercial hybrid (Tainung 01). Plants were arranged in rows, spaced 3.60 m between rows and 1.50 m between plants using eight holes per row. Three seedlings were planted per hole and three months after planting the plants were sexed, maintaining only the hermaphrodite plants. The plants were conducted according to the pedigree method, which proposes the selection of plants within and between lines in segregating  $F_{2,3}$  progenies, making the identification of lines and plants within the rows, taking individual data and registers in the spreadsheets to keep information about kinship.

The study was carried out using the randomized complete block experimental design with 30  $F_{2,3}$  progenies, four blocks, and eight plants per plot, totaling 32 plants per progeny. Two assessments were performed six and nine months after transplanting (MAT).

A methodology based on digital image analysis, which was validated and described by Cortes et al. (2017a) to phenotyping of papaya plants, was used to measure the following traits: plant height (PH) and first fruit-insertion height (FFIH), both expressed in cm; stem diameter (SD), expressed in mm; number of commercial fruits (NCF); number of deformed fruits (NDF); and number of fruitless leaf axils (FLLA).

The following traits were also measured: mean fruit mass (FM), expressed in kg and measured in an analytical balance; fruit length (FL) and fruit diameter (FD), expressed in cm and measured in a caliper; soluble solid contents (SS), expressed in °Brix and measured in a digital refractometer; fruit firmness (FF) and pulp firmness (PF) expressed in N and measured using a digital bench-mounted penetrometer; pulp thickness (PT), expressed in cm and measured using a ruler. These traits were measured in fruits presenting characteristics of the one maturity stage (green skin with a light yellow stripe; pulp exhibits some areas with orange color, is very hard and contains large amounts of latex) determined according to Basulto et al. (2009). Besides, it was estimated the plant production (PROD), expressed in kg and determined by multiplying NCF by FM.

### Statistical analyses

Estimation of variance components and genetic parameters

The statistical analyses of traits NCF, NDF, FLLA, and PROD, took into consideration the sum of the two assessment periods (six MAT + nine MAT). In turn, the statistical analyses of traits FFIH, PH, and SD took into account the mean of the two periods. On the other hand, the statistical analyses of FM, FL, FD, SS, FF, PF, and PT took into account the second period. The variance components and genetic parameters were estimated through the REML procedure, whereas the genotypic values were predicted through the Blup procedure.

The genetic assessment used the following statistical model (Eq. 1):

$$y = Xb + Za + Wc + \varepsilon \quad (1)$$

where:  $y$  is the vector of observation;  $b$  is the vector of block effects (assumed as fixed) added to the general mean;  $a$  is the vector of individual additive genetic effects (assumed as random);  $c$  is the vector of plot effects; and  $\varepsilon$  is the vector of errors or residues (random). The capital letters ( $X$ ,  $Z$ , and  $W$ ) represent the incidence matrices of the aforementioned effects. The distributions and variance structures associated with the model's terms were set through:

$$y | b, V \sim N(Xb, V); a | A\sigma_a^2 \sim N(0, A\sigma_a^2); \\ c | \sigma_c^2 \sim N(0, I\sigma_c^2); \varepsilon | \sigma_\varepsilon^2 \sim N(0, I\sigma_\varepsilon^2).$$

The random effects were set through:

$$\text{Cov}(a, c') = 0; \text{Cov}(a, e') = 0; \text{Cov}(c, e') = 0$$

The variance structures in the model were set through:

$$V = ZA\sigma_a^2Z' + WI\sigma_c^2W' + I\sigma_e^2;$$

wherein  $A$  is the additive genetic relationship matrix comprising all the individuals.

The mixed model equations are (Eq. 2):

$$\begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{c} \end{bmatrix} \begin{bmatrix} X'X & & X'Z \\ Z'X & Z'Z + A^{-1}((1 - h^2 - c^2)/(h^2)) & \\ W'X & & W'Z \end{bmatrix} \begin{bmatrix} X'W \\ Z'W \\ W'W + I^{-1}((1 - h^2 - c^2)/(c^2)) \end{bmatrix}^{-1} = \begin{bmatrix} X'y \\ Z'y \\ W'y \end{bmatrix} \quad (2)$$

where  $\hat{h}^2 = \hat{\sigma}_a^2 / \hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2$  is narrow-sense heritability in the block;  $\hat{\sigma}_a^2$  = additive genetic variance;  $\hat{\sigma}_c^2$  = variance between plots;  $\hat{\sigma}_e^2$  = residual variance (environmental + non-additive);  $c^2 = \hat{\sigma}_c^2 / \hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2$  is the correlation resulting from the common environment between plots. The variance component estimators via EM algorithm were set through:

$$\hat{\sigma}_e^2 = [y'y - \hat{b}'X'y - \hat{a}'Z'y - \hat{c}'W'y] / [N - r(X)];$$

$$\hat{\sigma}_a^2 [\hat{a}'A^{-1}\hat{a} + \sigma_e^2 \text{tr}(A^{-1}C^{22})/q]; \hat{\sigma}_c^2 = [\hat{c}'c + \sigma_e^2 \text{tr}(C^{33})/s].$$

wherein:  $C^{22}$  and  $C^{33}$  result from:

$$C^{-1} = \begin{bmatrix} C_{11} & C_{12} & C_{13} \\ C_{21} & C_{22} & C_{23} \\ C_{31} & C_{32} & C_{33} \end{bmatrix}^{-1} = \begin{bmatrix} C^{11} & C^{12} & C^{13} \\ C^{21} & C^{22} & C^{23} \\ C^{31} & C^{32} & C^{33} \end{bmatrix};$$

$C$  = matrix of the mixed model equation coefficients;  $\text{tr}$  = matrix trace operator given by the sum of the diagonal matrix elements;  $r(X)$  = place or number of linearly  $X$ -independent columns;  $N, q, s$  = total number of observations, number of individuals and number of plots, respectively.

The following variances and genetic parameters were also estimated through the following estimates, according to Viana and Resende (2014):

Individual phenotypic variance:  $\hat{\sigma}_f^2 = (\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2)$ ; narrow-sense individual heritability between progenies,

adjusted to plot effects:  $\hat{h}_{aj}^2 = \hat{\sigma}_a^2 / \hat{\sigma}_a^2 + \hat{\sigma}_c^2$ ; mean progeny heritability:

$$\hat{h}_m^2 = \hat{\sigma}_a^2 / \left[ \hat{\sigma}_a^2 + \frac{1}{4}\hat{\sigma}_c^2 + \left( \frac{1}{2}\hat{\sigma}_a^2 + \hat{\sigma}_e^2 / 32 \right) \right].$$

where 4 is the number of blocks, and 32 is the number of blocks multiplied by the number of plants per plot (8); progeny selection accuracy:  $Ac = \sqrt{h_m^2}$ ; additive heritability estimate within the plot ignoring the dominance genetic variance fraction (1/4):

$$\hat{h}_{ad}^2 = \frac{1}{2}\hat{\sigma}_a^2 / \left( \frac{1}{2}\hat{\sigma}_a^2 + \hat{\sigma}_e^2 \right);$$

individual additive genetic variation coefficient ignoring the dominance genetic variance fraction (1/4):

$$CVg = 100\sqrt{\hat{\sigma}_a^2 / \hat{m}};$$

residual variation coefficient:

$$Cve = \left( 100\sqrt{\hat{\sigma}_a^2 + \frac{1}{8}\hat{\sigma}_e^2 + \hat{\sigma}_c^2} \right) / \hat{m};$$

relative variation coefficient:

$$Cvr = Cvg / Cve.$$

The values of the coefficients associated with the number of blocks ( $b = 4$ ) and a number of plants per plot ( $c = 8$ ) of the expressions are obtained assuming 100% plant survival. However, due to the loss of 35% of the plants caused by the incidence of the disease, the coefficient values were adjusted to perform the statistical analysis. The incidence of Papaya Ringspot Virus may have contributed to reducing the number of experimental units since this virus led to plants loss.

The heritabilities between and within progenies were inflated by the dominance genetic variance fraction (1/4). However, it should not affect the selection of individuals, since the two heritabilities were used to calculate the genetic values (Resende 2016). The genotypic values of all progenies, estimated through Blup, were found by adding each genotypic effect ( $g$ ) to the overall mean of the experiment ( $\mu$ ).

The analyses were performed in the Selegen-REML/Blup software, model 59 (Resende 2016). This model was

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designed to assess individuals of  $F_3$  progenies in autogamous plants derived from  $F_2$  generations considering multiple observations per plot.

### Selection between and within superior progenies

The combined selection was carried out using the IG2 weight index, which was developed to select papaya genotypes, according to Ramos et al. (2014a). Also, the combined selection was performed between progenies in order to select superior progenies, as well as based in the plants in order to identify the best lines. A selection intensity of 20% was used to indicate the best progenies and lines. The index estimator was expressed through Eq. 3:

$$IG_2 = \sum_{i=1}^n (v_{gi} \times p_i) \quad (3)$$

where  $v_{gi}$  = standardized additive genetic value of trait  $i$ ;  $p$  = economic weight attributed to trait  $i$  with positive or negative sign according to the selected direction. The weights assigned to the 13 traits were: PH (1), FFIH (1), SD (5), NCF (100), NDF (-20), FLLA (-20), FM (1), FL (1), FD (1), SS (100), FF (100), PF (100) and PT (70). The weight relation was experimentally set based on the agronomic importance of the traits, as described by Silva et al. (2008).

Besides, the genetic gains obtained with the selection of the best progenies and the best lines were estimated. The gains were estimated using the following estimator (Eq. 4): where  $\hat{G}_s$  = genetic gain;  $\hat{y}_s - \mu_0$  = selection differential;  $\hat{h}^2$  = heritability based on the mean of the progenies ( $\hat{h}_m^2$ )

$$\hat{G}_s = (\hat{y}_s - \mu_0) \hat{h}_m^2 \quad (4)$$

for the selection of best progenies, or additive heritability within plots for the selection of lines within best progenies ( $\hat{h}_{ad}^2$ ).

## RESULTS

### Genetic parameter estimates

The magnitudes of the experimental variation coefficient (CVe) ranged from 4.55% to 35.41% (Table 1). Traits such as PF, FL, FF, PT, SS, SD, and PH showed low magnitudes (CVe  $\leq$  10), thus indicating high experimental accuracy. On the other hand, FFIH, FM, and FD showed medium magnitudes (10  $\leq$  CVe  $\leq$  20), thus evidencing good accuracy. NCF and FLLA showed high magnitudes (20  $\leq$  CVe  $\leq$  30), whereas NDF and PROD showed very high magnitude

**Table 1.** Variance components and Genetic parameters estimated for 14 morpho-agronomic traits in papaya  $F_{2:3}$  progenies.

Trait	$\hat{\sigma}_a^2$	$\hat{\sigma}_e^2$	$\hat{\sigma}_f^2$	$\hat{h}_a^2$	$\hat{h}_{aj}^2$	$c^2$	$\hat{h}_m^2$	Ac	$\hat{h}_{ad}^2$	CVg	CVe	CVr	$\hat{m}$
FFIH	49.25	127.10	213.36	0.23	0.28	0.17	0.79	0.89	0.19	11.22	11.63	0.96	62.56
PH	152.50	476.50	812.32	0.18	0.24	0.23	0.72	0.85	0.17	7.520	9.49	0.80	164.20
SD	0.38	1.16	1.99	0.19	0.25	0.23	0.72	0.85	0.17	5.77	7.21	0.80	10.72
NCF	20.29	50.29	85.78	0.24	0.29	0.18	0.79	0.89	0.20	22.84	23.51	0.97	19.72
NDF	0.75	8.19	9.50	0.08	0.08	0.06	0.65	0.81	0.05	24.25	35.41	0.68	3.56
FLLA	11.39	49.12	63.93	0.18	0.19	0.05	0.83	0.91	0.12	22.71	20.81	1.09	14.86
FM	88.66	80.32	204.33	0.43	0.52	0.17	0.89	0.94	0.55	17.35	12.42	1.40	1.72
FL	579.20	1035.00	1620.40	0.36	0.36	0.00	0.94	0.97	0.28	9.46	4.57	2.07	254.50
FD	9.50	534.50	716.52	0.01	0.02	0.24	0.14	0.37	0.01	2.57	12.91	0.20	119.80
FF	4.17	110.90	126.56	0.03	0.04	0.09	0.4	0.63	0.02	1.88	4.65	0.41	108.30
PF	4.41	54.26	65.77	0.07	0.08	0.11	0.56	0.75	0.04	2.56	4.55	0.56	81.93
PT	0.02	0.05	0.09	0.30	0.29	0.22	0.79	0.89	0.26	5.54	5.62	0.98	2.80
SS	0.32	0.68	1.20	0.30	0.32	0.18	0.81	0.90	0.24	7.45	7.11	1.04	7.58
PROD	46.34	213.3	346.76	0.13	0.18	0.25	0.62	0.79	0.11	20.24	31.71	0.64	33.64

$\hat{\sigma}_a^2$  = additive genetic variance;  $\hat{\sigma}_e^2$  = residual variance;  $\hat{\sigma}_f^2$  = individual phenotypic variance;  $\hat{h}_a^2$  = broad-sense individual heritability between progenies;  $\hat{h}_{aj}^2$  = narrow-sense individual heritability between progenies, adjusted to plot effects;  $c^2$  = correlation resulting from the common environment between plots;  $\hat{h}_m^2$  = mean progeny heritability; Ac = progeny selection accuracy;  $\hat{h}_{ad}^2$  = additive heritability within the plot; CVg% = individual additive genetic variation coefficient; CVe% = residual variation coefficient; CVr = relative variation coefficient;  $\hat{m}$  = mean. PH = plant height (cm); FFIH = first fruit-insertion height (cm); SD = stem diameter (cm); NCF = number of commercial fruits; NDF = number of deformed fruits; FLLA = number of fruitless leaf axils; FM = fruit mass (g); FL = fruit length (mm); FD = fruit diameter (mm); SS = soluble solid contents ( $^{\circ}$ Brix); FF = fruit firmness (N); PF = pulp firmness (N); PT = pulp thickness (cm); PROD = plant production (Kg).

( $CV_e > 30$ ). The magnitudes of the determination coefficient of plot effects ( $c^2$ ) ranged from low to high (0% to 25%). The  $c^2$  measures the variability within blocks and it may be classified as low ( $c^2 < 0.10$ ) and high ( $c^2 > 0.10$ ) (Sturion and Resende 2010). Low  $c^2$  magnitudes were found in FL, FF, NDF, and FLLA, whereas high magnitudes were found in the other traits. The magnitudes of the individual additive genetic variation coefficient (CVg) ranged from low to high (1.88% to 24.25%). All the herein assessed traits showed low to moderate magnitudes (1.88% to 17.35%), except for PROD, NCF, FLLA, and NDF, which showed high magnitudes. The relative variation coefficient (CVr) values ranged from 0.2 to 2.08. Magnitudes higher than the unity were found in FL, FM, SS, and FLLA. On the other hand, magnitudes close to the unit were found in PT, NCF, FFIH, PH, and SD. The other traits showed moderate magnitudes, except for FD and FF.

The heritability in the selection within progenies ( $\hat{h}_{ad}^2$ ) showed magnitudes similar to those of the narrow-sense individual heritability ( $\hat{h}_a^2$ ) in all traits. Low magnitudes ( $0.01 \leq h^2 \leq 0.15$ ) were found in FD, FF, PF, PROD, NDF, and FLLA. On the other hand, medium magnitudes ( $0.15 \leq h^2 \leq 0.50$ ) were found in FFIH, NCF, PH, SD, SS, PT, and FL, whereas high magnitude ( $h^2 > 0.50$ ) was found in FM. The standard deviations around each  $\hat{h}_{ad}^2$  estimate ranged from low to moderate; it showed that the estimated heritability values were statistically different from zero.

The heritability based on the mean of the progenies ( $\hat{h}_m^2$ ) showed magnitudes significantly higher than those of the narrow-sense individual heritability. Low  $\hat{h}_m^2$  magnitudes were estimated in FD (0.14), whereas median magnitudes were estimated in FF (0.40). The other traits showed high  $\hat{h}_m^2$  coefficients, which ranged from 0.56 (in PF) to 0.94 (in

FL). The accuracy values in the present study ranged from 0.37 to 0.97; they were considered high, except for FD (0.37).

### Selection between and within progenies

The genetic gains of the six progenies indicated through the  $IG_2$  index ranged from 3.07% to 26.61% for PROD, from 15.02% to 29.78% for NCF, from -27.98% to -12.62% for NDF, from -12.25% to 20.41% for FLLA, and from -3.44% to 3.43% for PT (Table 2). These values were satisfactory if one takes into consideration the mean gains estimated for each trait. It is worth highlighting that these gains were positive in PROD, NCF and PT, and negative in FLLA and NDF. The highest mean gains were found in PROD; mean increases of 13.28%, 21.67%, -18.12%, and 0.83% were predicted for PROD, NCF, NDF, and FLLA, respectively.

The  $IG_2$  index was also used in the present study to select the best plants within the best progenies, taking into consideration the 20% selection intensity. The gains predicted for the individual selection decreased when they were compared to the analysis based on the mean of the progenies in traits such as PROD and NCF (Table 3). The highest gain was estimated in NCF ( $-0.32\% \leq G_s \leq 2.62\%$ ), followed by PROD ( $-0.23\% \leq G_s \leq 1.05\%$ ). Progenies 6 and 5 presented the highest gains in PROD and NCF, although of low magnitude.

In addition to the genotypes selected through combined selection, the direct selection based on SS, FF, and PF was conducted to keep the SS, FF, and PF source genotypes in the breeding population. The gains for the direct selection ranged from 2.83% to 3.53% for FF and PF, and from 1.34% to 1.60% for SS (Table 4).

**Table 2.** Genetic gains ( $G_s$ ) and new predicted averages ( $\bar{X}_s$ ) in five traits of great importance in papaya breeding for the progenies selected by the  $IG_2$  index.

Progeny	Plant production (kg)		Number of commercial fruits		Number of deformed fruits		Fruitless leaf axils		Pulp thickness (cm)	
	$G_s$	$\bar{X}_s$	$G_s$	$\bar{X}_s$	$G_s$	$\bar{X}_s$	$G_s$	$\bar{X}_s$	$G_s$	$\bar{X}_s$
6	26.61	57.03	15.02	26.43	-27.98	5.69	-12.25	2.45	3.43	3.02
16	13.92	45.88	17.60	27.58	-21.28	7.89	-10.22	2.64	0.82	2.85
14	12.82	44.91	23.41	30.18	-12.62	10.72	-7.88	2.85	-3.44	2.59
9	12.35	44.49	29.78	33.02	-14.69	10.05	4.09	3.93	-0.08	2.80
5	10.90	43.22	21.27	29.22	-16.25	9.53	20.41	5.40	-2.60	2.64
21	3.07	36.33	22.95	29.97	-15.92	9.64	10.80	4.54	2.00	2.93
Mean	13.28	45.31	21.67	29.40	-18.12	8.92	0.83	3.63	0.02	2.80
Tainung		44.37		27.85		7.13		14.19		2.83

**Table 3.** Genetic gains ( $G_s$ ) and new predicted averages ( $\bar{X}_s$ ) in plant production (PROD) and a number of commercial fruits (NCF) to plants selected within the superior progenies.

Progeny Plant	PROD		NCF		Progeny Plant	PROD		NCF	
	$G_s$ (%)	(Kg)	$G_s$ (%)	$\bar{X}_s$		$G_s$ (%)	(Kg)	$G_s$ (%)	$\bar{X}_s$
6-5	1.05	48.90	2.62	24.17	9-2	1.04	40.92	1.56	27.76
6-1	0.61	48.68	1.43	23.89	9-5	0.20	40.58	0.97	27.60
6-4	0.65	48.70	1.59	23.92	9-2	0.05	40.52	-0.01	27.33
6-3	0.16	48.47	0.22	23.60	9-7	0.23	40.59	1.56	27.76
6-3	0.33	48.55	0.74	23.72	9-1	0.44	40.67	-0.27	27.26
6-1	0.41	48.59	0.39	23.64	9-6	0.48	40.69	1.27	27.68
16-3	0.81	41.73	1.91	24.69	5-4	2.98	40.91	4.17	26.18
16-4	0.48	41.59	0.61	24.38	5-8	0.59	39.97	1.34	25.47
16-5	0.06	41.42	-0.26	24.17	5-3	0.52	39.94	1.79	25.58
16-3	0.61	41.64	1.99	24.71	5-6	-0.06	39.71	-0.30	25.06
16-2	0.13	41.44	0.32	24.30	5-1	0.32	39.86	1.66	25.55
16-7	0.23	41.49	0.61	24.38	5-7	0.45	39.91	1.19	25.43
14-1	0.50	41.24	0.20	25.98	21-2	0.09	35.83	0.64	25.87
14-2	0.25	41.14	0.40	26.03	21-2	-0.11	35.76	0.14	25.74
14-4	0.25	41.13	0.11	25.96	21-3	-0.10	35.77	1.07	25.98
14-3	-0.14	40.97	0.40	26.03	21-2	-0.23	35.72	-0.32	25.63
14-1	-0.07	41.00	0.09	25.95	21-1	-0.14	35.75	0.01	25.71
14-1	-0.04	41.02	0.42	26.04	21-1	0.14	35.85	0.44	25.82

**Table 4.** Direct selection for three traits of agronomic importance for papaya breeding.

Trait	Progeny	Plant	f	a	u + a	$G_s$	$\bar{X}_s$
SS	26	3	11.46	1.6	9.19	1.60	9.19
SS	26	1	10.42	1.33	8.91	1.47	9.05
SS	19	3	10.66	1.22	8.81	1.38	8.97
SS	19	4	9.51	1.2	8.79	1.34	8.92
PF	3	1	101.30	3.53	85.46	3.53	85.46
PF	3	5	98.33	3.41	85.34	3.47	85.40
FF	12	2	135.94	2.85	111.18	2.85	111.18
FF	12	6	135.05	2.82	111.15	2.83	111.17

SS = soluble solids content (°Brix); PF = pulp firmness (N); FF = fruit firmness (N); f = phenotypic value; a = additive genetic effect; u + a = additive genetic value;  $G_s$  = genetic gain (%);  $\bar{X}_s$  = new average of the selected individuals.

## DISCUSSION

Our team of plant breeding studied the combination Sekati × JS12 in studies of combining ability and testers for development of hybrids (Ide et al. 2009), diallel trials and heterosis studies (Cardoso et al. 2014) and resistance to fungal diseases (Vivas et al. 2013), since we are trying to obtain cultivars with characteristics of the Formosa group to reduce dependence on the import of hybrid seeds from Taiwan. Therefore, we propose optimizing

methods to generate advanced breeding lines to evaluate and select superior homozygous genotypes (recombinant inbred lines) for traits related to yield and fruit quality. We approach the conduction and selection of lines using the pedigree method and mixed models (REML/Blup procedure), simultaneously performing the selection and recombination phases in order to reduce the time necessary to obtain superior genotypes.

The high magnitudes of heritability based on the mean of progenies for most of the traits indicate high genetic

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variability among progenies. This variability observed in traits associated with fruit quality as soluble solids content, pulp firmness and pulp thickness in segregating progenies was an expected result, since it is  $F_{2,3}$  generation derivate from parents that are divergent for such traits. In contrast, the low  $\hat{h}_m^2$  magnitudes estimated in fruit diameter indicated low genetic variability between progenies. High  $\hat{h}_m^2$  coefficients in papaya-segregating progenies were also estimated for plant height, first fruit height, number of commercial fruits, mean fruit mass, soluble solid contents, pulp thickness (Ramos et al. 2014b) and production (Silva et al. 2008), whereas low coefficients were estimated for fruit diameter (Ramos et al. 2014b). The heritability is not only related to the traits, but also to the population and to the environmental circumstances the individuals are subject to. Thus the heritability magnitude may be affected if one of the phenotypic and genetic variance components changes (Falconer 1987).

The moderate heritability within the plot in traits as commercial fruits, pulp thickness, plant height, stem diameter, and solid soluble content and high heritability observed in fruit mass indicates favorable variability situation to the selection of lines within progenies and to obtaining considerable genetic gains. The selection within progenies did not allow significant gains in traits as production, pulp firmness, and fruit firmness due to low heritability; thus, the selection between progenies is more relevant in this case. The low magnitudes may be explained through the genetic nature of the herein assessed progenies. According to Hallauer et al. (2010), the expected genetic variance between  $F_3$  progenies is  $\sigma^2F_3 = \sigma^2A + 1/4\sigma^2D$ , whereas the expected genetic variance within progenies is  $\sigma^2F_3 = 1/2\sigma^2A + 1/2\sigma^2D$ . It means that there is more genetic variance between than within progenies. Therefore, the individual heritability magnitudes are smaller than those of the heritability when the mean of the progenies is taken into account. Also, the individual selection shows the strong environmental influence and less experimental accuracy because of the higher residual variance, since there is no replication of individuals. The heritability estimate based on the mean of the progeny is even more accurate because the residual variance is adjusted to the number of replications.

Genetic variability in the population is essential to select superior genotypes aiming to improve any crop (Nicolai et al. 2013). Furthermore, the selection method

adopted, the genetic correlations between traits, the type of inheritance involved and the experimental precision also have an essential role in the success of a breeding program (Paiva et al. 2002). The quality of the genotypic assessment should be inferred using the parameter known as accuracy. This parameter expresses the correlation between the real genotypic value and the value predicted according to information resulting from field experiments; the lower the absolute deviations between these values, the higher the accuracy (Resende and Duarte 2007). The accuracy values should be higher than 0.5 since high accuracy indicates high progeny-selection precision. Thus, high accuracy was found in all the herein assessed traits, except for fruit diameter.

The high CVe magnitudes observed in NCF, NDF, FFLA, and PROD indicate relatively low experimental accuracy. In papaya experiments, the ranges of values of the coefficient of variation differ among the traits showing wide variation, justifying the need to use specific evaluation range for each trait (Ferreira et al. 2016). High CVe values have been associated with long-cycle crops, large-sized experiments, different genotype responses to high temperature and drought-related stresses, as well as with different genotype responses to pests, diseases, winds and pruning (Ferrão et al. 2008).

In studies of estimation of genetic parameters to assess the genetic variability of breeding populations, the CVe should be analyzed together with the CVg for more appropriate analyzes. The CVr, which refers to the magnitude of the relation between CVg and CVe, indicates how much of the existing variation results from genetic causes; it measures the accuracy of the inferences that may result from phenotypic assessments. Thus, values above the unit allow making inferences with high and very high accuracy. Magnitudes higher than and close to the unit are favorable to the selection of progenies based on FL, FM, FLLA, SS, PT, NCF, FFIH, PH and SD, thus suggesting that simple methods may be used to provide considerable genetic gains. However, more refined methods should be used to select superior progenies based on traits such as FD, FF, PF, PROD, and NDF.

Breeding values for individuals enable candidates to establish the best strategy to increase the efficiency of breeding (Barbosa et al. 2014). The main advantage of the BLUP method lies in the estimation of the environmental effect-free genotypic values. These values are the most



important for breeders since they are the true values to be predicted and represent the individuals' genetic potential. Since the genetic improvement of papaya plants aims to select genotypes able to meet the highest number of desirable traits, it is essential to use indices able to help the combined selection of traits. The REML/Blup procedure allows ordering genotypes according to each trait through the exploration of all genotype variations within and between progenies (Resende 2016). However, papaya individuals showing favorable responses to several traits are considered superior in breeding programs. Thus, the REML/Blup procedure is not appropriate to select promising genotypes simultaneously based on several traits. It justifies the use of selection indices based on genotypic values in order to increase the efficiency of breeding programs.

The selected progenies showed no significant genetic gains in fruit quality-related traits such as FF, PF, and SS. Equivalent results were observed in competition trials comprising papaya hybrids (Ide et al. 2009). The aforementioned authors concluded that it is difficult combining the fruit production per plant and fruit quality-related traits such as total soluble solids and pulp firmness in the same genotypes. In addition, the authors consider that the negative correlation between total soluble solids and plant production requires breeders to make significant efforts such as generating large-sized segregating populations to identify recombinants showing both traits. Thus, despite the moderate and high heritability magnitudes estimated for FF and SS in the present study, it was not possible to select highly productive progenies showing high FF, PF, and SS.

The lower predicted gains found through the selection within superior progenies when compared to the gains found through the selection between progenies were expected since the heritability of the individual selection unit was lower than that estimated through the mean of the progenies. Despite the smaller gains obtained within progenies, it is necessary to select individuals in order to advance generations and continue the breeding program. However, it was possible to see that the means of most of the selected genotypes were higher than the overall mean of the most important agronomic traits. The FM may explain the negative PROD sign obtained in some plants since this trait results from the multiplication of the NCF by FM. Thus, plants with high NCF may produce

smaller fruits. On the other hand, plants with low NCF may produce larger fruits. The genetic gains obtained with the selection within progenies allow inferring that selection between progenies should be performed to increase the mean of the traits in the advanced generations.

The direct selection based on genetic values obtained by Blup method has been successfully applied to fruit species. However, although the combined selection presents lower gains in comparison to the direct selection, it has been framed as an appropriate strategy in papaya breeding programs. The choice for this strategy comes from the high expectation of achieving success in future generations since it simultaneously takes into consideration the favorable and unfavorable traits to the papaya crop (Silva et al. 2008; Pinto et al. 2013; Ramos et al. 2014b).

Significant genetic variability was observed in most of the traits between progenies, a fact that shows the genetic potential these segregating generations may present in the development of superior lines. It is worth highlighting that several progenies presented higher performances than the Tainung-01 hybrid in traits such as plant production, number of commercial fruits and pulp thickness, which are extremely important to crop improvement (Table 2). It is also worth emphasizing the genetic potential of these progenies; although they derived from self-generation, their agronomic performance was similar to that of the Tainung-01 hybrid, which is a cultivar planted worldwide and used as a reference for representatives of group Formosa.

The IG2 index showed consistency in the ordering of the progenies and facilitated the combined selection of the six superior progenies based on the assessed traits. It also provided promising predicted genetic gains in traits of significant importance to papaya breeding. Six lines were selected within the six superior progenies in order to continue the superior-line development program focused on the Formosa group as a way to help large, medium, and small papaya farmers.

## ACKNOWLEDGMENTS

The authors would like to thank the Caliman Agrícola S/A for financing the experiment. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001. The authors also thank the Coordenação de Aperfeiçoamento de Pessoal



de Nível Superior (CAPES) for granting scholarships to the students Cortes D.F.M., Santa Catarina R. and Vettorazzi J.C.F.

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Conceptualization, Cortes D.F.M. and Pereira M.G.; Methodology, Cortes D.F.M., Ramos H.C.C., Viana A.P., Santa-Catarina R. and Vettorazzi J.C.F.; Investigation, Cortes D.F.M., Santa-Catarina R. and Vettorazzi J.C.F.; Writing – Original Draft, Cortes D.F.M. and Pereira M.G.; Writing – Review and Editing, Cortes D.F.M.; Funding Acquisition, Pereira M.G.; Resources, Pereira M.G. and Ramos H.C.C.; Supervision, Pereira M.G.

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